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Treatment of chronic vulvovaginal candidiasis with posaconazole and ciclopiroxolamine

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ABSTRACT

Therapy of chronic recurrent vulvovaginal candidiasis (VVC) caused by Candida glabrata is still rare in comparison to C. albicans infection, but therapy remains more difficult. Combination therapy with topical antifungals may improve therapy outcome, but still standard agents as fluconazole or itraconazole often fail. Posaconazole is a new systemic triazole with a wide antifungal spectrum including rare Candida species. Up to now, no clinical trials with posaconazole in chronic recurrent VVC have been undertaken. Here, first results of the application of a new therapy regimen consisting of oral posaconazole in combination with topical ciclopiroxolamine are presented. 15 patients with chronic recurrent VVC caused by C. glabrata have been treated. 14 of these patients experienced successful therapy, clinical and mycological cure 30 days after begin of therapy has been observed. Long-term results are promising, as in 4 patients clinical and mycologic cure persists for more than 1 year up to now.

Keywords: Chronic Recurrent Vulvovaginal Candidiasis; VVC; Candida Glabrata; Posaconazole

1. INTRODUCTION

Vulvovaginal candidiasis (VVC) is termed chronic if it recurs four times or more per year at intervals of 8 weeks or less [1]. Literature data suggest that acute vulvovaginal candidiasis becomes chronic in 5-8 % of cases [2,3]. Possible, but heatedly debated, risk factors for vaginal thrush include prior antibiotic or corticosteroid therapy, intrauterine contraceptive devices (coil), high-sugar diet, and certain sexual practices [2]. The etiology of chronic recurrent VVC is little understood even today. Gastrointestinal tract as a reservoir for re-infection, re-infection from sexual partner(s), and recurrent disease as a result of persistent colonization have been postulated. This last postulate is supported by studies showing recurrent disease to be caused by identical strains in the vast majority of cases [2].

Though C. albicans is the main pathogen in more than 95% of cases of acute infection [4], other species are implicated in chronic infection, chiefly C. glabrata. The characteristic features of Candida albicans and Candida glabrata are listed in **Table 1**.

The pathogenic role of C. glabrata is disputed, but patients who present to a physician with fungal disease caused by these atypical pathogens always report symptoms. Though vaginal discharge is rare, redness, agonizing itching, and a sour-smelling sticky discharge are characteristic. A diagnosis of “harmless commensal” is therefore both inappropriate and scientifically inaccurate. Both organisms are biosafety class 1 organisms. Hence, though low-virulence, they are not apathogenic—unlike Saccharomyces cerevisiae.

2. TREATMENT OF PROBLEM FUNGI

The therapeutic goal in chronic recurrent VVC is the eradication of the pathogen. The chances of achieving that goal appear to be good. Unlike C. albicans, non-albicans Candida organisms are located predominantly vaginally. Measures to prevent recurrence should be initiated before starting therapy [5]. Oral and bowel contamination in the patient (3 fecal samples taken on 3 different days) should be investigated, which may result in measures such as professional dental and denture cleaning and eradication of pathogens in the mouth and bowel with amphotericin B or nystatin (C. glabrata) in the form of lozenges or sugar-coated tablets. A hormonal coil is a potential pathogen reservoir and should ideally be removed before therapy. The sexual partner should be involved in treatment too. Colonization of the prostate (sperm sample) and contamination of dentures (smear tests) in older partners should be investigated.

Table 1. Characteristics of C. albicans and C. glabrata as organisms causing vaginal candidiasis.

Property	C. albicans	C. glabrata
Etiology	Mostly intra partum	Frequently iatrogenic
Course	Acute	Chronic
Findings	Discharge Redness Pruritus	Redness Pruritus
Virulence class	2 (high)	1 (low)
Pseudomycelium	Yes	No
Blastospores	midsize, oval	Small-cell, round
Chlamydospores	Yes	No
Hormone situation	Dependent	No
Antifungal	Pathogen susceptibility (S= sensitive, R= resistant)	
Clotrimazole	S	R
Nystatin	S	S
Ciclopiroxolamine	S	S
Fluconazole	S	R
Posaconazole	S	S
Drug of first choice: - topical - systemic	 Clotrimazole Fluconazole	 Ciclopiroxolamine Posaconazole

Drug therapy should encompass both a topical and a systemic component. C. glabrata is located deep in vaginal tissue-up to 10 layers deep-which explains the high failure rate for local treatment attempts. C. glabrata also thrives in surface locations–under the foreskin, around the clitoris, in the anal folds and pubic hair–and so the effectiveness of systemic therapy is limited. Therefore, combination therapy seems to be necessary to appropriately treat the patient and avoid recurrence of infection. It needs to be stressed that in addition, patient cooperation is crucial, as rigorous, reliable local treatment is required in conjunction with properly administered systemic therapy.

C. glabrata isolates have very low sensitivity to fluconazole and may be resistant in many cases. Secondary resistance is mainly due to two factors: genetic variability of the pathogen, which develops resistance to fluconazole at doses below 800 mg, and the fact that many therapists administer fluconazole doses as low as 150 mg [6]. In contrast, in vitro sensitivity to posaconazole is high [7]. Until just a few years ago, systemic high-dose treatment with 800 mg fluconazole was the treatment of first choice [8]. Given the large number of current treatment failures, the treatment approach is not advisable today [5]. Nevertheless, the options for treating invasive fungal infection of the kind are better now than ever. The most promising agents according to the current state of scientific knowledge are the triazole derivative posaconazole and the echinocandins caspofungin and anidulafungin. The latter require intravenous dosing and are associated with high treatment costs. Since vaginal candidiasis is usually treated in an ambulatory setting, these agents are of lesser relevance for treatment.

Ciclopiroxolamine is the most effective local drug for treating clotrimazole- and nystatin-resistant organisms [8]. It is the antifungal with the broadest spectrum, as well as showing deep penetration and sporicidal activity. The mechanism of action is polyvalent. Ciclopiroxolamine's activity is not limited to the level of ergosterol synthesis. It also targets regions mitochondria and protein synthesis, making it the ideal combination partner for all systemic antifungals. Inhibition of catalase production is particularly effective, because it disables metabolization of toxic H_2O_2 occurring in the fungal cell. All clinically relevant Candida species are sensitive to ciclopiroxolamine [8].

Posaconazole is a triazole derivative whose mechanism of action is based on inhibiting ergosterol synthesis [9]. It is effective against a variety of fungal pathogens. These include all relevant Aspergillus species, the organisms responsible for mycetoma, coccidioidomycosis, chromoblastomycosis, and refractory pathogens like Mucor and Fusarium [10,11]. The spectrum of action also includes Candida and especially the problem pathogens C. glabrata and C. krusei [7,10] which are resistant to fluconazole, itraconazole and voriconazole. Posaconazole's molecular structure gives it multiple docking sites, so different mutations would have to occur simultaneously for it to be ineffective [12]. In contrast, a single mutation is enough to induce resistance to fluconazole [12]. Posaconazole has linear kinetics up to a dose of 800 mg [9]. Exposure can be increased by division into two divided doses of 400 mg (10 ml suspension BID), and is further enhanced by dosing together with high-fat food, e.g. custard made with full-fat milk. It has a high volume of distribution (1744 liters), suggesting very good distribution. Posaconazole is metabolized to a very slight extent and is primarily eliminated with the feces. Posaconazole has the lowest potential for interactions of all systemic azole derivatives, since interactions with the CYP system are limited to CYP3A4 inhibition. The most common side effects reported in studies involving a total of 2400 subjects and patients were headache and nausea [9]. Posaconazole is approved for prevention of systemic infection in high-risk hemato-oncology patients, for salvage therapy of certain

invasive mould infections and for the treatment of oropharyngeal candidiasis [9]. Up to now, there is no clinical data supporting efficacy in treatment of vaginal candidiasis. In a murine vaginal model, posaconazole reduced the fungal burden of both fluconazole-susceptible and fluconazole-resistant Candida albicans strains [4].

The difficulty to treat chronic vaginal infections caused by C. glabrata lead to the development of a concept comprising a posaconazole-ciclopiroxolamine combination therapy for patients, who seemed to have exhausted all conventional treatment options.

3. PATIENTS

Patients were eligible if they had a chronic vaginal candidiasis caused by C. glabrata, a history of therapy failure and reduced sensitivity towards licensed antifungals indicated by susceptibility testing.

All patients were carefully examined, including microbiologic and dermatologic diagnosis, to exclude non-fungal infections and a non-infectious dermatosis such as lichen ruber, neurodermitis or psoriasis inversa. To avoid recurrence, the patients' surroundings were carefully examined mycologically by taking swabs or samples and cultivation on appropriate media (see Diagnostic Procedures). This included the partner's oral cavity, penis and sperm, but also the patient's oral cavity, vagina, clitoris and faeces. Vaginal swabs of all patients were taken and cultured before start of therapy for species identification and susceptibility testing. Foreign objects as Nuva-Ring or hormonal coil were removed before start of therapy.

Therapy response was assessed by clinical and mycologic examination at 7 and 30 days after end of therapy, respectively. For some patients, long-term results up to 2 years after therapy are available. Patients who have been treated here come from all over Germany, so that long-term follow-up is not possible in every case. For mycologic examination, vaginal and clitoris swabs were taken and inoculated as described below. Further follow-up visits are conducted regularly, about every 30 days, the results are presented below.

A successful therapy outcome was defined as substantial improvement of the patient's clinical infection signs and symptoms 7 days after start of treatment, as well as persistent clinical and mycologic cure 30 days after begin of therapy.

All patients were informed about the compassionate use project in depth and about the existing database on posaconazole in antifungal treatment. Health insurance reimbursement was checked and agreed in advance for all patients.

4. DIAGNOSTIC PROCEDURES

For species identification, swabs were inoculated onto chromID™ Candida agar, bioMérieux Deutschland GmbH, Nürtingen, Germany, which is specific for yeast isolation and direct identification of Candida albicans. Candida albicans colonies are coloured blue by specific hydrolysis of a hexosaminidase chromogenic substrate after 2 day of incubation at 37°C. The species identification of non-blue-coloured colonies like C. glabrata and C. krusei were examined by their assimilation pattern with the ID 32C yeast identification system, bioMérieux Deutschland GmbH, Nürtingen, Germany. ID 32C system consists of a single-use disposable plastic strip with 32 wells to perform 29 assimilation tests (carbohydrates, organic acids, and amino acids), 1 assimilation test with a negative control, 1 susceptibility test (cycloheximide), and 1 colorimetric test (esculin) including a database of 63 different species. Results were recorded by direct reading after 48 h of incubation at 30°C.

The susceptibility of all isolates towards itraconazole and flucoanzole was tested routinely, as these are the only systemic antifungal agents licensed to treat VVC. FUNGITEST™, Bio-Rad, Marnes-la Coquette, France was used. In general, this test is used to study growth of yeasts in the presence of 6 antifungal agents at 2 different concentrations, among them fluconazole and itraconazole. Growth assessment is based on reduction of the coloured indicator which turns the medium from blue to pink. When growth is inhibited by the fungal agent, the medium remains blue. Two growth and 2 negative controls are included in the test system. The interpretation of the results was performed according the following colour characteristics: Blue-blue = no growth, strain inhibited by the antifungal agent, sensitive strain ("S"); Pink-blue = low growth, intermediate strain ("I"); Pink-pink = growth, strain not inhibited by the antifungal agent, resistant strain ("R"). The breakpoints have been chosen following the study of the distribution of the antifungal agents MIC obtained with prototype microplates used with the same procedure as FUNGITEST™. If susceptibility testing indicates intermediate susceptibility or resistance towards fluconazole or itraconazole, therapy attempts with these agents cannot expect to be successful because of the genetic haploidy of C. glabrata.

5. THERAPY

All patients received a combination of systemic and topical therapy (**Table 1**). For systemic therapy, posaconazole was administered for 15 days at a daily dose of 800 mg (10 ml BID). Topical therapy consisted of ciclopiroxolamine cream BID administered intravaginally

every morning and evening. 12 of the patients received additional topical treatment with Nystatin Ovula (100,000 IU q.d.). Further measures were initiated in addition, if required: shaving of pubic hair of both partners before initiating therapy; antifungal decontamination of both partners (mouth, bowel, sperm).

6. RESULTS

15 patients with chronic vaginal candidiasis caused by C. glabrata were treated (**Table 3**). On average, they were 46.2 years old (range 20-76 years). For all patients, vaginal control swabs at baseline were positive for Candida glabrata, and most of the patients also had evidence of C. glabrata below the clitoral hood. 13 of the 15 patients had a history of failed treatment with fluconazole, 2 of these patients received even more than one systemic antifungal. Remaining 2 of the 15 patients had both been pretreated topically with clotrimazole. Both patients were infected with C. glabrata-strains that showed only intermediate susceptibility to fluconazole and itraconazole.

In total, 7 of 15 C. glabrata strains were resistant to fluconazole, 6 showed intermediate susceptibility and 2 results indicated susceptibility, although both patients experienced clinical failure with fluconazole. All 7 strains resistant towards fluconazole were also itraconazole-resistant, and in addition, one of the strains exhibiting intermediate susceptibility towards fluconazole tested itraconazole-resistant. No strain was itraconazole-susceptible.

All patients received treatment with posaconazole and ciclopiroxolamine in accordance with the regimen in **Table 2**. 14 of the 15 patients were treated successfully with this regimen: they experienced substantial clinical improvement already after 7 days of treatment; no clinical signs and symptoms of the infection could be detected 30 days after begin of therapy, and all mycologic swabs were negative for Candida glabrata. 11 of the 14 patients with successful therapy outcome after 30 days could be monitored for a longer time to detect a possible recurrence of infection. No patient showed any sign or symptom of recurrent fungal infection, and culture diagnostics have always been negative since. Clinical and mycological cure persists for about 60 days in 4 patients, for 90 days in 3 patients and for more than 1 year in 4 of the patients. Clinical success of the applied therapy regimen is documented photographically. One patient (patient No. 7 in **Table 3**) experienced therapy failure. This patient evidently failed to implement the treatment processes. She was transferred to hospital for intravenous treatment with an echinocandin, and topical therapy has been stopped there. The patient experienced recurrence of infection. Posaconazole was well tolerated by all patients, similarly to fluconazole.

7. DISCUSSIONS

Modern systemic antifungals such as posaconazole open up prospects for successful and sustainable therapy. No clinical data are available to date on posaconazole in the treatment of chronic recurrent vaginal candidiasis. Preclinical data suggest that posaconazole may be an option for this difficult-to-treat infection [4,7,12]. Posaconazole activity against Candida species seems to be high [7].

The key to a successful cure seems to be the combination of the highly active substances posaconazole and ciclopiroxolamine. Since nystatin is effective against C. glabrata, it may be included in the treatment regimen as well.

Posaconazole has been favoured as systemic combination partner over an echinocandin, although the main indication of echinocandins is the therapy of Candida

Table 2. Regimen for treatment of refractory chronic vaginal fungal disease caused by C. glabrata (according to Tietz).

Treatment	Conduct
Oral	**Posaconazole**, **800 mg** (suspension in 3 105-ml bottles) 400mg BID = 10ml BID with high-fat food (e.g. custard made with full-fat milk)
Local [1]**:** A) morning	**Ciclopiroxolamine Cream 35 g** Introduce deep into the vagina and apply to labia, vaginal opening, under the foreskin/clitoral hood, and from perineum to anus
B) evening	**Ciclopiroxolamine Cream 35 g** (repeat as stated under A) *Additionally at night*: **Nystatin Ovula**, inserted at depth once daily
Supportive	A) Pubic shaving of both partners before starting therapy B) Mouth, bowel and sperm of both partners must be fungus-free C) Remove IUD beforehand

[1] Treatment takes place from treatment day 1 in parallel with systemic therapy. Patients are required to use up their full supply of drugs.

Table 3. Therapy of chronic recurrent VVC in 15 patients with posaconazole and ciclopiroxolamine*.

No	Age	Premedication		Suceptibility testing						Last negative culture result after	Clinical and myclogical cure
		Antimycotic	Daily dosage	Itraconazole			Fluconazole				
				S	I	R	S	I	R		
1	43	Fluconazol	800 mg, 2 weeks			X			X	39 days	+
2	76	Fluconazol Voriconazol Anidulafungin Caspofungin	400 mg, 10 days 400 mg, 6 days 50 mg, 21 days 100 mg, 10 days 50 mg, 14 days			X			X	2 years	+
3	45	Fluconazol	800 mg, 2 weeks			X			X	1 year	+
4	24	Clotrimazol	several times		X			X		92 days	+
5	20	Fluconazol	150 mg, once		X		X			2 years	+
6	52	Fluconazol Itraconazol	150 mg, once 400 mg, once		X		X			98 days	+
7	72	Clotrimazol	Several times		X			X		non	non
8	51	Fluconazol	800 mg, 2 weeks		X			X		56 days	+
9	44	Fluconazol	800 mg, 2 weeks			X			X	90 days	+
10	63	Fluconazol	400 mg, 2 weeks			X		X		1 year	+
11	34	Fluconazol	400 mg, 2 weeks		X			X		60 days	+
12	51	Fluconazol	800 mg, 2 weeks			X			X	60 days	+
13	30	Fluconazol	800 mg, 2 weeks			X			X	35 days	+
14	52	Fluconazol	150 mg, once		X			X		76 days	+
15	37	Fluconazol	800 mg, 2 weeks			X			X	35 days	+

* Patients had a history of treatment with 800 mg over a 2-week period

infections. Echinocandins are only available in intravenous formulation, hence their application is complicated and maybe too costly in an ambulatory setting.

On the basis of the current state of scientific knowledge, it was possible to develop a treatment concept for successful treatment of patients who had exhausted conventional treatment options. From an ethical point of view, too, it was appropriate not to withhold the drug until marketing authorization. Unlike C. albicans fungal disease, which typically is associated with a high risk of re-infection, disease caused by C. glabrata was successfully cured. 14 of 15 patients in this drug use evaluation achieved a cure. The follow-up period ranged from 90 days to 2 years in 11 of the 14 patients, no relapses occurred. Concomitant topical treatment deserves merit as the second mainstay of success; systemic antifungals on their own will probably not reach all the remote niches in which pathogens may be present.

Larger clinical trials should be conducted to further study the efficacy of posaconazole in the treatment of chronic recurrent vaginal candidiasis caused by rare Candida species as C. glabrata and C. krusei.

REFERENCES

[1] Mendling, W. (2006) Vaginose, vaginitis, zervizitis und salpingitis. 2nd Edition, Springer Medizin Verlag, Heidelberg.

[2] Watson, C. and Calabretto, H. (2007) Comprehensive review of conventional and non-conventional methods of management of recurrent vulvovaginal candidiasis. *Aust N Z J Obstet Gynaecol*, **47(4)**, 262-272.

[3] Eschenbach, D.A. (2004) Chronic vulvovaginal candidiasis. *New England Journal of Medicine*, **351(9)**, 851-852.

[4] González, G.M., *et al.* (2007) Therapeutic efficacy of posaconazole against isolates of Candida albicans with different susceptibilities to fluconazole in a vaginal model. *Medical Mycology*, **45 (3)**, 221-224.

[5] Mendling, W., *et al.* (2008) Vulvovaginalkandidose. Guideline of the German Society of Gynecology and Ob-

stetrics, German Dermatologic Society, German-Speaking Mycologic Society, Berlin.
[6] Czaika, V., *et al.* (2000) Antifungal susceptibility testing in chronically recurrent vaginal candidosis as basis for effective therapy. *Mycoses*, **43(Suppl 2)**, 45-50.
[7] Pfaller, M.A., *et al.* (2001) Vitro activities of posaconazole (Sch 56592) compared with those of itraconazole and Fluconazole against 3,685 clinical isolates of Candida spp. and Cryptococcus neoformans. *Antimicrobial Agents and Chemotherapy*, **45(10)**, 2862-2864.
[8] Tietz, H.J. and Sterry W. (2006) Antimykotika von A-Z., Thieme, 4.Auflage, Stuttgart.
[9] Smith, W.J., Drew, R.H. and Perfect, J.R. (2009) Posaconazole's impact on prophylaxis and treatment of invasive fungal infections: an update. *Expert Review of Anti-Infective Therapy*, **7(2)**, 165-181.
[10] Herbrecht, R. (2004) Posaconazole: A potent, extended spectrum triazole antifungal for the treatment of serious fungal infections. *International Journal of Clinical Practice*, **58(6)**, 612-624.
[11] Schiller, D.S. and Fung, H.B. (2007) Posaconazole: An extended spectrum triazole antifungal agent. *Clinical Therapeutics*, **29(9)**, 1862-1886.
[12] Hof, H. (2008) Is there a serious risk of resistance development to azoles among fungi due to the widespread use and long-term application of azole antifungals in medicine? *Drug Resistance Updates*, **11(1-2)**, 25-31.

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Hematopoietic stem cells from peripheral blood the perspective of non-mobilized peripheral blood

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ABSTRACT

The peripheral blood is a major source of hematopoietic stem cells. Almost for two decades the peripheral blood has been mobilized, in order to enhance the $CD34^+$ concentration. The isolated stem cells from the mobilized peripheral blood are used as an alternative, or in addition to bone marrow derived stem cells. In this paper, a new perspective is being discussed; the use of non-mobilized peripheral blood as an alternative source for hematopoietic progenitor cells. The number of isolated hematopoietic stem cells is evaluated using flow cytometry. The viability can be evaluated using the trypan blue exclusion test, the flow cytometry or automated assays. The isolated hematopoietic stem cells could be used for ex vivo expansion either in static systems or in proper bioreactor systems, prior to cryopreservation and/or transplantation.

Keywords: Non-Mobilized Peripheral Blood; Hematopoietic Stem Cells; Ex Vivo Expansion

1. INTRODUCTION

Since the early 1990s, peripheral blood progenitor cells collected by apheresis have largely replaced bone marrow as a source of hematopoietic stem cells for autologous transplantation [1]. Peripheral blood cells produce more rapid hematopoietic recovery, thereby leading to reduced costs [2-5]. Furthermore, although follow-up is more limited in the PBSC group than in the BM group, no evidence was found that the use of PBSC was associated with an increased risk of chronic GVHD compared to results with BM [6].

2. CHARACTERIZATION OF HEMATOPOIETIC STEM CELLS

Hematopoietic stem cells are normally found in very limited numbers in the peripheral circulation (less than 0.1% of all nucleated cells). It is logical that progenitor cells circulate in the periphery, as this ensures an even distribution of hematopoiesis within the BM [7]. CD34 antigen expression is used as a surrogate marker for hematopoietic stem cells and enumeration of $CD34^+$ cells has been used to quantify progenitor and stem cell content [8].

PBSCs represent a subpopulation of all $CD34^+$ cells ($CD34^+/CD38^-$) found in the circulation [9]. $CD34^+$ cell viability was measured by an established flow cytometric method [10]. The method is based on triple staining with anti-CD34-PE, anti-CD45-FITC, and the viability marker 7-actinomycin D, and it allows the calculation of the absolute numbers of viable $CD34^+$ cells. Recently, a new rapid and accurate method has been developed for the viability evaluation based on luminometry [11].

3. CRYOPRESERVATION AND THAWING OF PBPCS

PBPCs are usually harvested and stored in liquid N_2 until reinfusion. Storage at this low temperature will block all enzymatic pathways and metabolism in the cell [12]. Cryopreservative(s) must be added to the PBSC before freezing in order to protect the cells. The concentration of the cryoprotectant and the rate at which the cells are frozen are the main factors governing the survival of the cells. Thereafter the cells are stored in liquid N_2 [13]. Due to their different cell membrane composition and higher osmotic inactive volume, $CD34^+$ cells are better protected from hypertonic shock and ice crystal formation and should be more resistant to cryopreservation damage than the remaining nucleated cell population [14].

A computer-controlled freezer is used for the cryopreservation. In order to ensure rate-controlled freezing an optimized program is developed and adjusted accordingly.

In controlled rate freezing, the concentrated stem cells are frozen down at a rate of $1\text{-}2^0$C/min up to a tempera-

ture point of about –40˚C. Then, the freezing process down to a target of –120˚C is performed at a faster pace, about 3-5˚C/min. For PBSCs the controlled rate freezing process is considered standard [15,16], and was in different reports found to be superior to uncontrolled freezing approaches. This procedure is time consuming and requires staff with a specific expertise. Hence, the use of uncontrolled rate freezing in which the specimen is first cooled down to –4˚C and then directly deposited into a freezer at –80˚C or put into liquid phase nitrogen has been evaluated. Several reports [17-19] established that the uncontrolled method is safe and reveals comparable results to the controlled rate process for PBSCs. A controlled study performed by Perez-Oteyza *et al*. [20] showed that the controlled and uncontrolled rate freezing approach are comparable in terms of viability testing and that only a statistically significant decrease in the CFU-GM clonality assay could be detected in the uncontrolled freezing situation. Recent studies suggested that uncontrolled freezing is also a viable approach for UCB stem cells [21,22].

As a cryoprotectant, a solution containing 50% DMSO in HAES-steril® 10% is used. Prior to freezing, a part of pre-cooled cryosolution (with 50% DMSO) is mixed with three parts of the buffy coat (pre-cooled), to achieve DMSO concentration of 5 or 10% in the final solution. Cryopreservation is then carried out in aliquots in cryogenic vials.

Current protocols for cryopreservation of PBPCs are usually based on the use of 10 percent DMSO in the freezing medium [23]. HPCs can be preserved with 5% DMSO, and such autografts can be safely used for stem cell rescue even after long-term nitrogen storage [24].

Several techniques for the thawing procedure have been proposed. The standard method is warming in a water bath at 37˚C until all ice crystals disappear [19]. A German study compared the thawing of cryopreserved units in a warm water bath with dry heat applied by gel pads at 37˚C. The viability and clonogenic potential were comparable, with a trend towards less infectious contamination in the dry method [25]. Different studies examined the preservation of function when thawed units were incubated at 0-37˚C [19,26].

Akkök *et al.* [25] suggest that even simple single-wash DMSO depletion causes significant $CD34^+$ cell loss. Despite a beneficial impact on the frequency of adverse effects during and after stem cell infusion, this time-consuming procedure caused a delayed PLT recovery and increased requirement for PLT transfusions. The $CD34^+$ cell loss, however, was never critically low, that is, never lower than 2×10^6 per kg. They concluded that single manual washing of autografts is a simple and safe procedure that decreases the frequency of adverse events during and after stem cell infusion. The procedure should be recommended especially for patients with an increased risk of serious toxicity, for example, patients with cardiac amyloidosis.

4. THERAPEUTIC DOSES OF PBSC

Typical doses of $CD34^+$ stem cells used for PBSCs are 2×10^6 cells/kg of recipient body weight or greater. Doses lower than this threshold is associated with prolonged cytopenias and increased early mortality [27]. The use of higher doses of $CD34^+$ cells may lead to quicker engraftment, particularly when doses are greatly increased [28,29]. Platelet recovery appears to be more sensitive to $CD34^+$ doses than neutrophil recovery [29]. Efforts to enrich PBSCT by ex-vivo $CD34^+$ cell selection (positive selection) have resulted in increased rates of GVHD, possibly by altering the cytokine expression patterns of transplanted cells or changing lymphocyte subsets delivered with the graft [30].

Autologous stem cell grafting has been used with varying degrees of success in chronic myelogenous leukemia (CML) [31,32], acute leukemia [33], myelodysplasia [34], and multiple myeloma [35].

Niwa *et al*. [36] reported successful autologous peripheral blood stem cell transplantation with a double-conditioning regimen for recurrent hepatoblastoma after liver transplantation.

Nevskaya *et al*. [37] with preliminary results suggested the feasibility of therapeutic angiogenesis by local implantation of $CD34^+$ and MNC from PB for Systemic Sclerosis ischemic ulcers. Improved endothelial function, stimulatory effects on circulating endothelial precursors kinetics and augmentation of microcirculatory blood flow may contribute to therapeutic potential of the implanted cells.

5. COST ANALYSIS

The cost method involved two sets of data: a data set including patient-related or direct costs, and a data set including nonpatient-related or indirect costs [38]. The patient-related costs comprise the followings: 1) hospitalization and basic medical service, including medical and nursing staff; 2) pharmacy and blood products; 3) procedures such as operating theatre, leukapheresis and cryopreservation. On the other hand, the indirect costs comprise the clinical service department costs, for instance, radiology, clinical chemistry, pharmacy and the nonclinical service departments such as transportation, housekeeping and kitchen services.

A study by Mishra *et al*. [38] reported a cost analysis using mobilized peripheral blood at 2001 prices and the

costs had been recalculated into US$ by using the exchange rates of 1st January 2001. The mean cost for the mobilization/cryopreservation phase per patient was US$ 6544 (range 5114-7273). The mean cost of high dose chemotherapy followed by hospitalization was US$ 25616 (13978-43277). This amounts to total running costs of US$ 32160 (19092-50550). Taken together, staffing, medication and blood products contributed to 74% of total costs. On average, 53% of total costs comprised staff costs, ranging from 39 to 76%. Personnel resources varied from one center to another, from US$ 12608 to US$ 26038 per patient. Pharmacy and blood products contributed 16 and 5%, respectively, of the total costs.

A study by van Agthoven [39] documented total costs of PBSC transplantation at Euro 33742. The author applied a unit cost method where staff costs accounted for 42% of the transplant cost. This relatively large difference in staff costs between Van Agthoven and Mishra *et al*. is notable, and may indicate there are cost variations between different countries, for example, related to wages. Van Agthoven [39] reported a remarkably low cost per patient for the stem cell harvesting and cryopreservation procedures, an average of €4982, and a blood component cost (during the induction chemotherapy regimen, harvesting and transplantation phase), respectively, of €904, €376 and €1680, a total of €2960.

In another study, Ghosh *et al.* [40] reported a PBSCT cost for patients with plasma cell leukemia that ranged from US$ 20000 to US$ 25000. The major part of the costs related to hospitalization, growth factors, blood products, collection and cryopreservation of PBSC.
Hopefully, the use of non mobilized peripheral blood could eliminate the cost, since there are no mobilization drugs and no any special equipment required.

6. EX VIVO EXPANSION OF HSC

The $CD34^+$ surface antigen, which is a glycoprotein expressed on early progenitor cells is present on less than 0.1% of the mononuclear cells in peripheral blood [41]. Many studies have shown that the minimum acceptable dose of HPCs for successful transplantation ranges between $2 – 5 \times 10^6$ $CD34^+$ cells per kg of recipient weight [42]. Furthermore, transplantation of higher doses of $CD34^+$ cells seems to improve haematopoietic recovery and overall survival [43,44]. To try and overcome the problem of low progenitor cell dose, ex vivo expansion of CB-derived cells has been attempted. The true test of this method is whether an expansion technology will be able to provide a reliable, reproducible increase in the number of progenitor cells available from a single unit of CB, resulting in superior rates of engraftment and overall survival in adult patients. A significant hurdle of presently available methods for graft production is the ability to generate an expanded population of committed hematopoietic progenitor cells without compromising the numbers of less differentiated progenitor cells ($CD34^+$ $CD38^-$ or $CD34^+$ Lin^- cells), which are important functional hematopoietic repopulating cells [45].

In order to consistently achieve an adequate cell dose, the processing methods must minimize cell losses. Each additional manipulation of a cellular product potentially leads to further loss of cells. In most studies, $CD34^+$ cell selection is done before initiating cell culture [46], but the $CD34^+$ cell selection itself is associated with a substantial loss of progenitor cells. This cell loss, which may not be significant for smaller children, may become critical in reaching a suitable dose for transplant in older children and adults [47].

To achieve adequate cell doses, many researcher used different ex vivo expansion protocols, either the traditional way, or using a bioreactor system. Beshlawy *et al*. [48] used three cytokine combinations, *i.e.* cell factor alone, IL-3 alone, and both stem cell factor and IL-3. Interleukin-3 enhances the amplification of early and committed progenitor cells without impairing the long-term engraftment of stem cells [49].

Several investigators reported significantly decreased cell viability after cryopreservation [50-51] and attributed this to the effect of thawing and washing to remove the cryoprotectant. Laroche *et al*. [47] stated that thawing and washing result in loss of cells approaching 20% when compared with pre-freeze counts, with the wash step responsible for nearly half of this cell loss. However, Beshlawy *et al.* [48], using umbilical cord blood derived hematopoietic stem cells, detected mean fold expansion of 6.64 ± 3.34 with stem cell factor alone, 7.38 ± 2.86 with both stem cell factor and IL-3, and 8.11 ± 4.49 with IL-3 alone after 2 days culture of the samples frozen for 2 weeks. There were no statistically significant differences in fold expansion between the 3 cytokine combinations before freezing and after 1 week and 2 weeks of freezing. They concluded that although preservation procedures could decrease the count and viability of cord blood HSCs, freezing does not impair their ex vivo expansion potential; however, it results in a significant loss of cell viability.

In a similar study, Moezzi *et al*. [51] used stem cell factor, IL-3, and thrombopoietin and reported levels of expansion of (4.2-4.7 fold) after 7 days of culture of samples cryopreserved for 1 month.

It was shown that a combination of early- and late-acting cytokines, including SCF, thrombopoietin (TPO), G-CSF and IL-3, resulted in only a marginal-fold expansion of late ($CD34^+$) and early ($CD34^+CD38^-$) progenitor

cells, probably due the fact that the late-acting cytokines drive the cultures mainly toward accelerated differentiation [52,53].

On the other hand, cultures with only early-acting cytokines (SCF, TPO, IL-6 and FLT-3 ligand) resulted in better and prolonged expansion of both late and early progenitors [54], which are important for short-term early trilineage engraftment [55-57].

Peled *et al*. [58] suggested that TEPA supports the self renewal division cycle without compromising differentiation capacity of hematopoietic stem cells.

A number of serum-free media have been used over the last few years with different results [59-62]. For obtaining sufficient numbers of progenitor cells for transplant, FCS [63] and autologous plasma [60,61] have been used in clinical expansion protocols. However, Lam *et al*. [61] suggested that, with the appropriate serum-free media and cytokines, FCS may be excluded in clinical expansions. On the other hand, human plasma, which may contain factors that promote cell maturation [64,65] is thus unlikely to add significant value to the expansion.

It has been reported that MSC constitutively secrete various hematopoietic cytokines, among them stem cell factor (SCF), Flt-3 ligand (FL), thrombopoietin (TPO), leukemia-inhibiting factor (LIF), interleukin (IL)-6, IL-7, IL-8, IL-11, IL-12, IL-14, and IL-15 [66-68]. Addition of MSC as a feeder layer has been shown to improve expansion of cord blood HSC, inhibit their differentiation, and decrease their rate of apoptosis [66,69-71]. Li *et al*. [66] demonstrated that bone marrow MSC can increase human adult PBSC expansion as compared with culture in the presence of cytokine alone.

Conventional culture systems such as T-flasks and gas permeable blood bags are the most widely used devices for expanding hematopoietic cells. However, such static culture systems have several inherent limitations. Firstly, lack of mixing results in concentration gradients for dissolved oxygen (DO), pH, cytokines and metabolites. Secondly, the environmental conditions in well-plate and T-flask are not readily monitored or controlled online. Thirdly, static systems require repeated changes of culture medium, which significantly increases the risk of contamination. Hence there is an urgent need for developing bioreactors for HSCs expansion, which overcomes the limitation of mass transport, keeps culture parameters constant and controls differentiation [72].

Several kinds of bioreactors have been applied in the field of HSCs expansion, including stirred tank bioreactor, fixed bed bioreactor and perfusion chamber [73-75]. It is known that hematopoietic cells are extremely sensitive to shear force, hence cells may suffer some physical damage under shear environment like in a stirred tank bioreactor and perfusion chambers [73]. In stirred tank bioreactor, agitation may affect cell surface marker expression, including cytokine receptors, which can have a profound effect on which cells expand and to what extent expansion occurs [73]. A condition with low shear but low concentration gradients is highly desirable for hematopoietic cell expansion [72].

Rotating wall vessel (RWV) bioreactor may provide a technical solution. The RWV bioreactor has several key characteristic features as follows [76]: firstly, fluid flow is near solid body and is laminar at most operating conditions, which avoids the large shear stresses associated with turbulent flow and allows introduction of controlled and nearly homogenous shear fields; secondly, the culture medium is gently mixed by rotation, avoiding the necessity for stirring vanes, which may damage cells by both local turbulence at their surface and the high flow rates created between the vessel walls and the vanes; thirdly, there is no headspace in the RWV bioreactor while in roller bottles, due to incomplete filling of the vessel, the air in the headspace creates turbulence and secondary bubble formation in the culture medium, which are both potent sources of extra shear and turbulence; finally, the RWV bioreactor supports coculture efficiently by bringing different cell types of different size and density together simply and efficiently. So by optimizing the geometry of the bioreactor and operational condition, it is possible to provide a uniform and low shear condition within the bioreactor. At the same time concentration gradient can be minimized.

RWV bioreactors have been used to simulate microgravity in space flight to study how microgravity affects the hematopoiesis of astronauts [77,78], to proliferate BM cells [79]. The RWV bioreactor can provide a 3D suspension culture environment and all hematopoietic cells are suspended in the culture medium effectively, which overcomes the concentration gradients in T-flasks and makes the utilization of cytokines more effective.

The National Aeronautics and Space Administration (NASA) developed two RWV bioreactors for tissue mass culture [80]. The slow turn lateral vessel (STLV) bioreactor has been used to culture several kinds of cells both on Earth and in space. The STLV was operated at 15-30 rpm on Earth and slower in space allowing a free-fall state, reducing the shear stress. The high aspect ratio vessel (HARV) bioreactor has a similar design, but the rotating speed can be slower than STLV. The NASA RWV systems have been used to study the effects of microgravity on murine HSC and evaluating the hematopoietic homeostasis during long space expeditions [81].

The elucidation of mechanisms governing self-renewal and differentiation of HSC is needed to control the in

vitro expansion. Results from pilot clinical trials of transplants using expanded UCB-HSC have shown no adverse effects in the patients. However, more clinical trials must be conducted using expanded HSC for guarantying the safety [82]. Very recently, Delaney *et al*. (2010) claimed that when cord blood progenitors expanded ex vivo in the presence of Notch ligand we infused in a clinical setting after a myeloablative preparative regimen for stem cell transplantation, the time to neutrophil recovery was substantially shortened. This is the first instance of rapid engraftment derived form ex vivo expanded stem/progenitor cells in humans [83].

7. AUTHORS' CONTRIBUTION

All authors contributed substantially to this research. V.K., J.G., and N.G. designed research and collected the data; V.K., Z.P., A.P., E.N., I.K., and K.A.A. performed literature revision; V.K. analysed and interpreted data, and wrote the manuscript. All authors drafted the manuscript, revised it critically and approved it.

REFERENCES

[1] Champlin, R.E., Schmitz, N., Horowitz, M.M., Chapuis, B., Chopra, R., Cornelissen, J.J., Gale, R.P., Goldman, J.M., Loberiza, F.R., Hertenstein, B., Klein, J.P., Monstserrat, E., Zhang, M.J., Ringdeon, O., Tomany, S.C., Rowlings, P.A., Van Hoef, M.E. and Gratwohl, A. (2000) Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. *Blood*, **95(12)**, 3702-3709.

[2] Bensinger, W.I., Clift, R., Martin, P., Appelbaum, F.R., Demirer, T., Gooley, T., Lilleby, K., Rowley, S., Sanders, J., Storb, R. and Buckner, C.D. (1996) Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: A retrospective comparison with marrow transplantation. *Blood*, **88(7)**, 2794-2800.

[3] Smith, T.J., Hillner, B.E., Schmitz, N., Linch, D.C., Dreger, P., Goldstone, A.H., Boogaerts, M.A., Ferrant, A., Link, H., Zander, A., Yanovich, S., Kitchin, R. and Erder, M.H. (1997) Economic analysis of a randomized clinical trial to compare filgrastrim-mobilized peripheral-blood progenitor cell transplantation and autologous bone marrow transplantation in patients with Hodgkin's and non-Hodgkin's lymphoma. *Journal of Clinical Oncolology*, **15(1)**, 5-10.

[4] Beyer, J., Schwella, N., Zingsem, J., Strohscheer, I., Schwaner, I., Oettle, H., Serke, S., Huhn, D. and Siegert, W. (1995) Hematopoietic rescue after high-dose chemotherapy using autologous peripheral-blood progenitor cells or bone marrow: A randomized comparison. *Journal of Clinical Oncolology*, **13(6)**, 1328-1335.

[5] Hartmann, O., Le Corroller, A.G., Blaise, D., Michon, J., Philip, I., Norol, F., Janvier, M., Louis Pico, J., Baranzelli, M.C., Rubie, H., Coze, C., Pinna, A., Meresse, V. and Benhamou, E. (1997) Peripheral blood stem cell and bone marrow transplantation for solid tumors and lymphomas: Hematologic recovery and costs—a randomized, controlled trial. *Annals of Internal Medicine*, **126(8)**, 600-607.

[6] Schmitz, N., Linch, D.C., Dreger, P., Goldstone, A.H., Boogaerts, M.A., Ferrant, A., Demuynck, H.M., Link, H., Zander, A. and Barge, A. (1996) Randomised trial of filgrastim-mobilised peripheral blood progenitor cell transplant- tation versus autologous bone-marrow transplantation in lymphoma patients. *Lancet*, **347(8998)**, 353-357.

[7] Cutler, C. and Antin, J.H. (2001) Peripheral blood stem cells for allogeneic transplantation: A review. *Stem Cells*, **19(2)**, 108-117.

[8] Sutherland, H.J., Hogge, D.E., Landsdorp, P.M., Phillips, G.L., Eaves, A.C. and Eaves, C.J. (1995) Quantitation, mobilization, and clinical use of long-term culture-initiating cells in blood cell autografts. *Journal of Hematotherapy*, **4(1)**, 3-10.

[9] Siena, S., Schiavo, R., Pedrazzoli, P. and Carlo-Stella, C. (2000) Therapeutic relevance of CD34 cell dose in blood cell transplantation for cancer therapy. *Journal of Clinical Oncolology*, **18(6)**, 1360-1377.

[10] Keeney, M., Chin-Yee, I., Weir, K., Popma, J., Nayar, R. and Sutherland, D.R. (1998) Single platform flow cytometric absolute $CD34^+$ cell counts based on the ISHAGE guidelines. International Society of Hematotherapy and Graft Engineering. *Cytometry*, **34(2)**, 61-70.

[11] Katsares, V., Petsa, A., Felesakis, A., Paparidis, Z., Nikolaidou, E., Gargani, S., Karvounidou, I., Ardelean, K.A., Grigoriadis, N. and Grigoriadis, J. (2009) A rapid and accurate method for the stem cell viability evaluation. The case of the thawed umbilical cord blood. *Lab Medicine*, **40(9)**, 557-560.

[12] Makino, S., Harada, M., Akashi, K., Taniguchi, S., Shibuya, T., Inaba, S. and Niho, Y. (1991) A simplified method for cryopreservation of peripheral blood stem cells at –80°C without rate-controlled freezing. *Bone Marrow Transplantation*, **8(4)**, 239-244.

[13] Bakken, A.M. (2006) Cryopreserving Human Peripheral Blood Progenitor Cells. *Current Stem Cell Research & Therapy*, **1(1)**, 47-54.

[14] Tarasov, A.I., Petrenko, A.Y. and Jones, D.R. (2004) The osmotic characteristics of human fetal liver-derived hematopoietic stem cell candidates. *Cryobiology*, **48(3)**, 333-340.

[15] Lewis, J.P., Passovoy, M., Conti, S.A., McFate, P.A., Trobaugh, F.E. (1967) The effect of cooling regimens on the transplantation potential of marrow. *Transfusion*, **7(1)**, 17-32.

[16] Meryman, H.T., Williams, R.J. and Douglas, M.S. (1977) Freezing injury from "solution effects" and its prevention by natural or artificial cryoprotection. *Cryobiology*, **14(3)**, 287-302.

[17] Cilloni, D., Garau, D., Regazzi, E., Sammarelli, G., Savoldo, B., Caramatti, C., Mangoni, L., Rizzoli, V. and Carlo-Stella, C. (1999) Primitive hematopoietic progenitors within mobilized blood are spared by uncontrolled rate freezing. *Bone Marrow Transplantation*, **23(5)**, 497-503.

[18] Halle, P., Tournilhac, O., Knopinska-Posluszny, W., Justyna, K., Gembara, P., Boiret, N., Rapatel, C., Berger,

M., Travade, P., Angielski, S., Bonhomme, J. and Déocq, F. (2001) Uncontrolled-rate freezing and storage at –80 ^{0}C, with only 3.5% DMSO in cryoprotective solution for 109 autologous peripheral blood progenitor cell transplantations. *Transfusion*, **41(5)**, 667-673.

[19] Katayama, Y., Yano, T., Bessho, A., Deguchi, S., Sunami, K., Mahmut, N., Shinagawa, K., Omoto, E., Makino, S., Miyamoto, T., Mizuno, S., Fukuda, T., Eto, T., Fujisaki, T., Ohno, Y., Inaba, S., Niho, Y. and Harada, M. (1997) The effects of a simplified method for cryopreservation and thawing procedures on peripheral blood stem cells. *Bone Marrow Transplantation*, **19(3)**, 283-287.

[20] Perez-Oteyza, J., Bornstein, R., Corral, M., Hermosa, V., Alegre, A., Torrabadella, M., Ramos, P., Garcia, J., Odriozola, J. and Navarro, J.L. (1998) Controlled-rate versus uncontrolled-rate cryopreservation of peripheral blood progenitor cells: A prospective multicenter study. Group for cryobiology and biology of bone marrow transplantation (CBTNO), Spain. *Haematologica*, **83(11)**, 1001-1005.

[21] Paczkowska, E. (2002) Freezing of umbilical blood cells in mechanical freezers (–80˚C). *Annales Academiae Medicae Stetinensis*, **48**, 117-133.

[22] Walter, Z., Szostek, M., Weglarska, D., Raguszewska, D., Jabłoński, M., Lorenz, F. and Skotnicki, A.B. (1999) Methods for freezing, thawing and viability estimation of hemopoietic stem cells. *Przeglad Lekarski*, **56(Suppl 1)**, 34-39.

[23] Goldman, J.M., Th'ng, K.H., Park, D.S., Spiers, A.S., Lowenthal, R.M. and Ruutu, T. (1978) Collection, cryopreservation and subsequent viability of haemopoietic stem cells intended for treatment of chronic granulocytic leukaemia in blast-cell transformation. *British Journal of Haematology*, **40(2)**, 185-195.

[24] Liseth, K., Ersvær, E., Abrahamsen, J.F., Nesthus, I., Ryningen, A., and Bruserud, Ø. (2009) Long-term cryopreservation of autologous stem cell grafts: A clinical and experimental study of hematopoietic and immunocompetent cells. *Transfusion*, **49(8)**, 1709-1719.

[25] Akkök, Ç.A., Holte, M.R., Tangen, J.M., Østenstad, B. and Bruserud, Ø. (2009) Hematopoietic engraftment of dimethyl sulfoxide–depleted autologous peripheral blood progenitor cells. *Transfusion*, **49(2)**, 354-361.

[26] Rollig, C., Babatz, J., Wagner, I., Maiwald, A., Schwarze, V., Ehninger, G. and Bornhäuser, M. (2002) Thawing of cryopreserved mobilized peripheral blood-comparison between water bath and dry warming device. *Cytotherapy*, **4(6)**, 551-555.

[27] Mavroudis, D., Read, E., Cottler-Fox, M., Couriel, D., Molldrem, J., Carter, C., Yu, M., Dunbar, C. and Barrett, J. (1996) CD34^{+} cell dose predicts survival, posttransplant morbidity, and rate of hematologic recovery after allogeneic marrow transplants for hematologic malignancies. *Blood*, **88(8)**, 3223-3229.

[28] Miflin, G., Russell, N.H., Hutchinson, R.M., Morgan, G., Potter, M., Pagliuca, A., Marsh, J., Bell, A., Milligan, D., Lumley, M., Cook, G. and Franklin, I. (1997) Allogeneic peripheral blood stem cell transplantation for haematological malignancies—an analysis of kinetics of engraftment and GVHD risk. *Bone Marrow Transplantation*, **19(1)**, 9-13.

[29] Shpall, E.J., Champlin, R. and Glaspy, J.A. (1998) Effect of CD34+ peripheral blood progenitor cell dose on hematopoietic recovery. *Biology of Blood and Marrow Transplantation*, **4(2)**, 84-92.

[30] Vij, R., Brown, R., Shenoy, S., Haug, J.S., Kaesberg, D., Adkins, D., Goodnough, L.T., Khoury, H. and DiPersio, J. (2000) Allogeneic peripheral blood stem cell transplantation following CD34+ enrichment by density gradient separation. *Bone Marrow Transplantation*, **25(12)**, 1223-1228.

[31] McGlave, P.B., De Fabritiis, P., Deisseroth, A., Goldman, J., Barnett, M., Reiffers, J., Simonsson, B., Carella, A. and Aeppli, D. (1994) Autologous transplants for chronic myelogenous leukaemia: results from eight transplant groups. *Lancet*, **343(8911)**, 1486-1488.

[32] Reiffers, J., Goldman, J., Meloni, G., Cahn, J.Y. and Gratwohl, A. (1994) On behalf of the Chronic Leukemia Working Party of the EBMT. Autologous stem cell transplantation in chronic myelogenous leukemia: A retrospective analysis of the European Group for Bone Marrow Transplantation. *Bone Marrow Transplantation*, **14 (3)**, 407-410.

[33] Burnett, A.K., Goldstone, A.H., Stevens, R.M., Hann. I.M., Rees, J.K., Gray, R.G. and Wheatley, K. (1998) Randomised comparison of addition of autologous bone-marrow transplantation to intensive chemotherapy for acute myeloid leukaemia in first remission: results of MRC AML 10 trial. *Lancet*, **351(9104)**, 700-708.

[34] De Witte, T., Van Biezen, A., Hermans, J., Labopin, M., Runde, V., Or, R., Meloni, G., Mauri, S.B., Carella, A., Apperley, J., Gratwohl, A. and Laporte, J.P. (1997) Autologous bone marrow transplantation for patients with myelodysplastic syndrome (MDS) or acute myeloid leu kemia following MDS. *Blood*, **90(10)**, 3853-3857.

[35] Harousseau, J.L., Cahn, J.Y., Pignon, B., Witz, F., Milpied, N., Delain, M., Lioure, B., Lamy, T., Desablens, B., Guilhot, F., Caillot, D., Abgrall, J.F., Francois, S., Briere, J., Guyotat, D., Casassus, P., Audhuy, B., Tellier, Z., Hurteloup, P. and Herve, P. (1997) Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. *Blood*, **90(8)**, 2978-2986.

[36] Niwa, A., Umeda, K., Awaya, T., Yui, Y., Matsubara, H., Hiramatsu, H., Watanabe, K.I., Adachi, S., Takashi, I., Shinji, U. and Tatsutoshi N. (2009) Successful autologous peripheral blood stem cell transplantation with a double-conditioning regimen for recurrent hepatoblastoma after liver transplantation. *Pediatric Transplantation*, **13(2)**, 259-262.

[37] Nevskaya, T., Ananieva, L., Bykovskaia, S., Eremin, I., Karandashov, E., Khrennikov, J., Mach, E., Zaprjagaeva, M., Guseva, N. and Nassonov, E. (2009) Autologous progenitor cell implantation as a novel therapeutic intervenetion for ischaemic digits in systemic sclerosis. *Rheumatology*, **48(1)**, 61-64.

[38] Mishra, V., Andresen, S., Brinch, L., Kvaløy, S., Ernst, P., Lønset, M.K., Tangen, J.M., Wikelund, J., Flatum, C., Baggerød, E., Helle, B., Vaaler, S. and Hagen, T.P. (2005) Cost of autologous peripheral blood stem cell transplantation: the Norwegian experience from a multicenter cost study. *Bone Marrow Transplantation*, **35(12)**, 1149-1153.

[39] Van Agthoven, M., Vellenga, E., Fibbe, W.E., Kingma, T. and De Groot, C.A.U. (2001) Cost analysis and quality of

life assessment comparing patients undergoing autologous peripheral blood stem cell transplantation or autologous bone marrow transplantation for refractory or relapsed non-Hodgkin's lymphoma or Hodgkin's disease: A prospective randomised trial. *European Journal of Cancer*, **37(14)**, 1781-1789.

[40] Ghosh, K., Gosavi, S., Pathare, A., Madkaikar, M., Rao, V.B. and Mohanty, D. (2002) Low cost autologous peripheral blood stem cell transplantation performed in a municipal hospital for a patient with plasma cell leukaemia. *Clinical and Laboratory Haematology*, **24(3)**, 187-190.

[41] Reich-Slotky, R., Colovai, A.I., Semidei-Pomales, M., Patel, N., Cairo, M., Jhang, J. and Schwartz, J. (2008) Determining post-thaw CD34+ cell dose of cryopreserved haematopoietic progenitor cells demonstrates high recovery and confirms their integrity. *Vox Sanguinis*, **94 (4)**, 351-357.

[42] Haas, R., Witt, B., Goldschmidt, H., Hohaus, S., Fruehauf, S., Wannenmacher, M. and Hunstein, W. (1995) Sustained long-term hematopoiesis after myeloablative therapy with peripheral blood progenitor cell support. *Blood*, **85(12)**, 3754-3761.

[43] Ketterer, N., Salles, G., Raba, M., Espinouse, D., Sonet, A., Tremisi, P., Dumontet, C., Moullet, I., Eljaafari-Corbin, A., Neidhardt-Berard, E.-M., Bouafia, F. and Coiffier, B. (1998) High $CD34^+$ cell counts decrease hematologic toxicity of autologous peripheral blood progenitor cell transplantation. *Blood*, **91(9)**, 3148-3155.

[44] Bittencourt, H., Rocha, V., Chevret, S., Socie, G., Esperou, H., Devergie, A., Dal Cortivo, L., Marolleau, J-P., Garnier, F., Ribaud, P. and Gluckman, E. (2002) Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. *Blood*, **99(8)**, 2726-2733.

[45] De Lima, M., McMannis, J., Gee, A., Komanduri, K., Couriel, D., Andersson, B.S., Hosing, C., Khouri, I., Jones, R., Champlin, R., Karandish, S., Sadeghi, T., Peled, T., Grynspan, F., Daniely, Y., Nagler, A. and Shpall, E.J. (2008) Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: A phase I/II clinical trial. *Bone Marrow Transplantation*, **41(9)**, 771-778.

[46] Briddell, R.A., Kern, B.P., Zilm, K.L., Stoney, G.B. and McNiece, I.K. (1997) Purification of $CD34^+$ cells is essential for optimal ex vivo expansion of umbilical cord blood cells. *Journal of Hematotherapy*, **6(2)**, 145-150.

[47] Laroche, V., McKenna, D.H., Moroff, G., Schierman, T., Kadidlo, D. and McCullough, J. (2005) Cell loss and recovery in umbilical cord blood processing: A comparison of postthaw and postwash samples. *Transfusion*, **45(12)**, 1909-1916.

[48] El Beshlawy, A., Metwally, H.G., El Khalek, K.A., Zayed, R.A., Hammoud, R.F. and Mousa, S.M. (2009) The effect of freezing on the recovery and expansion of umbilical cord blood hematopoietic stem cells. *Experimental and Clinical Transplantation*, **7(1)**, 50-55.

[49] Rossmanith, T., Schröder, B., Bug, G., Müller, P., Klenner, T., Knaus, R., Hoelzer, D. and Ottmann O.G. (2001) Interleukin 3 improves the ex vivo expansion of primitive human cord blood progenitor cells and maintains the engraftment potential of SCID repopulating cells. *Stem Cells*, **19(4)**, 313-320.

[50] Yang, H., Zhao, H., Acker, J.P., Liu, J.Z., Akabutu, J. and McGann, L.E. (2005) Effect of dimethyl sulfoxide on post-thaw viability assessment of $CD45^+$ and $CD34^+$ cells of umbilical cord blood and mobilized peripheral blood. *Cryobiology*, **51(2)**, 165-175.

[51] Moezzi, L., Pourfathollah, A.A., Alimoghaddam, K., Soleimani, M. and Ardjmand A. (2005) The effect of cryopreservation on clonogenic capacity and in vitro expansion potential of umbilical cord blood progenitor cells. *Transplant Proceedings*, **37(10)**, 4500-4503.

[52] McNiece, I., Jones, R., Cagnoni, P., Bearman, S., Nieto, Y. and Shpall, E.J. (1999) Ex vivo expansion of hematopoietic progenitor cells: Preliminary results in breast cancer. *Hematological Cellular Therapy*, **41(2)**, 82-86.

[53] Koller, M.R., Manchel, I., Maher, R.J., Goltry, K.L., Armstrong, R.D. and Smith, A.K. (1998) Clinical-scale human umbilical cord blood cell expansion in a novel automated perfusion culture system. *Bone Marrow Transplantation*, **21(7)**, 653-663.

[54] Piacibello, W., Gammaitoni, L., Bruno, S., Gunetti, M., Fagioli, F., Cavalloni, G. and Aglietta, M. (2000) Negative influence of IL3 on the expansion of human cord blood in vivo long-term repopulating stem cells. *Journal of Hematotherapy & Stem Cell Research*, **9(6)**, 945-956.

[55] Henon, P., Sovalat, H., Becker, M., Arkam, Y., Ojeda-Uribe, M., Raidot, J.P., Husseini, F., Wunder, E., Bourderont, D. and Audhuy, B. (1998) Primordial role of $CD34^+/38^-$ cells in early and late trilineage haemopoietic engraftment after autologous blood cell transplantation. *British Journal Haematology*, **103(2)**, 568-581.

[56] Henon, P.H., Sovalat, H. and Bourderont, D. (2001) Importance of $CD34^+$ cell subsets in autologous PBSC trans-plantation: The mulhouse experience using $CD34^+$. *Journal of Biological Regulators and Homeostatic Agents*, **15**, 62-67.

[57] Ishikawa, F., Livingston, A.G., Minamiguchi, H., Wingard, J.R. and Ogawa, M., (2003) Human cord blood long-term engrafting cells are $CD34^+CD38^-$. *Leukemia*, **17(5)**, 960-964.

[58] Peled, T., Mandel, J., Goudsmid, R.N., Landor, C., Hasson, N., Harati, D., Austin, M., Hasson, A., Fibach, E., Shpall, E.J. and Nagler, A. (2004) Pre-clinical development of cord blood-derived progenitor cell graft expanded ex vivo with cytokines and the polyamine copper chelator tetraethylenepentamine. *Cytotherapy*, **6(4)**, 344-355.

[59] Lam, A.C., Li, K., Zhang, X.B., Li, C.K., Fok, T.F., Chang, A.M.Z., James, A.E., Tsang, K.S. and Yuen, M.P. (2001) Preclinical ex vivo expansion of cord blood hematopoietic stem and progenitor cells: Duration of culture; the media, serum supplements, and growth factors used and engraftment in NOD/SCID mice. *Transfusion*, **41 (12)**, 1567-1576.

[60] Kögler, G., Nürnberger, W., Fischer, J., Niehues, T., Somville, T., Göbel, U. and Wernet, P. (1999) Simultaneous cord blood transplantation of ex vivo expanded together with non-expanded cells for high risk leukemia. *Bone Marrow Transplantation*, **24(4)**, 397-403.

[61] Bertolini, F., Battaglia, M., Pedrazzoli, P., Prada, G.D., Lanza, A., Soligo, D., Caneva, L., Sarina, B., Murphy, S., Thomas, T. and Della Cuna, G.R. (1997) Megakaryocytic

progenitors can be generated ex vivo and safely administered to autologous peripheral blood progenitor cell transplant recipients. *Blood*, **89(8)**, 2679-2688.

[62] Williams, S.F., Lee, W.J., Bender, J.G., Zimmerman, T., Swinney, P., Blake, M., Carreon, J., Schilling, M., Smith, S., Williams, D.E., Oldham, F. and Van Epps D. (1996) Selection and expansion of peripheral blood CD34+ cells in autologous stem cell transplantation for breast cancer. *Blood*, **87(5)**, 1687-1691.

[63] Stiff, P., Chen, B., Franklin, W., Oldenberg**,** D., Hsi**,** E., Bayer, R., Shpall**,** E., Douville, J., Mandalam**,** R., Malhotra**,** D., Muller**,** T., Armstrong**,** R.D. and Smith A. (2000) Autologous transplantation of ex vivo expanded bone marrow cells grown from small aliquots after high-dose chemotherapy for breast cancer. *Blood*, **95(6)**, 2169-2174.

[64] Ruggieri, L., Heimfeld, S. and Broxmeyer, H.E. (1994) Cytokine-dependent ex vivo expansion of early subsets of $CD34^+$ cord blood myeloid progenitors is enhanced by cord blood plasma, but expansion of the more mature subsets of progenitors is favored. *Blood Cells*, **20(2-3)**, 436-454.

[65] Bertolini, F., Lazzari, L., Lauri, E., Corsini, C. and Sirchia, G. (1994) Cord blood plasma mediated ex vivo expansion of hematopoietic progenitor cells. *Bone Marrow Transplantation*, **14(3)**, 347-353.

[66] Li, N., Feugier, P., Serrurrier, B., Latger-Cannard, V., Lesesve, J.F., Stoltz, J.F. and Eljaafari, A. (2007) Human mesenchymal stem cells improve ex vivo expansion of adult human $CD34^+$ peripheral blood progenitor cells and decrease their allostimulatory capacity. *Experimental Hematology*, **35(3)**, 507-515.

[67] Devine, S.M. (2002) Mesenchymal stem cells: Will they have a role in the clinic? *Journal of Cellular Biochemistry-Supplement*, **28**, 73-79.

[68] Deans, R.J. and Moseley, A.B. (2000) Mesenchymal stem cells: Biology and potential clinical uses. *Experimental Hematology*, **28(8)**, 875-884.

[69] Kadereit, S., Deeds, L.S., Haynesworth, S.E., Koc, O.N., Kozik, M.M., Szekely, E., Daum-Woods, K., Goetchius, G.W., Fu, P., Welniak, L.A., Murphy, W.J. and Laughlin, M.J. (2002) Expansion of LTC-ICs and maintenance of p21 and BCL-2 expression in cord blood $CD34^+/CD38^-$ early progenitors cultured over human MSCs as a feeder layer. *Stem Cells*, **20(6)**, 573-582.

[70] Koh, S.H., Choi, H.S., Park, E.S., Kang, H.J., Ahn, H.S. and Shin, H.Y. (2005) Co-culture of human $CD34^+$ cells with mesenchymal stem cells increases the survival of $CD34^+$ cells against the 5-aza-deoxycytidine-or trichostatin A-induced cell death. *Biochemical and Biophysical Research Communications*, **329(3)**, 1039-1045.

[71] Bennaceur-Griscelli, A., Pondarre, C., Schiavon, V., Vainchenker, W. and Coulombel, L. (2001) Stromal cells retard the differentiation of $CD34^+/CD38^{low/neg}$ human primitive progenitors exposed to cytokines independent of their mitotic history. *Blood*, **97(2)**, 435-441.

[72] Liu, Y., Liu, T. , Fan, X., Ma, X. and Cui, Z. (2006) Ex vivo expansion of hematopoietic stem cells derived from umbilical cord blood in rotating wall vessel. *Journal of Biotechnology*, **124(3)**, 592-601.

[73] Nielsen, L.K. (1999) Bioreactors for hematopoietic cell culture. *Annual Review of Biomedical Engineering*, **1(1)**, 129-152.

[74] Cabrita, G.J., Ferreira, B.S., da Silva, C.L., Goncalves, R., Almeida-Porada, G. and Cabral, J.M. (2003) Hematopoietic stem cells: from the bone to the bioreactor. *Trends in Biotechnology*, **21(5)**, 233-240.

[75] Cabral, J.M.S. (2001) Ex vivo expansion of hematopoietic stem cells in bioreactors. *Biotechnology Letters*, **23 (10)**, 741-751.

[76] Hammond, T.G. and Hammond, J.M. (2001) Optimized suspension culture: the rotating wall vessel. *American Journal of Physiology-Renal Physiology*, **281(1)**, F12-F25.

[77] Plett, P.A., Abonour, R., Frankovitz, S.M. and Orschell, C.M. (2004) Impact of modeled microgravity on migration, differentiation, and cell cycle control of primitive human hematopoietic progenitor cells. *Experimental Hematology*, **32(8)**, 773-781.

[78] Sytkowski, A.J. and Davis, K.L. (2001) Erythroid cell growth and differentiation in vitro in the simulated microgravity environment of the NASA rotating wall vessel bioreactor. *In Vitro Cellular and Developmental Biology-Animal*, **37(2)**, 79-83.

[79] Plett, P.A., Frankovitz, S.M., Abonour, R. and Orschell-Traycoff, C.M. (2001) Proliferation of human hematopoietic bone marrow cells in simulated microgravity. *In Vitro Cellular and Developmental Biology-Animal*, **37(2)**, 73-78.

[80] Martin, Y. and Vermette, P. (2005) Bioreactors for tissue mass culture: design, characterization, and recent advances. *Biomaterials*, **26(35)**, 7481-7503.

[81] Ohi, S., Roach, A.N., Ramsahai, S., Kim, B.C., Fitzgerald, W., Riley, D.A. and Gonda, S.R. (2004) The hematopoi- etic stem cell therapy for exploration of deep space. New Frontiers & Future Concepts. *AIP Conference Proceedings*, **699**, 938-950.

[82] Andrade-Zaldívar, H., Santos, L. and Antonio De León Rodríguez, A. (2008) Expansion of human hematopoietic stem cells for transplantation: trends and perspectives. *Cytotechnology*, **56(3)**, 151-160.

[83] Delaney, C., Heimfeld, S., Brashem-Stein, C., Voorhies, H., Manger, R.L. and Bernstein, I.D. (2010) Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nature Medicine*, **16**, 232-236.

GLOSSARY OF ABBREVIATIONS AND INITIALISMS

BM: Bone Marrow
DMSO: Dimethyl sulfoxide
GVHD: Graft versus Host Disease
HARV: High Aspect Ratio Vessel
hESC: human Ebryonic Stem Cell
HLA: Human Leukocyte Antigen
HPC: Hematopoietic Progenitor Cell
HSC: Hematopoietic Stem Cell
LTC-IC: Long-term culture initiating colony
NASA: National Aeronautics and Space Administration
PBSC: Peripheral Blood Stem Cell
PBSCT: Peripheral Blood Stem Cell Transplantation
PPC: Primitive progenitor cell
RWV: Rotating Wall Vessel
STLV: Slow Turn Lateral Vessel

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Local cerebral blood perfusion correlates with nerve fibre integrity in transient ischemic attack patients with middle cerebral artery stenosis: a pilot study

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ABSTRACT

Recent advances in neuroimaging contribute a lot to the accurate diagnosis and evaluation of cerebrovascular diseases. To explore the relationship among blood perfusion, metabolism and brain structure integrity, 6 Chinese transient ischemic attack (TIA) patients with middle cerebral artery (MCA) stenosis were examined by xenon-enhanced computed tomography (Xe-CT), magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI) to compare cerebral blood flow (CBF) values, (choline + creatine)/N-acetyl aspartate [(Cho + Cr)/NAA] values and fractional anisotropy (FA) values in the MCA territory. Our results showed that CBF values significantly decreased in the ipsilateral basal ganglion regions in all 5 cases with unilateral MCA stenosis, with a corresponding decrease of FA values in the same region. In conclusion, decreased blood perfusion may indicate nerve fibre damage in the dominating regions of stenosed arteries.

Keywords: Transient Ischemic Attack;
Magnetic Resonance Imaging;
Xenon-Enhanced Computed Tomography

1. INTRODUCTION

Knowledge on recurrence, optimal evaluation and effective prevention strategy of transient ischemic attack (TIA) is still lacking. An increasing body of evidence has revealed that TIA in Chinese population is more related to intracranial artery stenosis [1]. Recent advances in neuroimaging contribute a lot to the accurate diagnosis and evaluation of TIA and cerebrovascular stenosis. To address the relation between blood perfusion, structure integrity and metabolism in the brain, we enrolled Chinese TIA patients with middle cerebral artery (MCA) stenosis to compare cerebral blood flow (CBF), (choline + creatine)/N-acetyl aspartate [(Cho + Cr)/NAA] and fractional anisotropy (FA) values of MCA territory measure by xenon-enhanced computed tomography (Xe-CT) and magnetic resonance imaging (MRI).

2. SUBJECTS AND METHODS

A total of 6 consecutive TIA patients with MCA stenosis detected by TCD and confirmed by MRA, were enrolled to undertake Xe-CT, MRS and DTI examinations 3 days after the most recent attack in the department of radiology from January to June in 2009. TIA was as previously defined [2]. Study protocol and informed consent were obtained from all participants in the study, which was conducted in accordance with institutional guidelines. Patients with lacunar infarction in the basal ganglion of CT or MRI were excluded from this research.

All patients had a full clinical assessment, including electrocardiogram (ECG), carotid duplex Doppler Ultrasonography, and mini mental status examination (MMSE) and National Institutes of Health Stroke Scale (NIHSS). American Nicolet TC8080 TCD machine, German Siemens MAGNETOM Avanto 1.5 T MR machine was used to perform MRI, MRA, DTI and MRS examination, and American Diversified Diagnostic Products Xe-CT CBF system with German Simens PLUS4 CT were used to perform Xe-CT examination.

MRI Protocol: MRI scanning, including conventional MR images, including T2-weighted, T1-weighted, and

fluid-attenuated inversion recovery (FLAIR) images, MRA, MRS and DTI were performed with the use of a clinical 1.5 T whole-body MR system Siemens MAGNETOM Avanto with a conventional gradient system (Magnetom Vision, Siemens Medical System). Parameters are as follows: TSE/T1WI: TR 400 ms, TE 7.8 ms; TSE/T2WI: TR 3250 ms, TE 99 ms; Time-of-flight (TOF)-MRA, scanning time 6′01″, TR 25 ms, TE 4.6 ms, thickness 0.9 mm, band width 85 Hz; MRS(^{1}H): two-dimensional MRS of basal ganglion, scanning time 7′12″, TR 1500 ms, TE 135 ms, band width 35 Hz; DTI: TR 3900 ms, TE 76 ms; DTI: TR 3900 ms, TE 76 ms, matrix 64 × 64, in-plane resolution was 1.875 × 1.875 mm.

Xe-CT protocol: Xe-CT studies used a 28 to 33% concentration of medical-grade xenon gas mixed with O_2. At the start of each study, a face mask was used to deliver the mixed gas and the patient was closely monitored. The procedure used within the DDPI system involved obtaining two baseline scans before Xe inhalation and six scans during Xe inhalation for each of four axial planes of the brain, each 20 mm thick. CT scanning was performed at intervals of 1 min at the basal ganglial level. The resulting image was shown as a CBF map, on which CBF values could be extracted by placing regions of interest (ROIs) on corresponding brain tissue.

The results of TCD and MRA were independent of the patients' clinical manifestations, given by professional TCD and MRI technicians. And the (Cho + Cr)/NAA values, FA values and CBF values of the basal ganglion regions were measured and calculated by three respecttive professional radiological physicians blinded to the clinical data of the subjects. Results are presented as mean ± SD. The data of FA and (Cho + Cr)/NAA values were analysed with nonparametric analysis, Wilcoxon Signed Ranks Test by software of SPSS 11.5 (for Windows OS). And CBF values were analysed with PEMS 3.0 to compare means of two samples. A probability value of < 0.05 was considered significant.

3. RESULTS

All 6 subjects were male, aged 55.7 ± 10.3; ECG, carotid duplex Doppler ultrasonography, MMSE and NIHSS showed negative results. Average CBF values in bilateral ROIs were different, with the maximum difference of 12.2 ml/(100 g·min), and the minimum of 1.5 ml/(100 g·min); CBF values significantly decreased in the ipsilateral basal ganglion regions of stenosed MCA in all 5 cases with unilateral MCA stenosis ($P < 0.05$). (**Table 1**) FA values in stenosed MCA territory were significantly lower than those in the contra-lateral counterparts in 5 subjects with unilateral MCA stenosis. There was no difference when comparing (Cho + Cr) /NAA values of bilateral MCA territory.

Table 1. CBF values measured by Xe-CT [ml/(100 g·min)].

Subject No.	Ipsilateral	Contralateral	*p* value
1	45.6 ± 17.0	51.9 ± 19.9	0.0000
2	45.2 ± 17.1	56.7 ± 20.9	0.0000
3	59.0 ± 20.3	63.4 ± 24.8	0.0000
4	32.9 ± 17.7	40.0 ± 14.7	0.0003
5	52.7 ± 17.5	64.9 ± 23.0	0.0000
*6	56.1 ± 18.4	57.6 ± 23.3	0.1270

* Bilateral MCA stenosis.

4. REPRESENTATIVE CASE

A 53-years old male with a history of untreated hypertension and diabetes mellitus, presented ictal left hemiparesis for the first time. Dizziness, vertigo, nausea and dysarthria were absent. TCD suggested a reduced blood flow velocity of right MCA. Stenosed right MCA was confirmed by MRA. Xe-CT showed decreased CBF in the right basal ganglion, with CBF of 45.6 ± 17.0 ml/ (100 g·min) compared with 51.9 ± 19.9 ml/(100 g·min) in the right counterpart ($p < 0.05$) FA values in stenosed MCA territory were significantly lower than the counterpart. ($p < 0.05$) There was no significant difference between bilateral (Cho + Cr) /NAA values ($p > 0.05$) (**Figure 1**).

5. DISCUSSION

Xe-CT is a quantitative method of CBF analysis, and multiple studies have validated the accuracy of CBF values obtained with Xe-CT [3]. MRS can evaluate the metabolism of brain tissues in vivo [4]. Abnormalities of neuronal structures lead to reductions in NAA quantity [5]. An increase in the Cho peak is associated with conditions such as demyelinating disease and brain tumors [6]. Also alterations in the NAA/Cho ratio have been reported with findings suggestive of neuronal damage caused by neuronal disorders [7]. In theory, the chronic or acute ischemia of brain tissue may result in the changes of (Cho + Cr)/NAA due to axonal degeneration or demyelination secondary to artery stenosis, while in the present study, no significant changes in (Cho + Cr)/NAA value could be seen. The similar results can be seen in a previous MRS study in TIA without stenosed cerebral artery [8]. One reason may be argued that although there is a reduction of CBF in the territory of

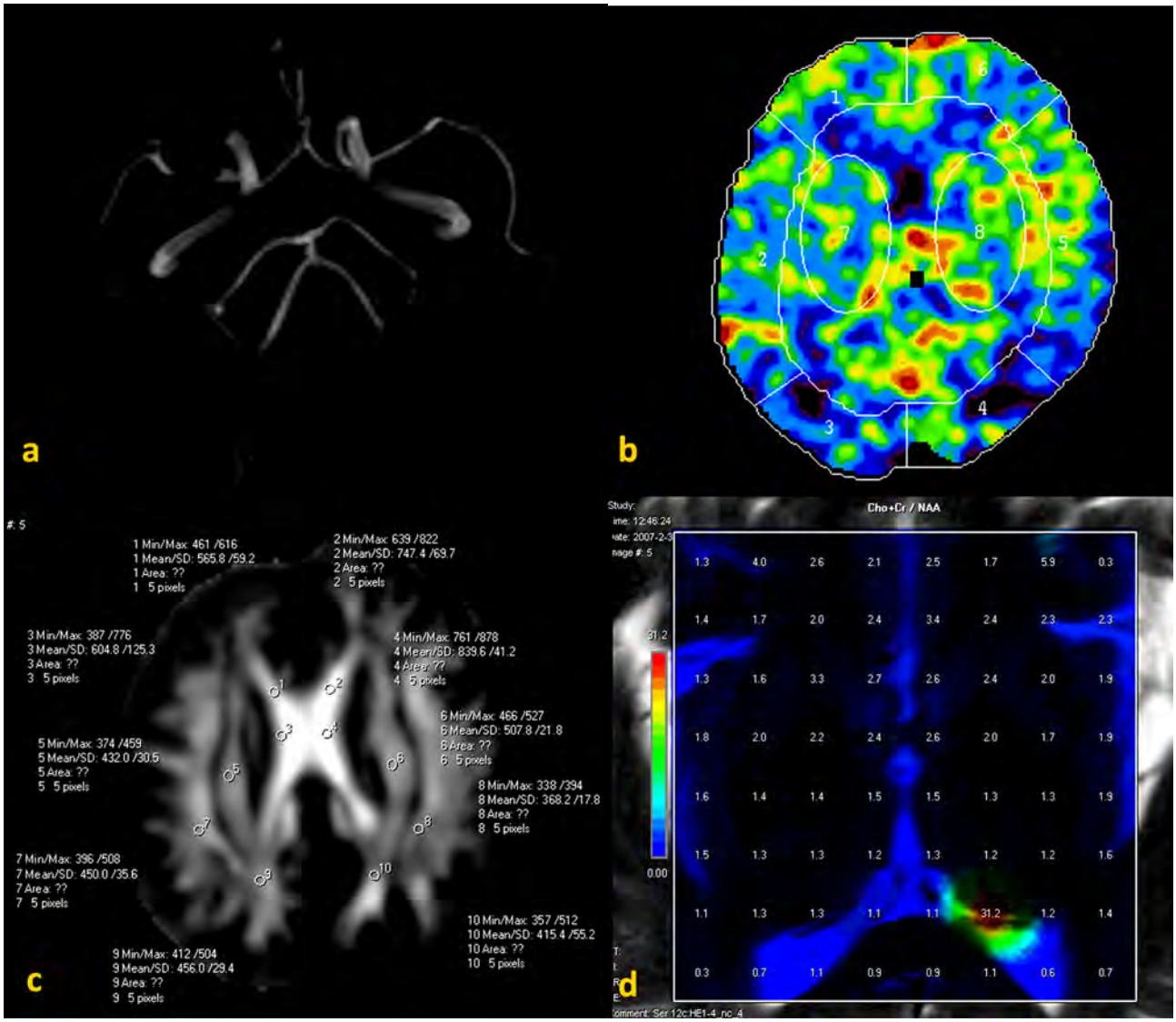

Figure 1. (a) MRA showed stenosed right MCA in the representative case; (b) Xe-CT showed decreased CBF in the right basal ganglion, with CBF of 45.6 ± 17.0 ml/(100 g·min) compared with 51.9 ± 19.9 ml/(100 g·min) in the right counterpart. (p = 0.000); (c) DTI showed a decrease of FA values in stenosed MCA territory. (p = 0.008); (d) MRS showed no significant difference in bilateral ROIs. (p = 0.866).

stenosed MCA compared with the contralateral counterpart, the relative ischemia of the brain tissue may not necessarily result in a significant metabolic change. DTI may be used to map and characterize the three-dimensional diffusion of water as a function of spatial location. Chronic ischemia of brain is postulated to possibly cause demyelination of white matter, which can result in the reduction of FA values. And our data confirmed such a hypothesis.

Although only 6 patients contributed to this study, a preliminary conclusion of the present study may be drawn: local changes of CBF may be used to estimate the integrity of nerve fibres in TIA patients with stenosed MCA.

As regards the limitation of our study, the primary one is the small number of the subjects; Secondly, although blind method was used, there still involved subjective deflection in the measurement and calculation of CSF, FA value and (Cho + Cr)/NAA values; Thirdly, the relatively low field intensity of 1.5 T MRI is another weakness in performing MRS and DTI examination; Also, lactic acid, which can directly reflect the degree of hypoperfusion and hypoxemia, was not detected.

In summary, the combined use of CT and MR tech-

niques to quantitatively assess blood perfusion, metabolism and water molecule diffusion may be a first step towards accurate diagnosis and evaluation of TIA and intracranial artery stenoses and may in the future allow effective prevention of stroke and monitoring of treatment strategy.

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REFERENCES

[1] Li, D., Wang, M.L., Li, S.M. and Ling, F. (2008) Distribution and risk factors of steno-occlusive lesions in patients with ischemic cerebrovascular disease. *National Medical Journal of China*, **88(17)**, 1158-1162.

[2] Albers, G.W., Caplan, L.R., Easton, J.D., Fayad, P.B., Mohr, J.P., Saver, J.L., Sherman, D.G. and TIA Working Group (2002) Transient ischemic attack-proposal for a new definition. *New England Journal of Medicine*, **347 (21)**, 1713-1716.

[3] Latchaw, R.E., Yonas, H., Hunter, G.J., Yuh, W.T., Ueda, T., Sorensen, A.G., Sunshine, J.L., Biller, J., Wechsler, L., Higashida, R., Hademenos, G. and Council on Cardiovascular Radiology of the American Heart Association (2003) Guidelines and recommendations for perfusion imaging in cerebral ischemia: a scientific statement for healthcare professionals by the writing group on perfusion imaging, from the Council on Cardiovascular Radiology of the American Heart Association. *Stroke*, **34(4)**, 1804-1104.

[4] Alkan, A., Sarac, K., Kutlu, R., Yakinci, C., Sigirci, A., Aslan, M. and Baysal, T. (2003) Early and late-state subacute sclerosing panencephalitis: chemical shift imaging and single-voxel MR spectroscopy. *American Journal of Neuroradiology*, **24(3)**, 501-506.

[5] Cecil, K.M. and Jones, B.V. (2001) Magnetic resonance spectroscopy of the pediatric brain. *Top Magn Reson Imaging*, **12(6)**, 435-452.

[6] Irwan, R., Sijens, P.E., Potze, J.H. and Oudkerk, M. (2005) Correlation of proton MR spectroscopy and diffusion tensor brain MR imaging. *Magn Reson Imaging* **23(8)**, 851-858.

[7] Kadota, T., Horinouchi, T. and Kuroda C. (2001) Development and aging of the cerebrum: assessment with proton MR spectroscopy. *American Journal of Neuroradiology*, **22(1)**, 128-135.

[8] Giroud, M., Walker, P., Guy, F., Lemesle, M., Lalande, A., Baudouin, N., Martin, D., Couveur, G. and Brunotte, F. (1999) Cerebral metabolism after transient ischemic attack. A 1H MR spectroscopy study. *Neurological Research*, **21(6)**, 563-565.

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Health

Arterial pulse impact on blood flow

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ABSTRACT

Numerous pathophysiologic observations in humans and animals led to the formulation of the response-to-injury hypothesis of atherosclerosis, which proposed that endothelial denudation by the blood flow was the first step in atherosclerosis. At present it is impossible to describe hemodynamics only by the Navier-Stokes or Oldroyd-B equations because in the large arteries blood flow is unsteady, with the flow separation and waveform propagation of the thyxotropic mass. The purpose of this paper is to study the impact of the arterial pulse wave on the blood flow and initial factors of atherosclerosis. In 12 healthy men (25-39 years of age) peak velocity, mean velocity, mean flow and net flow in the aorta have been investigated by MR angiography. Initial velocity was registered after 43msec of the ECG-R wave, and it differed from zero at all sites of the aorta, although net flow was equal to zero. Womersley's number from the ascending to the thoracic aorta decreased from 12.5 ± 1.5 to 7.3 ± 1.2; flow modified from inertio-elastic to viscous. In the aortic arch in protodiastole blood flow separated into the opposite directed streams resulting in wave superposition with the high net flow. At the isthmus area separated waves interferences and reflects to anterograde direction. Here flow acceleration in protodiastole is 6 times higher than in systole. Pulse waves move on artery walls fifteen or more times more rapidly than the blood flow. Pulse oscillation increases strain rate to the contiguous vessel wall flow layers. At the sites with the flow wave negative interference vessel pulse oscillation attenuates and at the boundary reflection flow wave can shift the vessel wall.

Keywords: Arterial pulse; Blood flow; Wave Propagation; Blood/Arterial Wall Viscoelasticity; Endothelial Denudation; Magnetic Resonance Imaging; Atherosclerosis

1. INTRODUCTION

The lesions of atherosclerosis occur principally in large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain, or extremities, resulting in infarction [1]. They may be present throughout a person's lifetime. In fact, the earliest type of lesion, the so-called fatty streak, which is common in infants and young children, is a pure inflammatory lesion [2,3].

Local hemodynamic temporal pressure and wall shear stress are important for understanding the mechanisms leading to various complications in cardiovascular function [4].

At present blood motion has been studied as a continuum with the steady flow and has been described by the Navier-Stokes equations. These equations arise from applying Newton's second law to fluid motion and are nonlinear partial differential equations [5]. In a real situation blood flow in the large arteries is unsteady, with the flow separation and waveform propagation of the thyxotropic mass.

Polymer solutions are convenient for experimental studies of viscoelastic flows. The Couette-Taylor flow is often chosen because of its geometrical simplicity and its diversity of instability modes and turbulent states. The most striking elastic property of the polymer solutions is, probably, the dependence of mechanical stresses in flow on the history of the flow. So, the stresses do not immediately become zero when fluid motion stops, but rather decay with some characteristic relaxation time. Equations with the expression of time derivative of the polymer stress constitute the Oldroyd-B model of polymer solution rheology [6].

At very small scales or under extreme conditions, real fluids made out of discrete molecules will produce results different from the continuous fluids modeled by the equations cited above. Up today it is impossible to describe blood flow only by the fluid mechanics. Depending on the Knudsen number of the problem, statistical mechanics or possibly even molecular dynamics may be a more appropriate approach.

For the complicacy of the problem we are discussing

the theoretical basis for the waveform propagation of the viscoelastic substances and the ways of its correlation.

2. MATHERIALS AND METHODS

We have investigated 12 healthy men (25-39 years of age) with a 1.5-T MR imager with the breath hold (18 sec) and ECG triggering in different sites of the aorta. Pulse rate 72 to 78 beats per minute. Kinematic viscosity of the blood 3.8×10^{-6} m^2/s [7,8].

Volunteers were preliminarily examined by the cardiologist, angiologist and hematologists. Flow quantification (mean velocity, peak velocity, mean flow, net flow) was carried out in every 43 sec at different sites of the ascending aorta, aortic arch and thoracic aorta in 1 mm slices of 7 cm^2 area. All data are given below as graphs. (**Figures 1-5**) Examinations were performed with surface coil technology, with a gradient strength 40 mT/m and a maximum slew rate 200 mT/m/msec.

3. RESULTS

In our cases the initial flow velocity was registries after 43msec of the ECG-R wave, and it was different from zero at all sites of the aorta, although net flow was equal to zero. During the heart cycle blood systolic velocity varies in sinusoid shape, whereas net flow increases gradually at the diastole when blood velocity was low. Initial flow acceleration was lower than of the next one, although gradient pressure much higher initially and gradually decreased at the flow. Womersley's number from ascending to thoracic aorta decreased from 12.5 ± 1.5 to 7.3 ± 1.2 and flow modified from the inertio-elastic to viscous. Specificity, sensitivity and accuracy of the MRA to the explored area were 95%, 97%, and 96% (**Figures 1-5**).

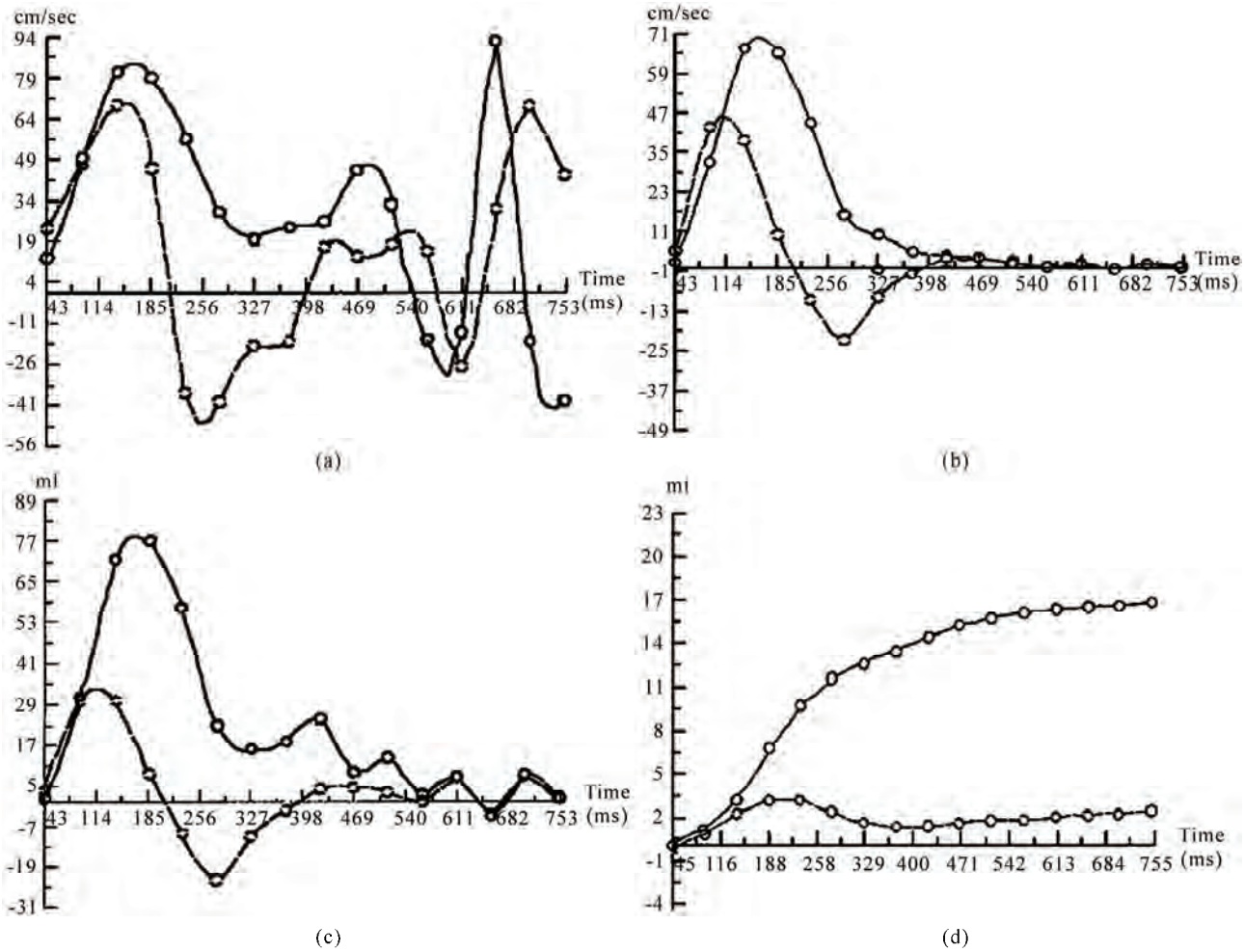

Figure 1. Peak velocity (a), mean velocity (b), mean flow(c) and net flow (d) graphs at the ascending aorta. (Dotted line - flow at the internal wall). Velocity and flow graph starts after ECG-R wave with the 43msec delay time. Blood acceleration for the initial systole (43msec) is lower than that for the next time (43-90msec). Net flow increases at the end diastole.

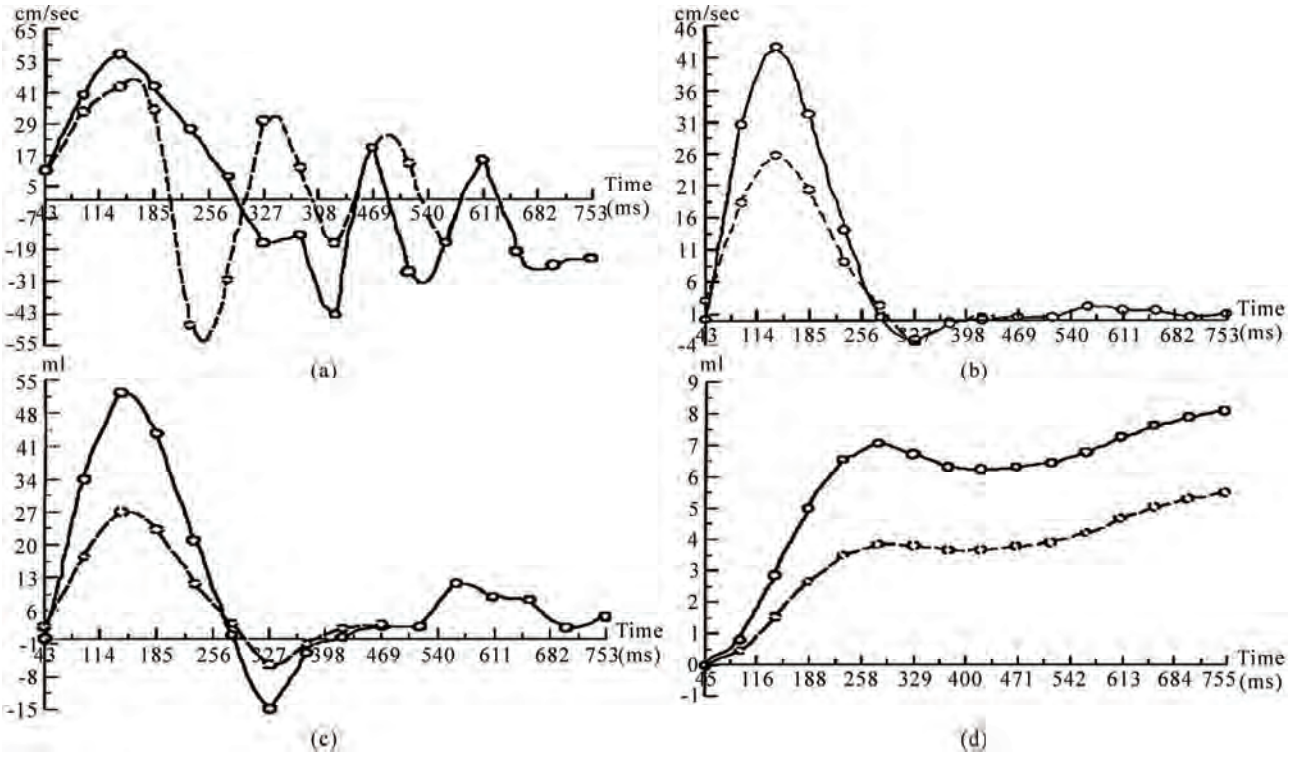

Figure 2. Peak velocity (a), mean velocity (b), mean flow(c) and net flow (d) graphs at the aortic arch. (Dotted line-flow at the external wall). At peak velocity graph separated stream flows in opposite direction. Net flow increases at the end diastole.

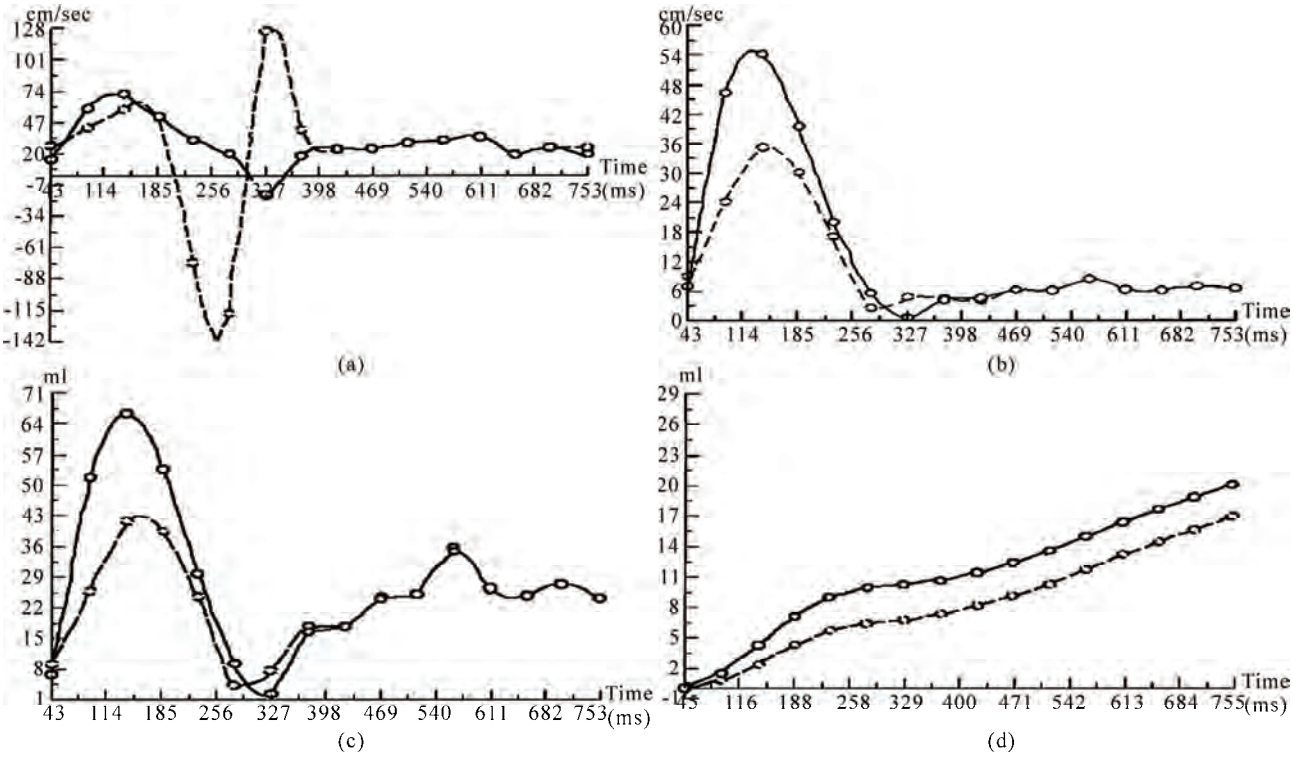

Figure 3. Peak velocity (a), mean velocity (b), mean flow(c) and net flow (d) graphs at the isthmus of the aorta. (Dotted line-flow at the external wall). Peak velocity at the 300msec is zero (negative interference) and then sharply increases to downstream. Here acceleration is 6 times higher than that in systole. Reflected wave changes polarity (phase change). Net flow increases at the end diastole.

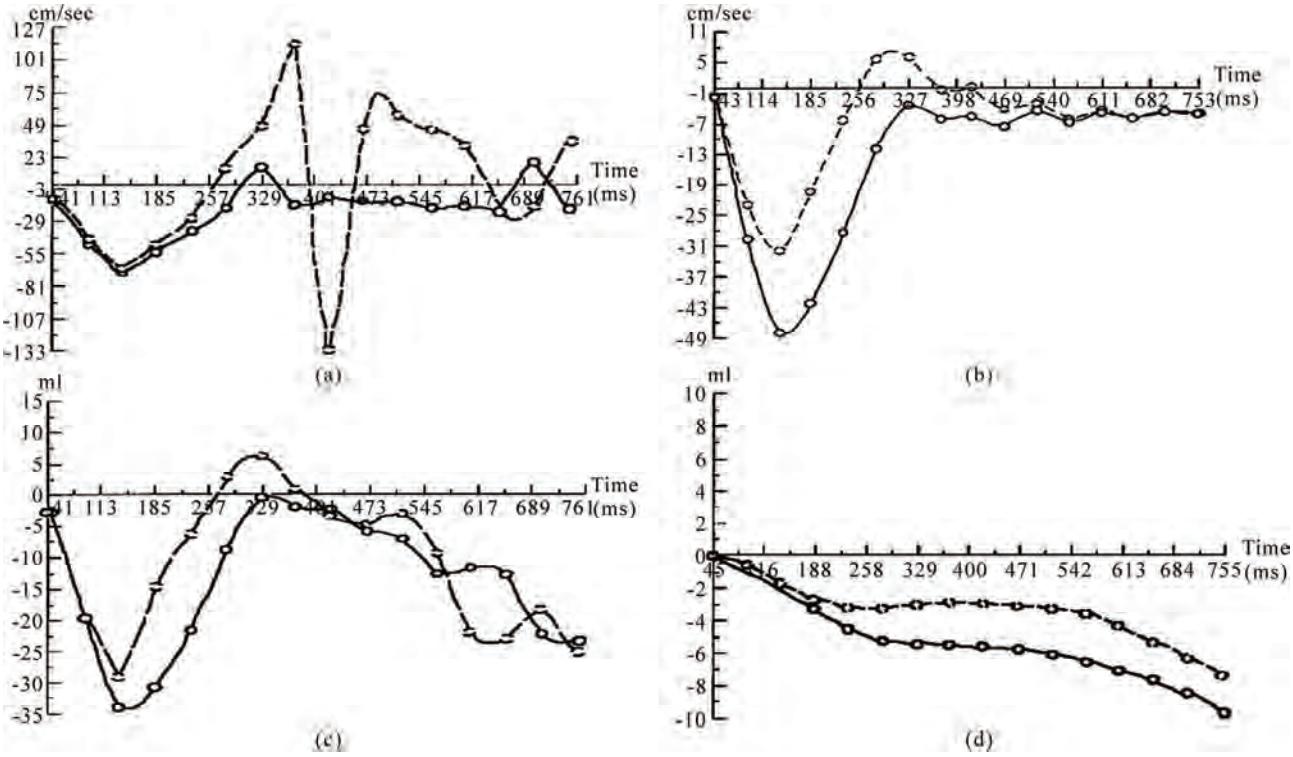

Figure 4. Peak velocity (a), mean velocity (b), mean flow(c) and net flow (d) graphs at the end of aortic arch (a). (Dotted line-flow at the internal wall). Flow direction at the graphs inverted due to slice position at MRI. Wave oscillation is transmits from external to internal wall. Fotmation of the peak velocity delays to the same at the isthmus area. Net flow increases at the end diastole.

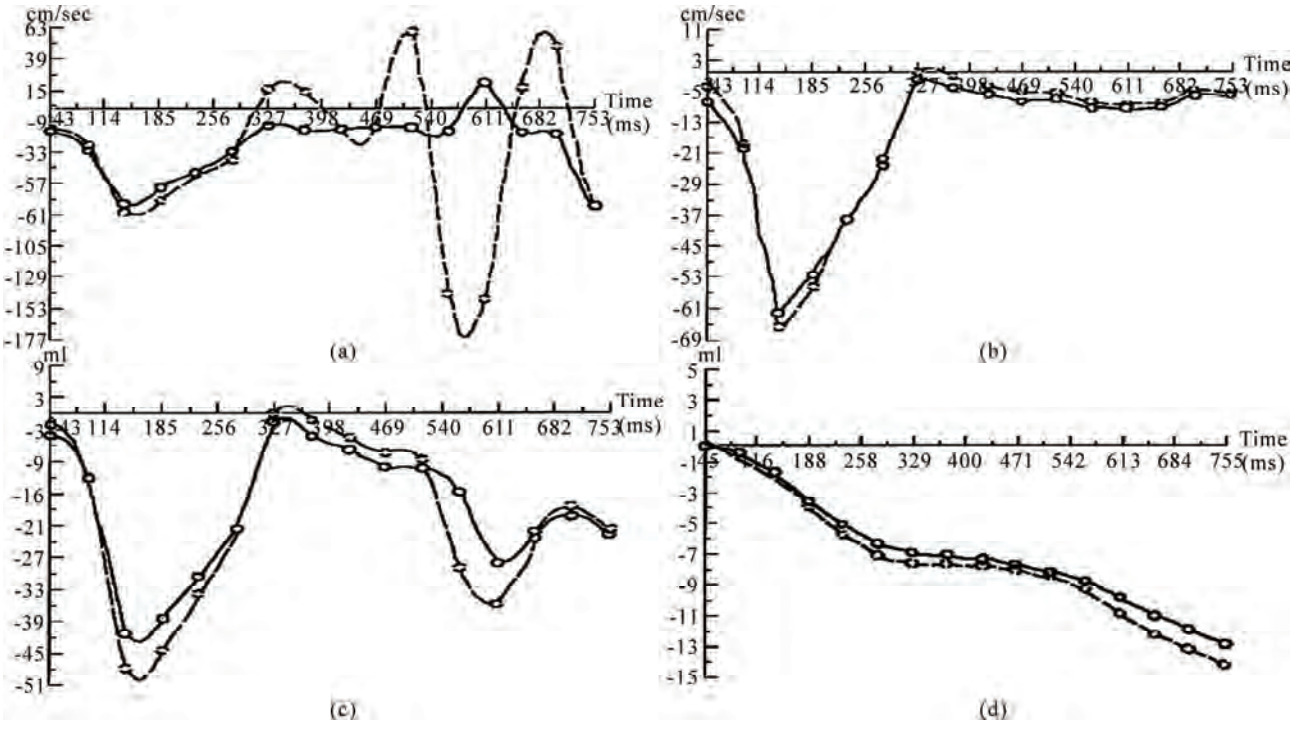

Figure 5. Peak velocity (A), mean velocity (B), mean flow(C) and net flow (D) graphs at the thoracic aorta. (Dotted line-flow at the internal wall). Flow direction at the graphs inverted due to slice position at MRI. There is no flow separation at the protodiastole. Net flow increases at the end diastole.

4. DISCUSSION

Inertial flow is characteristic for the Newtonian fluids. Blood is non Newtonian [9], thyxotropic [10,11] and as a viscoelastic substance must be expressed in some phenomena:

1) If the stress is held constant, the strain increases with the time (creep);

2) If the strain is held constant, the stress decreases with time (relaxation);

3) Effective stiffness depends on the rate of application of the load;

4) If cyclic loading is applied, hysteresis (phase lag) occurs, leading to a dissipation of mechanical energy;

5) Wave experience attenuation;

6) Rebound of the object following an impact is less than 100% [12].

With the viscoelastic hydrocolloids as a blood, some deformation caused by shear stress is elastic and will return to zero when force is removed. The remaining deformation will not return to zero forming creep flow of the substance. Under the constant force elastic displacement remains constant whereas the sliding displacement increases. If the force varies in sinusoid shape, shear strain rate lags behind the changes in the causative force by the phase.

Systolic sinusoid pulse pressure in the vessel wall and inside the blood propagates as a wave [13]. Wave is the disturbance that runs through the space and time, transferring energy, little/no associated with the mass transport; instead there are oscillations around almost fixed positions. Within wave, phase of the oscillation is different for adjacent points in space because the vibration reaches these points at different times [14].

The mechanical wave in substance propagates as elastic deformation and velocity shown in phase velocity. Wave phase velocity (v_p) is given by the equation: $v_p = \lambda f$ (λ-wave length, f-fluctuation frequency). The phase velocity for waves in a medium is determined in part by intrinsic properties of the medium. For all mechanical waves in elastic media, the square of the phase velocity is proportional to the ratio of the appropriate elastic property of the medium to the appropriate inertia property. Wave velocity within the material is independent of the wave amplitude [15].

When a wave encounters a boundary which is neither rigid (hard) nor free (soft) but instead somewhere in between, part of the wave is reflected from the boundary and part of the wave is transmitted across the boundary. The exact behavior of reflection and transmission depends on the material properties on both sides of the boundary. If a pulse crosses the boundary from a denser medium into a less dense medium, the speed and the wavelength are both increased. The frequency of the incident pulse is the same as the frequency of the transmitted pulse. At the soft boundary, the restoring force is zero and the reflected wave has the same polarity (no phase change) as the incident wave [16].

Arterial pulse pressure evaluating in the initial systole, move on arteries and it is not caused by the forward movement of the blood: blood stroke volume forming distension of the medium and pulse pressure propagates through the aorta in waveform by elastic deformation of the vessel wall and the blood [17,18]. Further to elastic, sliding deformation of the blood is accompanying. The large arteries, *i.e.*, aorta, pulmonary artery and their major branches are referred to elastic arteries because of the presence of relatively large amounts of elastin. The structural features of various segments of the arterial tree have a functional significance in the conversion of discontinuous ventricular output to a continuous flow in the peripheral circulation. Due to the dispensability of the large arteries and the resistance encountered in the peripheral circulation, a portion of the blood ejected by the ventricle is stored in the large arteries during systole. In diastole stored blood is released, thus maintaining the continuity of peripheral blood flow and preventing excessive fall in arterial pressure [19].

Pulse oscillation in arterial wall increases strain rate to the contiguous wall flow layers. In our cases in image area at the time 0.043 sec initial net flow = 0, mean oscillation velocity v = 3-5 cm/sec, distance to the wall r = 0.5 cm. Oscillatory strain rate = 6-10 sec^{-1}. Due to viscosity/strain rate graph, at the strain rate up to 1 sec^{-1} blood viscosity sharply (more than x30) decreased.

The changes in viscosity are a result of modification in arrangement, orientation and stretching of the red blood cells. In the low shear rate region the cells are encountered in large aggregates and as the shear rate increases, the size of the aggregates diminish. At the mid shear rates, near unit strain internal stress due to pressure is sufficient to separate the aggregated cells causing breakage of aggregates (creep). Increasing shear rate causes the cells to orient in the direction of flow. Above a unit strain, a cell is forced to move past its adjacent neighbour. At the high shear stress rates blood forms layers of stretched and packed red blood cells sliding on layers of plasma.

Pulse wave velocity from aorta to small arterial wall is ≈ 7-15-35 m/sec. Pressure wave velocity in blood is ≈ 6-8 m/sec. Peak flow velocity in aorta is ≈ 1.2-1.4 m/sec. [20]. At the identical frequency, wave length to the vessel wall and adjoining flow blood slice must be different. Initiation of the strain rate and blood flow is shown on the stream surface and gradually involves the whole blood mass. So, phase delayed creep flow becomes sim-

pler. These correlations are more expressed at the proximal aorta. Here pulse wave oscillation amplitude is high and blood in systole reveals inertio-elastic properties. To the distal pulse wave attenuates and boundary layer enlarges, flow becomes viscous. Blood viscoelastic transformation is one of the main reasons for the low flow velocity at the high systolic pressure at the distal arterial tree (**Figure 6**).

Blood flow (creep/relaxation) delays to the pressure wave oscillation by the phase. Pressure and flow phase difference in viscoelastic flow is shown in Womersley's number-α. ($\alpha = d/2\sqrt{2\pi f\rho/\mu}$. d-diameter, f-fluctuation frequency, ρ-density, μ-viscosity). It defines relationship between elastic and viscous stress during the pressure oscillation and shows which part of the pipe is occupies by the boundary layer's viscous flow. In the large arteries, boundary layer is less than the blood volume provided by the pressure [5].

At the 43 msec. distances propagating by the pressure wave is about 28-60 cm. and covers all of the imaged places.

At the ascending aorta Flow is inertio-elasatic. Elasticity is promoted by the blood and vessel wall structure. Womerslay's number is 12.5 ± 1.5. In the elastic material phase angle between causative force-pulse pressure and strain rate (blood systolic oscillation) is low (**Figure 7**). Blood viscous stress exceeds to elastic and pulse energy stored mostly in the substance. In systole blood mostly oscillates; there is no/low creep flow. Creep flow phase delay to pressure is about 85˚; oscillatory (creep) flow amplitude is low. Flow amplitude is characterized by the relation of the maximum volumetric flow rate Qmax (volume of the fluid which passes through a given surface per unit time), to the stationary volumetric flow rate-Qst (flow rate which would be established at the maximum gradient pressure at the Poiseuille's flow) (**Figure 7**). This is main reason for the low initial systolic flow acceleration.

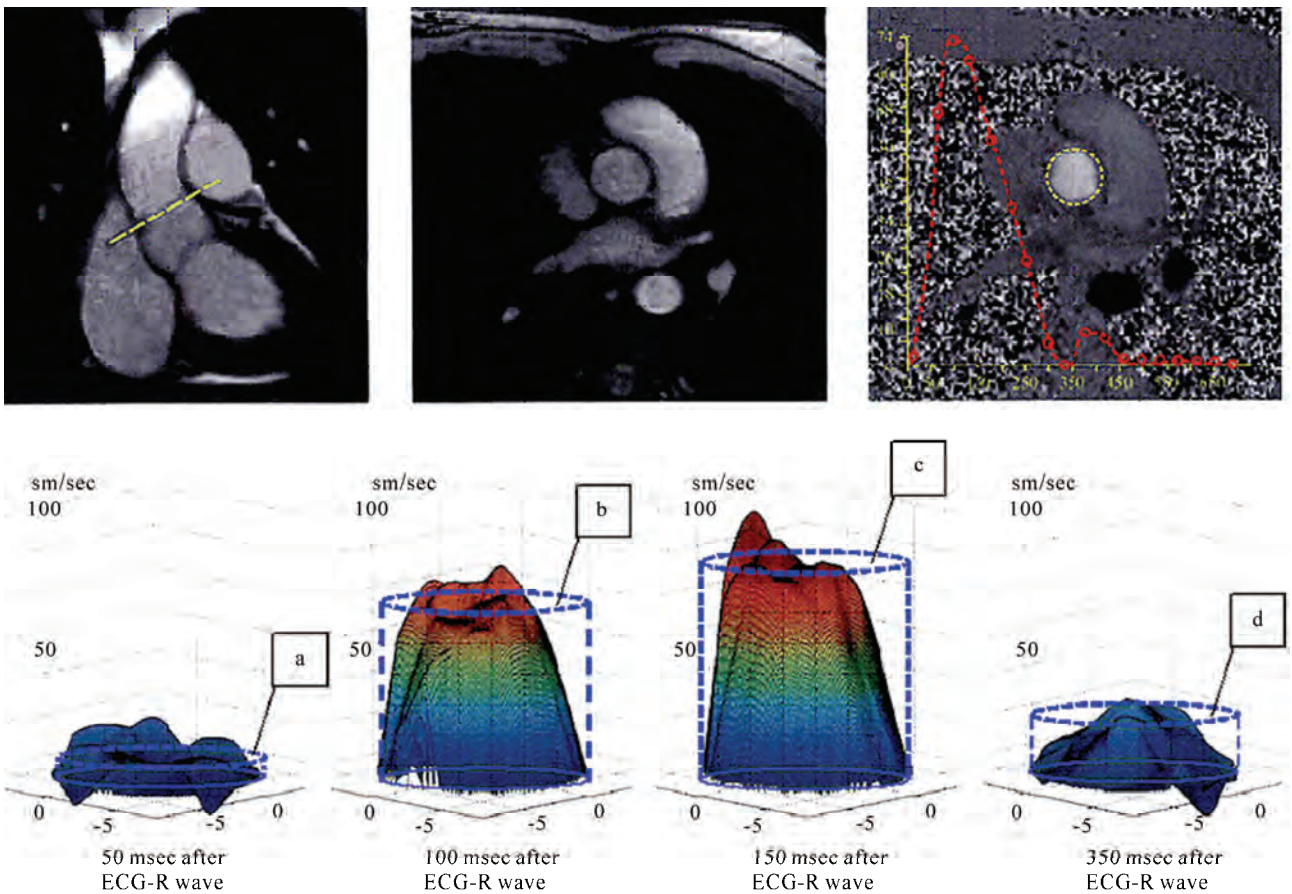

Figure 6. Transformation of the inertio-elastic flow to viscous through the time; boundary layer formation at the ascending aorta (3D reconstruction of the mean velocity in voxels inside the imaging slice). At the in initial systole pulse oscillation is noted at the stream surface. b and c. Inertio-elastic flow with the flat profile (plane wave at high Womerersley's number). d. Viscous (Poiseuille's) flow with the lower Womersley's number at 350msec. In difference to–a, flow profile is parabolic, although flow time is the same–50msec. (at 300msec velocity is zero).

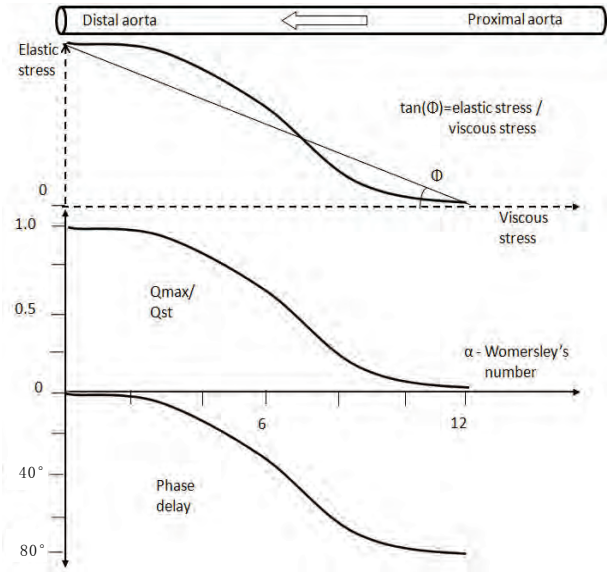

Figure 7. Pressure and flow phase delay in viscoelastic flow at the different sites of aorta. Elastic/viscous stress correlation in the material (above), flow amplitude (middle-comparable with the net flow) and pressure/flow phase delay (below) at the sinusoidal varying pressure with the increasing Womersley's number-α.

Blood is thyxotropic and decreases in the apparent viscosity under shearing, followed by a gradual recovery when the shear is removed. The effect is time dependent.

In diastole pressure is less pulsating. Pressure exposition time for the viscoelastic creep flow is higher than that for the systole. These correlations are more expressed at the distal aorta. Here pressure dissipates in blood structural rearrangement. Womerslay's number is 7.3 ± 1.2. In the viscous material phase angle between the causative force - pulse pressure and strain rate (creep flow) is high (**Figure 7**). Flow is more viscous/less inertial. Creep flow phase delay to pulse pressure decreases (about 40˚). Flow amplitude and net flow is high. Ratio of mean/net blood flow shows wiscoelastic properties of the local circulatory system.

Ratio of the (dynamic) viscous force to the inertial force (fluid density) is characterized by the kinematic viscosity. Kinematic viscosity with diameter of the vessel defines Womerslay's number. At the lower Womersley's number flow profile is parabolic and volumetric flow rate is determined by the instant pressure. Aforesaid is especially importance at the end diastole and to the distal arterial tree.

At the aortic arch blood circular movement facilitates flow velocity dispersion. In initial protodiastole blood flow is separated into the opposite flowing streams. Later streams have identical direction (flow recirculation). (**Figure 8**).

In line of our date, protodiastolic and systolic flow waves initially have different frequencies and at the superposition do not resist to each other [20]. Wave superposition facilitates to increase strain rate: blood particles at the same time participating in different oscillations. It causes to high net flow at the diastole as shown in graphs and can simplify blood outflow in the different arterial branches. Here stress must be sufficient to causing breakage of the red cell aggregates. (**Figures 1(d)-5(d)**).

At the isthmus area (end/entrance of the circle flow) separating flow waves at 300-400 msec have identical frequencies and phase (antegrade directed waves are formed at the same places of Walsalva sinuses), so they can interfere. (**Figure 3(a)**). Here at the destructive interference flow velocity is zero and further sharply increases. Flow anterograde acceleration in protodiastole is 6 times higher than in systole. At zero velocity systolic kinetic energy of the blood passes in to potential energy of the vessel wall. Wall elastic stress increases, wall oscillation basic frequency changes and systolic pulse wave can be attenuates.

At the isthmus area flow wave with the high acceleration changes position from external to internal wall. Circumstances can be promoted by the presence of the recirculation zones. Wave propagation on the graphs is similar to the wave reflection at the boundaries (**Figure 8**).

Obviously, systolic energy of the heart transforms in space and time by the elastic oscillation and/or viscous sliding of the blood elements. Movement is always in waveform. At the wave destructive interference high elastic stress facilitates initial recirculation at the external aortic wall.

By our dates, in protodiastole blood peak velocity at the external wall is much higher than that of systolic and peak shear stress is about 25 N/m^2. But just before the flow local pressure is much higher than at the flow and is equal to shear stress, it exceeds thresholds of the elastic deformation and as the outer slice of the blood is adhesive to the wall, forming endothelial denudation. At the place with the high local pressure flow wave reflects, *i.e.*, wave velocity vector changes in quantity and direction. At the hard boundary restoring force is high and the reflected wave has the different polarity to the incident wave (phase changes). But endothelial layer is not absolutely hard and wave can shift the vessel wall.

The ground substance, non-cellular components of extracellular matrix fibers in the human body, is thyxotropic and acts as a support for the cells. It seems that at the high shear stress to the viscoelastic transformation undergo not only blood cells, but extracellular matrix too.

The more so as cholesterol in experimental membranes

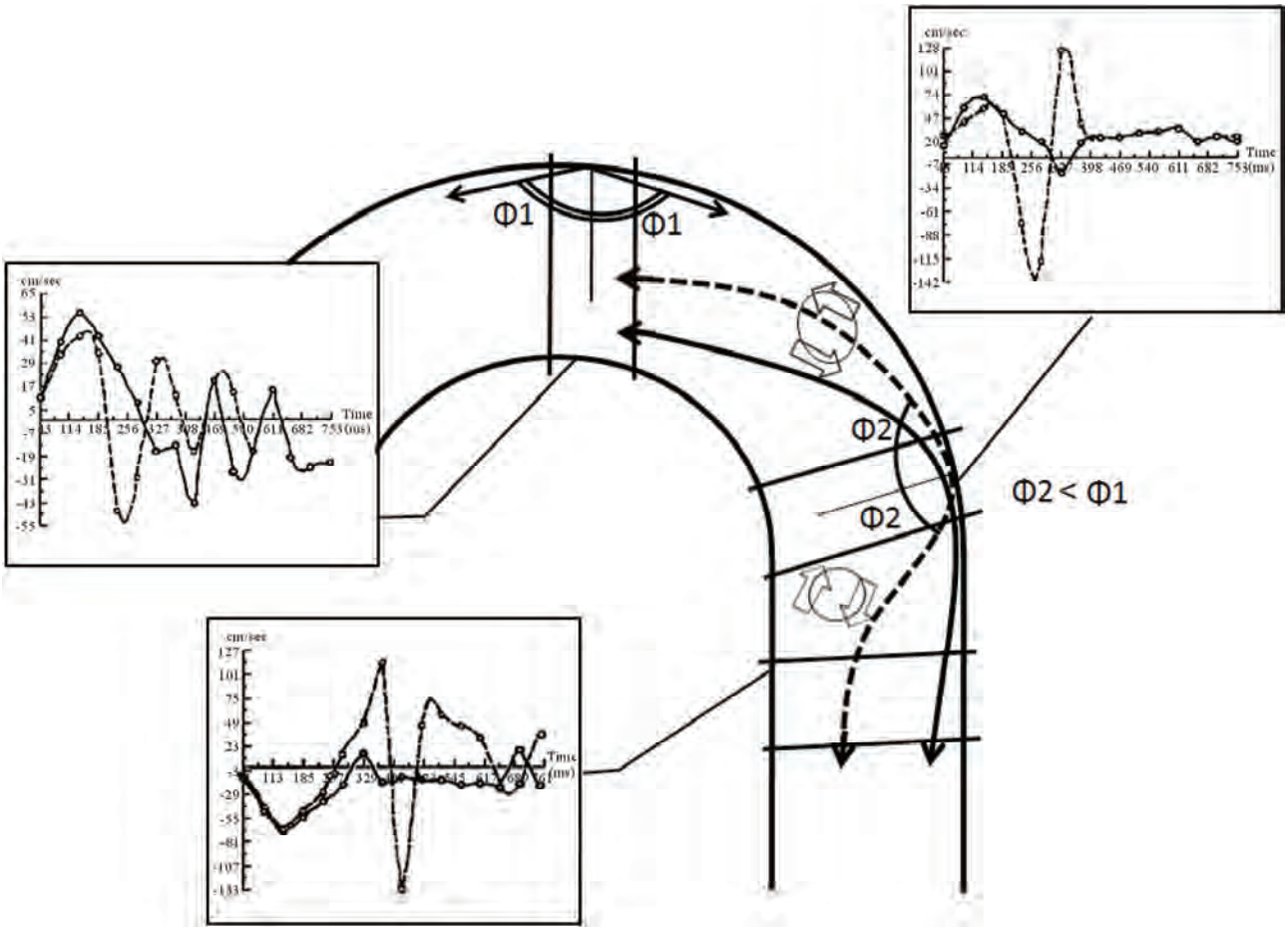

Figure 8. Flow wave reflection and recirculation sites at the different sites of the aortic arch. Dotted line – separated flow stream in retrograde (at 190-300msec) and anterograde (after 300msec) direction at the different walls of the aortic arch. At the isthmus wave node is displaced to the vessel wall.

can modify lateral tension and surface viscosity of the lipid bilayers [21]. In atherosclerosis it can be compensatory reaction of the vessel wall to the increased stress.

Flow wave interference with the reflection at the external wall also specified to the distal part of the ascending aorta (opposite to isthmus), but flow acceleration is much lower than at the isthmus area. Aortic dissection is characteristic for those areas.

It is significant that red blood cell aggregation affects blood viscoelasticity, but shear stress threshold to destruction of erythrocyte membrane is 6 times higher than for the endothelial sheet and erythrocyte membrane can move around the cell. Pathological data have shown that atherosclerotic change in children and young adults can be correlated with the presence of the same factors as it is identified in adults [22].

Circular movement at the arterial bifurcation can be facilitated by the same changes as at the aortic arch. It seems that at the vessel branching sites wave superposetion increases net flow, but is fraught with the endothelial denudation. Modified arterial pulse can be used as an atherosclerosis prevention factor. To obtain more specific information about the flow physiology in protodiastole and blood viscoelastic modification during the cardiac cycle, studies must be continued. Author hopes for cooperation with the other institutes.

5. CONCLUSIONS

Systolic pulse oscillation in arterial wall increases strain rate to the contiguous wall blood flow layers.

At the circular sites flow separation and wave superposition promotes to the blood viscoelastic modification and high net flow in diastole.

At the distinct circular area flow wave superposition forms destructive interference. Here wall systolic pulse oscillation attenuates and flow wave reflects.

In protodiastole anterograde directed viscous flow recirculation with the high acceleration and wave reflection shifts the endothelial layer.

REFERENCES

[1] Crowther, M.A. (2005) Pathogenesis of atherosclerosis**.** *Hematology*, **1(1)**, 436-441.

[2] Russel, R. (1999) Atherosclerosis–an inflammatory disease. *The New England Journal of Medicine*, **340(2)**, 115-126.

[3] Peter, L., Ridker, P.M. and Attilio, M. (2002) Inflammation and atherosclerosis. *Circulation*, **105(9)**, 1135-1143.

[4] Tuveli, M. (2001) Pathophysiological aspects of vascular disease: role of hemodinamic factors. *Technical Report. CRS4*, *Center for Advanced Studies*, Researche and development in Sardinia, Cagliari.

[5] Pedley, T.J. (1980) The fluid mechanics of large blood vessels. Cambridge University Press, Cambridge.

[6] Groisman, A. and Steinberg, V. (2004) Elastic turbulence in curvilinear flows of polymer solutions. *New Journal of Physics*, **6(29)**, 74437-74437.

[7] Almeder, C.R. (1997) Simulation of the Human Arterial System. Diplomarbeit ausgefuhrt ander Abteilung fur Simulationstechnik des Instituts fur Analysis, Technische Mathematik und Versicherungsmathematik an der Technische Universitat Wien, Wien.

[8] Gotz, J. (2006) Numerical Simulation of blood flow in aneurysms using the lattice boltzmann method. Master Thesis, Lehrstuhl für Informatik, Erlangen.

[9] Mandal, P.K. (2005) An unsteady analysis of non- Newtonian blood flow through tapered arteries with stenosis. *International Journal of Non Linear MechAnics*, **40(1)**, 151-164.

[10] Dintenfass, L. (1962) Thixotropy of the blood and proneness to thrombus formation. *Circulation Research*, **11 (2)**, 233-239.

[11] Brujan, E.A. (2000) Collapse of cavitation bubbles in blood. *Europhysics letters*, **50(2)**, 175-181.

[12] Lakes, R.S. (2009) Viscoelastic materials. Cambrige University Press, New York.

[13] Guyton, G. and Hall, J.E. (2006) Textbook of medical physiology. 11th Edition, Elsevier, Philadelphia.

[14] Billingham, J. and King, A.C. (2000) Wave motion. Cambridge Texts in Applied Mathematics, Cambridge University Press, Cambridge.

[15] Pain, H.J. (2005) The physics of vibration and waves. 6th Edition, John Wiley & Sons Ltd., England.

[16] Korpas, D., Halek, J. and Dolezaz, L. (2009) Parameters describing the pulse wave. *Physiological Research*, **58(4)**, 473-479.

[17] Mackenzie, I.S., Wilkinson, I.B. and Cockcroft, J.R. (2002) Assesment of arterial stiffness in clinical practice. *QJM*: *Oxford Journal Medicine*, **95(2)**, 67-74.

[18] Kitawaki, T. and Shimizu, M. (2005) Effect of the blood vessel viscoelasticity on the blood pressure wave propagation (Numerical analysis using one-dimen- sional viscoelastic tube model). *Transactions of the Japan Society of Mechanical Energies*, **71(707)**, 1768-1775.

[19] Aggoun, Y. and Beghetti, M. (2002) Noninvasive assessment of arterial function in children: Clinical application. *Images Pediatric Cardiology*, **13**, 12-18.

[20] Beraia, M., Todua, F. and Khomeriki, O. (2006) Protodiastole and vessel damage in atherosclerosis. *Biocybernetics and Biomedical Engineering*, **26(3)**, 65-75.

[21] Hiderbrand, M.F. and Bayerl, T.M. (2005) Differences in the modulation of collective membrane motions by ergosterol, lanosterol and cholesterol: A dynamic light scattering study. *Biophysical Journal*, **88(5)**, 3360-3367.

[22] Wennemann, P. (2008) Particle image velocimetry for microscale blood flow measurement. Gildeprint, Enschede.

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Determinants of self-rated private health insurance coverage in Jamaica

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ABSTRACT

The purpose of the current study was to model the health insurance coverage of Jamaicans; and to identify the determinants, strength and predictive power of the model in order to aid clinicians and other health practitioners in understanding those who have health insurance coverage. This study utilized secondary data taken from the dataset of the Jamaica Survey of Living Conditions which was collected between July and October 2002. It was a nationally representative stratified random sample survey of 25,018 respondents, with 50.7% females and 49.3% males. The data was collected by way of a self-administered questionnaire. The non-response rate for the survey was 29.7% with 20.5% not responding to particular questions, 9.0% not participating in the survey and another 0.2% being rejected due to data cleaning. The current research extracted 16,118 people 15 years and older from the survey sample of 25,018 respondents in order to model the determinants of private health insurance coverage in Jamaica. Data were stored, retrieved and analyzed using SPSS for Windows 15.0. A p-value of less than 0.05 was used to establish statistical significance. Descriptive analysis was used to provide baseline information on the sample, and cross-tabulations were used to examine some non-metric variables. Logistic regression was used to identify, determine and establish those factors that influence private health insurance coverage in Jamaica. This study found that approximately 12% of Jamaicans had private health insurance coverage, of which the least health insurance was owned by rural residents (7.5%). Using logistic regression, the findings revealed that twelve variables emerged as statistically significant determinants of health insurance coverage in this sample. These variables are social standing (two wealthiest quintile: OR = 1.68, 95% CI = 1.23 – 2.30), income (OR = 1.00, 95%CI = 1.00 – 1.00), durable goods (OR = 1.16, 95% CI = 1.12 – 1.19), marital status (married: OR = 1.97, 95% CI = 1.61 – 2.42), area of residence (Peri-urban: OR = 1.45, 95% CI = 1.199 – 1.75; urban: OR = 1.83, 95% CI = 1.40 – 2.40), education (secondary: OR = 1.57, 95% CI = 1.20 – 2.06; tertiary: OR = 9.03, 95% CI = 6.47 – 12.59), social support (OR = 0.64, 95% CI = 0.53 – 0.76), crowding (OR = 1.14, 95% CI = 1.02 – 1.28), psychological conditions (negative affective: OR = 0.97, 95% CI = 0.94 – 1.00; positive affective: OR = 1.11, 95% CI = 1.06 – 1.16), number of males in household (OR = 0.85, 95% CI = 0.77 – 0.93), living arrangements (OR = 0.62, 95% CI = 0.41 – 0.92) and retirement benefits (OR = 1.55, 95% CI = 1.03 – 2.35). This study highlighted the need to address preventative care for the wealthiest, rural residents and the fact that social support is crucial to health care, as well as the fact that medical care costs are borne by the extended family and other social groups in which the individual is (or was) a member, which explains the low demand for health insurance in Jamaica. Private health care in Jamaica is substantially determined by affordability and education rather than illness, and it is a poor measure of the health care-seeking behaviour of Jamaicans.

Keywords: Health Insurance;
Private Health Coverage;
Social Determinants of Health Insurance Coverage;
Jamaica

1. INTRODUCTION

Literature on private health insurance or health insurance in the Caribbean, and in particular Jamaica, has been substantially on 1) population density–*i.e.* coverage, 2) coverage offerings, 3) cost of care–*i.e.* health economics, and 4) acceptance (or lack of) by health service providers of certain insurance coverage. Having extensively perused the literature review on private health insurance and health care reform in Jamaica, it is obvious that no study has been conducted identifying the different factors that explain health insurance coverage in this nation. The individual utilization pattern of health insurance coverage is highly associated over time with older adults [1,2] as they prepare for the degeneration of the body; but, what else do we know about those who have private health insurance in Jamaica? Do insurers attract healthy patients, and are high risk individuals more likely to become insured as against their low risk (*i.e.* less health conditions) counterparts? Health insurance is a constituent of health seeking behaviour, suggesting that it is equally important in any study of health, quality of life, and wellbeing. In this study the researchers will critically examine factors that can be used to predict private health insurance coverage by using a logistic regression technique to explain the independent effect; and in the process the researchers will investigate the lives of respondents in order to understand those who reported having private health insurance coverage.

Instead of providing an elaborate and extensive description of 'health insurance', we will give a simplified meaning of this construct. Health insurance is protection against medical costs owing to the possibility of injuries, dysfunctions and other happenings that hinder the body from performing at some functional standard. In keeping with this definition, a health insurance policy is the contract that is signed by an insurer (*i.e.* insurance provider) and an individual or a group, in which the insurer agrees to pay a specific sum (*i.e.* a premium). Hence, the population's health service is partially dependent on health insurance coverage or the welfare system of the state. Jamaica does not have a public health insurance system, but one for the elderly and those who have particular chronic health conditions, such as diabetes mellitus, hypertension, cancer or a combination. In September 2001, the Cabinet of Jamaica accepted and approved a proposal for the establishment of a National Health Fund (NHF) that would assists patients as well as the elderly in Jamaicans. The individual benefits of the NHF (*i.e.* public health insurance options) for the elderly and for those with particular chronic health conditions was officially commenced in 2003 (*i.e.* August 1, 2003), and so there are only data on private health insurance coverage from 1988-2002. Despite the fact that Jamaica has instituted a free health-care service delivery programme for its child population (below 18 years in 2006), the quality of care which is relatively good is still surrounded by a certain socio-psychological milieu as well as inequality in health care offerings in the private versus the public sector. This explains the rationale why some people seek private health care and by extension private health insurance coverage [3] to meet the impending higher medical cost of care [1,4-7] and a particular quality of service–environment, customer service and length of service. The current study will be examined within the theoretical framework used by Franc, Perronnin, & Pierre [8].

1.1. Theoretical Framework

A South African Health Inequalities Survey (SANHIS) carried out in 1994 of 3,489 women ages 16 to 64 years was used to model the determinants of health insurance coverage. Kirigia *et al.*, [8] sought to model health insurance demand among South African women. They used binary logistic regression analyses to estimate health insurance coverage among the sample and various determinants of health insurance coverage. Health insurance coverage of the sample was determined by socio-demographic characteristics, health rating, environment rating, bad health choices (*i.e.* smoking and alcohol consumption), and contraceptives. These were embodied in the mathematical formula, **Eq. 1**:

$$P_{ij} = (\alpha + \beta_1 \text{Health rating} + \beta_2 \text{Environment rating} + \beta_3 \text{Residence} + \beta_4 \text{Income} + \beta_5 \text{Education} + \beta_6 \text{Age} + \beta_7 \text{Age squared} + \beta_8 \text{Race} + \beta_9 \text{Household size} + \beta_{10} \text{Occupation} + \beta_{11} \text{Employment} + \beta_{12} \text{Smoking} + \beta_{13} \text{Alcohol use} + \beta_{14} \text{Contraceptive use} = \beta_{15} \text{Marital status} + \varepsilon_i \quad (1)$$

where P_{ij} = 1 if individual I owns insurance (j = 1) and equal otherwise (j = 0); α is intercept terms; (β's) are the estimated coefficients; and ε_i is the stochastic error term.

The conceptual framework of Kirigia *et al.*'s work [8] was on two risks of health care. They believed that these risks are (1) the risk of becoming ill, with the associated loss in quality of life, cost of medical care, loss of productive times, more serious cases, mortality, and (2) the risk of total or incomplete or delayed recovery [8]. This denotes that a person's decision to buy health insurance would be based on differentials between the level of expected utility of the insurance and the expected utility without insurance. It is this binary nature dependent variable and the desire to determine the effect of particular independent variables that justified the binary logistic regression technique.

Eq. 1 allows for the estimation of the individual probability of having or not having health insurance by some explanatory variables. Kirigia *et al.*, [8] did not

stipulate whether health insurance was public or private coverage, and this was addressed in another research paper. Using the same principle of econometric analysis as Kirigia *et al*., a group of researchers used a single multiple regression equation that identified explanatory variables and the powers of particular factors that can be used to determine determinants of those who have private health insurance [9]. This captures a standard utility theory model of a demand for private health insurance coverage, **Eq. 2**:

$$Y = \beta_0 + \beta_1 P + \beta_2 I + \beta_3 Z \quad (2)$$

where the standard utility theory is expressed in the quantity demanded of health insurance, Y, can be written as a function of the user price of health insurance, P, income, I, and a vector of other factors, Z or (with time subscripts suppressed); and β_1 and β_2 represent, respectively, the price and income elasticity of the demand for private health insurance.

Like Kirigia *et al.*, [8] self-rated private health insurance coverage is a binary variable (1= yes and 0= otherwise), which denotes that a logistic regression model will be used to estimate the determinants and determine their impact on the dependent variable, as was done by Ahking, Giaccotto, and Santerre [9]-**Eq. 3**. Instead of having a vector factor which envelopes individual characteristics, this research isolates those factors including income, unlike **Eqs. 1** and **2**, and added more variables such as psychological conditions, living arrangements and social support.

$$HI_i = f(Y_i, HC_i, En_i, MS_i, AR_i, E_i, SS_i, O_i, P_i, G_i, NP_i, PP_i, M_i, F_i, D_i, EW_i, A_i, R_i, YP_i, Pmc_i, LL_i, CR_i) \quad (3)$$

where **Eq. 3** is Private Health Insurance coverage, HI_i, is a function of Y_i is average current income per person in household i; HC_i is health conditions of person i; En_i is physical environment of person i; MS_i is marital status of person i; AR_i is area of residence of person i; E_i is educational level of person i; SS_i is social support of person i; O_i is average occupancy per person i; P_i is property ownership of person i; G_i is gender per person i; NP_i is negative affective psychological conditions per person i; PP_i is positive affective psychological conditions per person i; M_i is number of males per household per person i; F_i is number of females per household per person i; D_i is the number of children per household per person i; EW_i is durable goods; A_i is age of person i; R_i is retirement benefits of person i; YP_i is social standing of person i; Pmc_i is cost of medical care of person i, LL_i is living arrangements of person i; and CR_i is crowding.

The current study found the following determinants of private health insurance of Jamaica (**Eq. 4**):

$$HI_i = f\ (Y_i, AR_i, MS_i, SS_i, E_i, \sum(NP_i, PP_i), M_i, EW_i, R_i, YP_i, LL_i, CR_i) \quad (4)$$

where **Eq. 4** is Private Health Insurance Coverage, HI_i, is a function of Y_i is average current income per person in household i; HC_i is health conditions of person i; AR_i is area of residence of person i; MS_i is marital status of person i; SS_i is social support of person i; G_i is gender per person i; E_i is educational level of person i; NP_i is negative affective psychological conditions per person i; PP_i is positive affective psychological conditions per person i; EW_i is durable goods of person i; D_i is the number of children per household per person i; Ri is retirement benefits of person i, YPi is social standing of person i, LL_i is living arrangements and CR_i is crowding.

2. MATERIALS AND METHODS

2.1. Method

This study utilized secondary data taken from the dataset of the Jamaica Survey of Living Conditions which was collected between July and October 2002. It was a nationally representative stratified random sample survey of 25,018 respondents, with 50.7% females (N = 12,675) and 49.3% males (N = 12,332). The data was collected by way of an administered questionnaire. The non-response rate for the survey was 29.7% with 20.5% not responding to particular questions, 9.0% not participating in the survey and another 0.2% being rejected due to data cleaning. The current research extracted a subsample of 16,118 people 15 years and older from the survey sample of 25,018 respondents in order to model the determinants of private health insurance coverage in Jamaica.

The rationale for the use of the 2002 data set instead of the 2007 is primarily because of the sample population. In 2002, the institutions that were principally responsible for the data collection used 10% of the national population to gather pertinent data on the labour force, and this was for the Survey of Living Conditions. It represents the largest data collected on the Jamaican population, and data was also collected on crime and victimization and the environment, these being included for the first time, and omitted in subsequent surveys. Given the nature of crime, violence and victimization in the nation, we opted to use a survey that had crime and the environment as among data collected. Another condition for the selection of this dataset was the fact that it was a large population, as against other years when the population was less than 3,000. Within the context of a non-response rate that ranges from 10 to 30 per cent, a larger rather than a smaller sample size coupled with some pertinent variables was preferred to a smaller sample size without the two critical aforementioned variables. Data were stored, retrieved and analyzed using

SPSS for Windows 15.0. A p-value of less than 0.05 was used to establish statistical significance. Descriptive analysis will be done on the sampled population in order to provide background information on the respondents; and the enter method of logistic regression will be used to establish the determinants of self-reported private health insurance in Jamaica. Using the principle of parsimony, the final model will consist of only those statistically significant variables. Where multicollinearity existed (r > 0.7), variables were independently entered into the model to aid in determining which one should be retained during the final model construction (*i.e.* the decision therefore was based on the variable's contribution to the predictive power of the model and the goodness of fit).

2.2. Measure

Health conditions: The summation of reported ailments, injuries or illnesses in the last four weeks, which was the survey period; where higher values denote greater health conditions; it ranges from 0 to 4 conditions. Health status is a dummy variable, where 1 (good health) = not reporting an ailment or dysfunction or illness in the last four weeks, which was the survey period; 0 (poor health) if there were no self-reported ailments, injuries or illnesses. While self-reported ill-health is not an ideal indicator of actual health conditions as people may under-report their health condition, it is still an accurate proxy of ill-health and mortality. Household crowding: This is the average number of persons living in a room. Physical Environment: This is the number of responses from people who indicated suffering landsides; property damage due to rains, flooding or soil erosion. Psychological conditions are the psychological state of an individual, sub-divided into positive and negative affective psychological conditions. 18-19 Positive affective psychological condition signifies the number of responses with regard to being hopeful and optimistic about the future and life generally. Negative affective psychological condition means number of responses from a person on having lost a breadwinner and/or family member, loss of property, being made redundant, or failing to meet household and other obligations.

Income is proxied by total individual expenditure in USD. During the survey period, United States $1.00 was equivalent to Jamaican $50.97. Average income (*i.e.* per person per household) is total expenditure divided by the number of persons in the household. Age: The number of years lived, which is also referred to age at last birthday. This is a continuous variable, ranging from 15 to 99 years. Age group is classified into three categories. These are young adults (ages 15 to 30 years), middle aged adults (ages 31 to 59 years) and the elderly (ages 60 + years). Retirement benefits were measured by those who recei- ved retirement income. Private Health Insurance Coverage: This is a dummy variable, where 1 denotes self- reported ownership of private health insurance coverage and 0 is otherwise.

Durable goods: This variable is the summation of the self-reported durable goods owned by an individual excluding houses, buildings and property. where D_i $EW = \sum_{i=1}^{28}(D_i)$ ranges from 1 to 28, where higher values denote greater ownership of durable goods.

Living arrangements are a dummy variable where, 1 = living alone, 0 = living with family members or relative.

Social support (or network) denotes different social networks with which the individual has been or is involved (1 = membership of and/or visits to civic organizations or having friends that visit one's home or with whom one is able to network, 0 = otherwise).

Crime:

$$\text{Crime Index}i = \sum_{i=1}^{n}(K_i T_j)$$

where K_i represents the frequency with which an individual has witnessed or experienced a crime, where i denotes 0, 1 and 2, in which 0 indicates not witnessing or experiencing a crime, 1 means witnessing 1 to 2, and 2 symbolizes seeing 3 or more crimes. T_j denotes the degree of the different typologies of crime witnessed or experienced by an individual (where j = 1…4, where 1 = valuables stolen, 2 = attacked with or without a weapon, 3 = threatened with a gun, and 4 = sexually assaulted or raped. The summation of the frequency of crime by the degree of the incident ranges from 0 and a maximum of 51.

Social standing is proxied by per capita population quintile (from poorest-to-wealthiest)

3. RESULTS

3.1. Demographic Characteristics of Sample

The sample was 16,619 respondents (*i.e.* 48.6% males and 51.4% females; with 39.2% young adults, 42.7% middle aged adults and 18.1% elderly). Some 25.8% of the sample resided in peri-urban areas; 60.2% in rural zones; 14.0% were from urban areas; 16.8% were below the poverty line (*i.e.* poorest 20%); while 18.2% were just above the poverty line compared to 21.2% in the wealthy quintile and 24.1% in the wealthiest 20%. Of the sample, 97.6% responded to the health status question. Of those who responded to the health status question, 80.6% indicated at least good health and 19.4% poor health. Ninety-seven percentage points of the sam-

ple (n = 16,118) responded to the health insurance coverage question, of that 11.9% revealed having health insurance coverage.

Based on **Table 1**, poverty is substantially a rural phenomenon. The findings revealed that 21.2% of rural residents were below the poverty line (*i.e.* poorest 20%) compared to 10.7% of peri-urban dwellers and 9.5% of urban settlers. Health insurance was greatest among urban residents: Some 20.8% of urban dwellers had health insurance compared to 17.6% for peri-urban settlers and 7.5% of rural residents. A significant statistical difference was found between area of residence and crime, and income in this sample.

Peri-urban residents spent the most statistically on medical care (USD 39.16 ± USD 85.77, 95% CI: USD 31.39 – USD 46.94) compared to urban (USD 30.25 ± USD 61.47, 95% CI: USD 22.66 – USD 37.83) and rural residents (USD 29.33 ± USD 54.15, 95% CI: USD 26.58 – USD 32.06) (**Table 1**)

On examination of the cross tabulation between good health status and social standing, a statistical correlation was found (P = 0.001) (**Table 2**). **Table 2** showed that the worst health was reported by those in the wealthiest quintile (21.8%), the poorest (19.9%), the poor (18.6%) and so on.

There is a positive statistical correlation between ageing and self-reported poor health (or health conditions) of Jamaicans (P = 0.001) (**Table 3**). Further examination of **Table 3** revealed that 10.3% of young adults reported poor health compared to 17.4% of middle aged adults and 43.6% of the elderly

Table 1. Demographic characteristic of sample by area of residence.

	Rural % (n)	Peri-urban % (n)	Urban % (n)	*P*
Age group				0.001
Young adults	38.3 (3833)	41.0 (1760)	39.7 (923)	
Middle age adults	41.6 (4160)	44.2 (1895)	44.6 (1039)	
Elderly	20.1 (2010)	14.8 (634)	15.7 (365)	
Health insurance coverage				0.001
Yes	7.5 (722)	17.4 (726)	20.8 (471)	
No	92.5 (8969)	82.6 (3442)	79.2 (1788)	
Gender				0.001
Male	50.4 (5041)	46.8 (2006)	44.3 (1031)	
Female	49.6 (4962)	53.2 (2283)	55.7 (1296)	
Per capita income quintile				0.001
1 = Poorest 20%	21.2 (2118)	10.7 (458)	9.5 (222)	
2	22.0 (2196)	13.3 (572)	11.2 (261)	
3	20.8 (2085)	18.7 (800)	16.7 (388)	
4	19.8 (1978)	22.7 (972)	24.3 (565)	
5 = Wealthiest 20%	16.2 (1625)	34.7 (1487)	38.3 (891)	
Marital status				0.001
Married	25.5 (2460)	26.9 (1115)	21.0 (475)	
Never married	66.6 (6433)	66.4 (2755)	71.6 (1619)	
Divorced	0.6 (56)	1.0 (41)	1.2 (26)	
Separated	1.1 (104)	1.2 (49)	1.4 (32)	
Widowed	6.3 (610)	4.5 (187)	4.8 (108)	
Crowding mean (SD)	1.77 ± 1.24	1.75 ± 1.28	1.72 ± 1.18	0.216
Crime index	1.74 ± 7.37	2.34 ± 8.08	2.83 ± 9.30	0.001
Medical expenditure[1] mean (SD)	$ 29.33±$54.15	$ 39.16 ± $85.77	$ 30.25± $61.47	0.012
Income[2] mean (SD)	$ 5496.12 ± $4860.97	$ 7534.74 ± $5544.26	$ 8779.26 ±$10568.69	0.001

[1]Medical Expenditure is expressed in USD: *United States* $1.00 *was equivalent to Jamaican* $50.97 (*during surveyed period*)

[2]Income is expressed in USD: *United States* $1.00 *was equivalent to Jamaican* $50.97 (*during surveyed period*)

Table 2. Good health status by social standing (Per capita population quintile).

Good health status	Social standing (Per Capita Population Quintile)					Total
	1=Poorest	2	3	4	5 = Wealthiest	
Poor	19.9	18.6	17.9	18.4	21.8	19.4
Good	80.1	81.4	82.1	81.6	78.2	80.6
Total	2738	2975	3208	3413	3883	16217

$\chi^2(4) = 23.273$, $P = 0.001$, contingency coefficient = 0.038

Table 3. Good health status by age group.

Good health status	Age group			Total
	Young age(15 to 30 years)	Middle age (31 to 59 years)	Elderly (60+ years)	
Poor	10.3	7.4	43.6	19.4
Good	89.7	82.6	56.4	80.6
Total	6283	6973	2961	16217

$\chi^2(2) = 1458.12$, $P = 0.001$, contingency coefficient = 0.287

3.2. Multivariate Analysis

Table 4 presents information on the variables which are correlated (or non-correlated) with private health insurance coverage in Jamaica of people 15 years and older. Using logistic regression, twelve variables emerged as statistically significant determinants of health insurance coverage in this sample (**Table 4**). These variables are social standing (two wealthiest quintiles: OR = 1.68, 95% CI = 1.23 – 2.30), income (OR = 1.00, 95% CI = 1.00 – 1.00), durable goods (OR=1.16, 95% CI = 1.12 – 1.19), marital status (married: OR=1.97, 95% CI = 1.61 – 2.42), area of residence (Peri-urban: OR = 1.45, 95% CI = 1.199 – 1.749; urban: OR = 1.831, 95% CI = 1.395 – 2.402), education (secondary: OR = 1.57, 95% CI = 1.20 – 2.06; tertiary: OR = 9.03, 95% CI = 6.47 – 12.59), social support (OR = 0.64, 95% CI = 0.53 – 0.76), crowding (OR = 1.14, 95% CI = 1.02 – 1.28), psychological conditions (negative affective: OR = 0.97, 95% CI = 0.94 – 1.00; positive affective: OR = 1.11, 95% CI = 1.06 – 1.16), number of males in household (OR = 0.85, 95% CI = 0.77 – 0.93), living arrangements (OR = 0.62, 95% CI = 0.41 – 0.92) and retirement benefits (OR = 1.55, 95% CI = 1.03 – 2.35).

The model [**Eq. 4**] had statistically significant predictive power (model $\chi^2 = 1604.389$, $P = 0.001$; Hosmer and Lemeshow goodness of fit $\chi 2 = 5.280$, $P = 0.727$), and correctly classified 91.3% of the sample (Correct classification of cases of reported health insurance coverage = 32.0% and correct classification of cases with no insurance coverage = 98.3%).

4. DISCUSSION

This study found that health insurance coverage is influenced by social standing, durable goods, income, marital status, area of residence, education, social support, crowding, psychological conditions, retirement benefits, living arrangements and the number of males in the household, and that those with good health are more likely to purchase health insurance than those with poor health. Continuing, rural residents, elderly and poorest, are the least likely to purchase health insurance coverage in Jamaica.

In the literature, it is well documented that the majority of uninsured workers in South Dakota were either employed or self-employed [6]. The poor, elderly and many rural residents are more likely to be employed on a seasonal basis in the informal sector, and these occupations and employment types do not have private health insurance, suggesting a further rationale for why unemployed people within a particular socio-economic status would be less likely to be holders of health insurance coverage. In this study, it was revealed that more uninsured Jamaicans were poor, elderly and from rural zones, and these were the ones most likely to be unemployed in Jamaica. The current study was not able to validate the direct claim of employability of the uninsured, but the elderly can indirectly validate the literature that more unemployed people do not have health insurance. In addition to the aforementioned fact, another finding was that poor health is associated with low income, owing to

Table 4. Logistic regression: Private health insurance coverage by some variables.

	P	Odds Ratio	95.0% C.I.	
			Lower	Upper
Age	0.443	1.00	0.99	1.00
Middle quintile	0.174	1.24	0.91	1.71
Two wealthiest quintiles	0.001	1.68	1.23	2.30
†Poorest 20%-to-poor		1.00		
Household Head	0.213	1.80	0.71	4.55
Logged medical expenditure	0.671	1.01	0.95	1.08
Average income	0.009	1.00	1.00	1.00
Durable goods	0.000	1.16	1.12	1.19
Separated or Divorced	0.608	0.90	0.61	1.33
Married	0.000	1.97	1.61	2.42
†Never married		1.00		
Peri-urban	0.000	1.45	1.10	1.75
Urban	0.000	1.83	1.40	2.40
†Rural area		1.00		
Environment	0.116	0.85	0.70	1.04
House tenure - rented	0.999	0.00	0.00	
House tenure - owned	0.950	1.04	0.27	4.03
House tenure - squatted*		1.00		
Secondary	0.001	1.57	1.20	2.06
Tertiary	0.000	9.03	6.47	12.59
†Primary and below		1.00		
Social support	0.000	0.64	0.53	0.76
Sex	0.722	1.03	0.86	1.24
Crowding	0.018	1.14	1.02	1.28
Crime index	0.652	1.00	0.99	1.01
Land ownership	0.665	0.96	0.79	1.16
Negative affective	0.034	0.97	0.94	1.00
Positive affective	0.000	1.11	1.06	1.16
Number of males in house	0.001	0.85	0.77	0.93
Number of females in house	0.622	0.98	0.89	1.07
Number of children in house	0.438	0.97	0.90	1.05
Living arrangement	0.017	0.62	0.41	0.92
Retirement benefits (1 = yes)	0.038	1.55	1.03	2.35
Poor health status	0.309	0.94	0.83	1.06

-2Log Likelihood = 3982.175

Nagelkerke R Square = 0.359

Model $\chi^2(8)$ = 1604.389, P-value =0.001

Hosmer and Lemeshow χ^2 = 5.280, P = 0.727

Overall correct classification = 91.3%: Correct classification of cases of reported health insurance coverage = 32.0%; Correct classification of cases with no health insurance coverage = 98.3%

†Reference group

the difficulties it creates with accessing crucial health care [6].

This research disagrees with the literature that the poor have lower health statuses, suggesting that they have more health-related conditions than the wealthy. The rich engage in highly involved particular lifestyle practices that expose them to health hazards, and this is not equally comparable to the poor environment of the poor, justifying why they reported the least health status. Pacione [10] has shown that the quality of the physical environment affects the quality of life (or health or wellbeing) of people, but that lifestyle behavioural practices play a significant role in determining one's health [11] like the physical milieu. [12,13] Moreover, the high cost of health care is a deterrent for the poor to have health insurance coverage; [6] and we concur with the literature as we found a positive statistical association between self-rated health insurance coverage and income. However, in this study we have refined the income variable, as there is a ceiling to income and its relation with the purchase of health coverage in Jamaica. The current work has revealed that those in the wealthy-to-wealthiest quintiles were twice as likely to purchase health insurance coverage as the poor-to-poorest people. Within the context that those in the wealthiest quintile purchased the most health insurance and indicated the lowest health status, it can be inferred that the purchase of health insurance is in keeping with their life style and the perceived role of income in buying good health, as against preventative behaviour.

Health insurance coverage is an elderly phenomenon, [6] and this work does not concur with the literature. The argument put forward is that younger people are healthier, and so do not see the need to invest in health coverage, as the risk of becoming ill is low, hence the willingness to engage in risky behaviour compared to their older counterparts, [6] suggesting that the futuristic end for health insurance coverage becomes even more critical after 30 years when more people will have families, as well as the fact that the purchase of health insurance may materialize owing to futuristic changes in the economic circumstances of the individual.

There is a statistical relationship between socioeconomic conditions and the health status of Barbadians, which is not the case in Jamaica. A study by Hambleton *et al.*, [11] of elderly Barbadians revealed that 5.2% of the variation in reported health status was explained by the traditional determinants of health. Furthermore, when this was controlled for current experiences, the percentage fell to 3.2% (a drop of 2%). When the current set of socioeconomic conditions was used, they accounted for some 4.1% of the variation in health status, while 7.1% were due to lifestyle practices compared to 33.5% which were as a result of current diseases. [11] Despite this fact, it is obvious from the data that there are other indicators which explain health status; people do not necessarily pay attention to this fact although they may have more income or access to more economic resources. This explains the rationale for more health conditions being reported by the wealthiest as well as the group that purchased the most health insurance, where the thinking is that money can buy health.

A study published in the Caribbean Food and Nutrition Institute on the elderly in the Caribbean found that 70% of individuals who were patients within different typologies of health services were senior citizens. [14-16] Among the many issues that the research reported on are the five major causes of morbidity and mortality, taken from the Caribbean Epidemiology Centre, which are of paramount importance to this discussion, and their influence on the elderly—cerebrovascular, cardiovascular, neoplasm, diabetes, hypertension and acute respiratory infection—and these dysfunctions are highly costly to treat. It should be noted that many of these dysfunctions are owing to lifestyle behaviour. Hence, the purchase of private health insurance coverage by these people when they become old and approach retirement is in keeping with the cost of health care and the high likelihood of becoming ill.

Eldemire, [17] on the other hand, opined that the elderly are not as sick as some people are making them out to be–"The majority of Jamaican older persons are physically and mentally well and living in family units" [17]; but the fact is they are preparing for the eventuality of health conditions owing to the principle of the degeneration of the body with the onset of old age. Eldemire is somewhat right. The current study found that for every 1 young adult who reported poor health, there were approximately 2 middle aged adults and 4 elderly persons. Simply put, there were elderly people with poorer health than other age cohorts; but of the elderly, more of them indicated good health status (56.4%). The mere fact of living longer (life expectancy post retirement is at least 15 years), suggests that the aged population will require more for medical care if they become ill. [18] With ageing the issue is not if they become ill but when. A group of scholars found that there is a direct association between ageing and health conditions, [19] a concept with which this study concurs. And this provides the explanation for the purchase of private health insurance more than other age cohorts, because they are at a different stage from other age cohorts in a population.

Health conditions are crucial to the purchase of primary health insurance coverage, and this is highlighted by ageing. Eldemire's works [17,18] have shown that ageing in an individual does not translate to high physi-

cal impairments, but that with ageing come particular changes in the profile of dysfunctions–Alzheimer's disease, dementia, cerebrovascular, cardiovascular, neoplasm, diabetes, hypertension and acute respiratory infection. [20] A study conducted by Costa [21], using secondary data drawn from the records of the Union Army (UA) pension programme that covered some 85% of all UA, shows that there is an association between chronic conditions and functional limitation–which includes difficulty walking and bending, blindness in at least one eye and deafness [21]. Among the significant findings is–(i) the predictability between congestive heart failure in men and functional limitation (*i.e.* walking and bending). Although Costa's study was on men, this applies equally to women, as biological ageing reduces physical functioning, and so any chronic ailment will only further add to the difficulties of movement of the aged, be it man or woman. One study has contradicted the works of Eldemire, and it showed that a large percentage of the elderly suffer from at least one health condition.

Women are more involved in health seeking behaviour, compared to their male counterparts, [22] irrespective of the age factor, and this is owing to the cultural background in which they live. Unlike women, across the world men have a reluctance to 'seek health-care' compared to their female counterparts. It follows in truth that women have bought themselves additional years in their younger years, and it is a practice that they continue throughout their lifetime which makes the gap in age differential what it is–approximately a 4-year differential in Jamaica. In keeping with the preventative care approach to health care, it would be expected that women would purchase more health insurance coverage than them, but this is not the case in Jamaica as gender was not a predictor of health status. However, the more men in a household, the less an individual will purchase health insurance coverage.

The Planning Institute of Jamaica in collaboration with the Statistical Institute of Jamaica has shown that while the general health status is commendable, chronic illnesses are undoubtedly eroding the quality of life enjoyed by people who are 65 years and older [23,24]. The JSLC report reveals that the prevalence of recurrent (chronic) diseases is highest among individuals 65 years and over. [23] The findings show that in 2000, the prevalence of self-reported illness/injury for people aged 65 years and over was 41.7%, for those 60 to 64 years it was 27.6% compared to 19.8% for children less than five years old. However, the prevalence of self-reported illness/injury for those 50 to 59 years was 18.8%. Some 36.6% of individuals 65 years and over reported injuries/illnesses in 2002 which is a 5.6% reduction in self-reported prevalence of illnesses/injuries over 2000, but the self-reported prevalence of illness/injuries rose by 25.8% to 62.4% in 2004. [25,26] It should be noted here that this increase in self-reported cases of injuries/ailments does not represent an increase in the incidence of cases, as according to the JSLC for 2004,the proportion of recurring/chronic cases fell from 49.2% in 2002 to 38.2% in 2004 [26]. In addition, the PIOJ and STATIN [23] in (JSLC 2000) opined that individuals 60-64 years of age were 1.5 times more likely to report an injury than children less than five years of age, and the figure was even higher for those 64 years of age and older (2.5 times more). In this paper, the findings concurred with the literature that health conditions are significantly greater; but other issues account for them not demanding more health insurance coverage than middle age adults. This is reinforced in the findings that showed that people who received retirement benefits were approximately twice as likely to purchase health insurance coverage as those who did not receive any retirement benefits. Embedded in this finding is the fact that health insurance is a matter of affordability and education, and not illness, which justifies why rural residents had the lowest health insurance coverage, yet still the poorest 20% good health status was greater than that of those in the wealthiest 20%. Statistics revealed that poverty in 2007 for the nation was 9.9%, and rural poverty was 15.3% compared to 4% in peri-urban and 6.2% in urban areas [27], accounting for the lowest private health insurance coverage in that group.

5. CONCLUSIONS

In summary, married Jamaicans are more likely to purchase health insurance coverage compared to those who were never married, with urban residents being more likely to purchase health insurance than rural dwellers. An individual who has attained tertiary level education was more likely to purchase health insurance than one with at most primary level education, and those who lived alone were less likely to purchase health insurance coverage than those who dwelled with relatives or family members. Moreover the wealthiest were more likely to purchase health insurance, but were less healthy, and this indicates that income does not buy good health. Therefore, this study highlighted the need to address preventative care for the wealthiest, and the fact that social support is crucial to health care, along with the fact that medical care costs are borne by the extended family and other social groups in which the individual is (or was) a member, which explains the low demand for health insurance in Jamaica.

6. DISCLAIMER

The researchers would like to note that while this study used secondary data from the Jamaica Survey of Living Conditions, none of the errors in this paper should be ascribed to the Planning Institute of Jamaica or the Statistical Institute of Jamaica, but to the researchers.

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REFERENCES

[1] Ettner, S.L. (1997) Adverse selection and the purchase of Medigap insurance by the elderly. *Journal of Health Economics*, **16(5)**, 543-562.

[2] Liu, T. and Chen, C. (2002) An analysis of private health insurance purchasing decisions with national health insurance in Taiwan. *Social Science & Medicine*, **55(5)**, 755-774.

[3] Dong, H., Kouyate, B., Cairns, J., Mugisha, F. and Sauerborn, R. (2003) Willingness-to-pay for community-based insurance in Burkina Faso. *Health Economics*, **12(10)**, 849-862.

[4] Carrin, G. (2003) Social health insurance in developing countries: A continuing challenge. *International Social Security Review*, **55(2)**, 57-69.

[5] Thomasson, M.A. (2006) Racial differences in health coverage and medical expenditure in the United States. *Social Science History*, **30(4)**, 529-550.

[6] The Lewin Group (2002) Health insurance coverage in South Dakota: Final report of the state planning grant program. South Dakota Department of Health.

[7] Varghese, R.K., Friedman, C., Ahmed, F., Franks, A.L., Manning, M. and Seeff, L.C. (2005) Does health insurance coverage of office visits influence colorectal cancer testing. *Cancer Epidemiology Biomarkers & Prevention*, **14(3)**, 744-747.

[8] Kirigia, J.M., Sambo, L.G., Nganda, B., Mwabu, G.M., Chatora, R. and Mwase, T. (2005) Determinants of health insurance ownership among South African women. *BMC Health Services Research*, **5(1)**, 1-17.

[9] Ahking, F.W, Giaccotto, C. and Santerre R. (2009) The aggregate demand for private health insurance coverage in the US. *Journal of Risk and Insurance*, The American Risk and Insurance Association, **76(2)**, 133-157.

[10] Pacione, M. (2003) Urban environmental quality of human wellbeing—a social geographical perspective. *Landscape and Urban Planning*, **65(1-2)**, 19-30.

[11] Hambleton, I.R., Clarke, K., Broome, H.L., Fraser, H.S., Brathwaite, F. and Hennis, A.J. (2005) Historical and current determinants of self-rated health status among elderly persons in Barbados. *Rev Panam Salud Publica* 2005, **17(5-6)**, 342-353.

[12] Bourne, P. (2007) Determinants of well-being of the Jamaican elderly. Master's Thesis, The University of the West Indies, Mona Campus.

[13] Bourne, P. (2007) Using the biopsychosocial model to evaluate the wellbeing of the Jamaican elderly. *West Indian Medical Journal*, **56(Suppl 3)**, 39-40.

[14] Caribbean Food and Nutrition Institute (1999) Health of the Elderly. *Caribbean Food and Nutrition Institute Quarterly*, **32**, 217-240.

[15] Caribbean Food and Nutrition Institute (1999) Focus on the elderly. *Caribbean Food and Nutrition Institute Quarterly*, **32**, 179-240.

[16] Anthony, B.J. (1999) Nutritional assessment of the elderly. *Caribbean Food and Nutrition Institute Quarterly*, **32**, 201-216.

[17] Eldemire, D. (1995) A situational analysis of the Jamaican elderly 1992. The Planning Institute of Jamaica, Kingston.

[18] Eldemire, D. (1997) The Jamaican elderly: A socioeconomic perspective & policy implications. *Social and Economic Studies*, **46(1)**, 75-93.

[19] Zimmer, Z., Martin, L.G. and Lin, H.-S. (2003) Determinants of old-age mortality in Taiwan. *Policy Research Division Working Papers Series*, **181**, Population Council, New York. (http://www.popcouncil.org/pdfs/wp/181.pdf)

[20] Eldemire, D. (1996) Level of mental impairment in the Jamaican elderly and the issues of screening levels, caregiving, support systems, carepersons, and female burden. *Molecular and Chemical Neuropathology*, **28(1-3)**, 115-120.

[21] Costa, D.L. (2002) Chronic diseases rates and declines in functional limitation. *Demography*, **39(1)**, 119-138.

[22] Rice, P.L. (1998) Health psychology. Wadsworth Publishing, Belmont.

[23] Planning Institute of Jamaica and Statistical Institute of Jamaica (2001) Jamaica survey of living conditions 2000. Kingston.

[24] Planning Institute of Jamaica and Statistical Institute of Jamaica (1998) Jamaica survey of living conditions 1997. Kingston.

[25] Planning Institute of Jamaica and Statistical Institute of Jamaica (2003) Jamaica survey of living conditions 2002. Kingston.

[26] Planning Institute of Jamaica and Statistical Institute of Jamaica (2005) Jamaica survey of living conditions 2004. Kingston.

[27] Planning Institute of Jamaica and Statistical Institute of Jamaica (2008) Jamaica survey of living conditions 2007. Kingston.

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Group training on the improvement of college students' career decision-making self-efficacy

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ABSTRACT

A group training was conducted on 17 college students to improve their career decision-making self-efficacy (CDMSE). The result showed that there was significant difference between the pre-test and the post-test for the experimental group (n = 17), whereas no significant difference was found between the pre-test and the post-test for the control group (n = 17). In the pre-test, there was no significant difference between the experimental group and the control group, and obvious difference between the two groups was found in the post-test. This indicated that the group training was effective on improving the CDMSE of the college students whose scores of CDMSE were below 27% point of the total students.

Keywords: College Student; Group Training; Attribution Training; Career Decision-Making Self-Efficacy

1. INTRODUCTION

Career decision-making self-efficacy (CDMSE) is the specific application of self-efficacy theory in the domain of career studies. Based on Bandura's self-efficacy theory and Crites's career maturity theory, Taylor and Betz define CDMSE as the extent to which individuals' believe that they can evaluate themselves, collect career information, select goals, make plans, and resolve problems relevant to career decision-making [1]. It has been found that CDMSE is of great importance to individuals career development. Individuals with higher level of CDMSE tend to be more active and positive in career decision-making, while individuals with lower level of CDMSE tend to be more passive and negative when choosing careers [2-4].

With the reform of college students' employment system in China in late 1990s, college graduates have to make their career choices by themselves, instead of waiting to be distributed by the government. In this situation, students are likely to feel confused and dazed and think that they are not capable of selecting a suitable career for themselves. Under many occasions, many graduates with lower CDMSE choose jobs just for the salary or only based on their parents' views, they usually choose some "hot" jobs beyond their abilities, interests and work values and exclude jobs that are actually in consistent with their abilities and interests. Career decisions made in this way are not mature and often lead to individuals' unsatisfaction with their jobs and other negative emotions, and result in a negative impact on their career development. In addition, individuals with lower CDMSE usually feel anxious and depressed during their career decision- making and their mental health is negatively affected. Therefore, it is urgent and necessary to design intervention which can improve students' decision-making self-efficacy.

Group training is a kind of psychological counseling conducted in a group context. Through means of observation, learning and experiencing during interpersonal interaction, group training enables individuals to explore, understand, and accept themselves, improve their interpersonal relationships, acquire new attitudes and behavioral styles, and develop good adaptation abilities. Group training has been found to be an effective way to improve college students' CDMSE [5-8]. In group training, by using such means as helping group members re-experience previous success, verbal persuasion, overcoming the anxiety occurring in career decision-making, providing modeling, and attributional training, counselors assist group members in self-evaluation, career information collection, goal setting, plan making, and solution of problems. However, little empirical studies based on the Chinese reality have been conducted on improving college students' CDMSE, which has blocked

subsequent studies in the domain of CDMSE. Moreover, existing studies on CDMSE improvement were conducted either by teaching students career decision-making skills or by attributional training, seldom studies have combined the two means. As argued by Borkowski, mere emphasis on individuals' effort is not enough, because great effort can not necessarily lead to progress when individuals are directed by incorrect cognitive strategies, which will decrease individuals' confidence [9]. Borkowski's statement has implied the importance of both internal attribution and career decision-making skills. Therefore, in this study, we consider the combination of attributional training and skills acquirement to improve college students' career decision-making self-efficacy.

2. METHOD

2.1. Participants

Students were recruited from the Southwest University in China through poster advertising. Career Decision-making Self-efficacy Scale (CDMSE) was administrated to the recruited students [10]. Students' scores on CDMSE scale were used as standard to select participants, 17 students whose scores were below 27% point of the total students were randomly selected into the experimental group (female = 12, male = 5), and another 17 students with the same condition were selected into the control group (female = 12, male = 5). No significant difference existed between the two groups on CDMSE.

2.2. Instrument

2.2.1. Career Decision-making Self-efficacy Scale

On the basis of Career Decision-making Self-efficacy Scale by Betz and Taylor, according to the interview and open-ended questionnaire, Peng developed this 39 items Career Decision-making Self-efficacy Scale, which included five dimensions: self-evaluation, information collection, goal selection, plan making, and problem solution [10]. The internal consistent coefficients of each dimension were between .68 and .81, the internal consistent coefficient of the total scale was .94.The retest reliability of each dimension were between 0.51 and 0.60, the retest reliability of the total scale was 0.66.

2.2.2. Internal verse External Locus of Control Scale

Individuals' CDMSE was found to be significantly related with their attributional style, with individuals with internal attribution having higher level of CDMSE while external attribution individuals having lower level of CDMSE. Attribution training has been shown to be effective in improving individuals' CDMSE [11-13]. Hence, in this study, we noticed the important role of positive attributional training and used the Internal verse External Locus of Control Scale as an assistant instrument. The Internal verse External Locus of Control Scale including 29 items in this study was developed by Rotter [14]. The score ranged from 0-23, with higher scores indicting higher level of external locus of control. The internal consistent coefficient was 0.70.

2.3. Intervention Program

The design of the intervention program was based on career decision-making self-efficacy theory by Taylor and Betz [1], while Bandura's self-efficacy theory [15], attribution theory, and other theories relevant to career were also taken into consideration. The whole intervention program can be divided into seven units, with each units owning a specific topic, including 'know about your achievement', 'learn to search career information', 'understand your interest and ability', 'set career goals', 'plan your career', 'overcome difficulties', and 'take job applications'. The group training included the following activities and forms such as brain storm, role play, modeling, verbal persuasion, group discussion, and games, etc. During the group training, the experimenter noticed to provide group members with encouragement and direction, and to help them form positive attribution style. At the end of each session, the group members were asked to summarize their feelings towards the training. The experimenter created an acceptable and supportive atmosphere. Through group activities and interpersonal interaction, the group members could attempt to feel the positive experience, rebuild rational knowledge relevant to career choice, learn career decision-making skills, change their career-decision style from the improper and immature one to the proper and mature one, which can be used in realistic situation and therefore improve group members' CDMSE.

2.4. Implementation Progress

As a first step, assign participants into control group and experimental group. Based on the concrete situation of the experimental group members, we made modifications on the initial intervention program in order to make the intervention more goal-directed and specific.

Second, the group training was conducted one session each week, with each session lasting 90 minutes. The participants in the control group received on training.

2.5. Follow up Study

The participants in the experimental group were investigated again 3 months after the end of CDMSE group training.

3. RESULTS

Career Decision-making Self-efficacy Scale and Internal verse External Locus of Control Scale were used in the pre-test and post-test. Data were analyzed with SPSS 12.0 software for Windows XP.

The comparison of CDMSE scores on pre-test and post-test between the experimental and control group was made (see **Table 1**).

As can be seen from **Table 1**, for the participants in the experimental group, the scores on all the dimensions and on the total scale in the post-test were significant higher than scores in the pre-test ($p < 0.001$). However, for the control group, except on the plan making dimension, no significant difference was found on other dimensions and on total score between the pre-test and the post-test ($p > 0.05$).In the pre-test, no matter on all the dimensions and on total scale, no significant difference was found between the experimental group and the control group. However, in the post-test, the scores on all dimensions and on total scale in the experimental group were significantly higher than those in the control groupmembers ($p < 0.001$). This indicated that the experimental group members' CDMSE was significantly improved after the group training.

The comparison of locus of control scores on pre-test and post-test between the experimental and control group was made (see **Table 2**).

As can be seen from **Table 2**, for the experimental group, there was a significant difference between the pre-test and post-test on scores of locus of control ($p < 0.01$), with the post-test scores higher than the pre-test scores. For the control group, no such significant difference was found between the pre-test and the post-test ($p > 0.05$). In the pre-test, there was no significant difference between the control group and experimental group on the scores of locus of control. But significant difference between the two groups was observed after the group training ($p < 0.001$), which indicated that the group training had significantly improved experimental group members' internal locus of control.

Three months after the group training, another survey of CDMSE using the same scale was conducted on the experimental group members to check up the sustained effects of the group training. The comparison between the scores obtained immediately after the training and the scores obtained three months later was made in **Table 3**.

Table 1. The comparison of CDMSE scores on pre-test and post-test between the experimental and control group.

Dimension	Group	Pre-test (n = 17) Mean ± SD	Post-test(n = 17) Mean ± SD	t value
Self-evaluation	Experimental group	2.44 ± 0.46	3.68 ± 0.63	5.61***
	Control group	2.39 ± 0.54	2.49 ± 0.55	2.28
	t value	–.29	–5.86***	
Information collection	Experimental group	2.33 ± 0.42	3.76 ± 0.57	7.13***
	Control group	2.43 ± 0.49	2.52 ± 0.54	2.02
	t value	.18	–6.56***	
Goal setting	Experimental group	2.12 ± 0.41	3.56 ± 0.55	8.62***
	Control group	2.15 ± 0.45	2.20 ± 0.42	2.16
	t value	0.60	–7.45***	
Plan making	Experimental group	2.14 ± 0.43	3.40 ± 0.65	6.25***
	Control group	2.13 ± 0.34	2.32 ± 0.36	4.95***
	t value	.66	–6.04***	
Problem solution	Experimental group	2.24 ± 0.37	3.63 ± 0.56	8.04***
	Control group	2.31 ± 0.36	2.41 ± .45	2.14
	t value	0.19	–7.00***	
Total scale	Experimental group	2.24 ± 0.33	3.60 ± 0.51	8.34***
	Control group	2.28 ± 0.32	2.30 ± 0.36	2.42
	t value	0.28	–7.89***	

Note: ´p<0.05; ´´p<0.01; ´´´p<0.001.

Table 2. The comparison of locus of control scores on pre-test and post-test between the experimental and control group.

Group	Pre-test (n = 17) Mean ±SD	Post-test (n = 17) Mean ± SD	t value
Experimental group	15.82 ± 2.834	9.82 ± 2.32	–19.41***
Control group	15.41 ± 1.906	15.59 ± 2.35	0.24
t value	–0.497	7.20***	

Note: ´p<0.05; ´´p<0.01; ´´´p<0.001.

Table 3. The comparison between the scores obtained immediately after the training and the scores obtained three months later.

Dimension	Immediate after training (n = 17) Mean ± SD	Three months after training (n = 17) Mean ± SD	t value
Self-evaluation	3.68 ± 0.63	3.58 ± 0.64	–1.89
Information collection	3.76 ± 0.57	3.67 ± 0.47	–1.93
Goal setting	3.56 ± 0.55	3.44 ± 0.53	–2.01
Plan making	3.40 ± 0.65	3.43 ± 0.55	–0.67
Problem solution	3.63 ± 0.56	3.63 ± 0.55	0.15
Total scale	3.60 ± 0.51	3.58 ± 0.52	–1.52

Note: ´p<0.05; ´´p<0.01; ´´´p<0.001.

As can be seen from **Table 3,** no significant difference was found between the scores obtained immediately after the training and the scores obtained three months later, despite a nonsignificant reduce on the dimension of self-evaluation, information collection, goal selection, plan making and on the total score (p > 0.05). This indicated that the group training in this study has a sustained effect on the improvement of CDMSE.

4. DISCUSSIONS

Group training is an assistant form of education on career development. In this study it was found that group training was effective on the improvement of CDMSE and could facilitate individuals' career planning development. The group members in the experimental group maintained a higher level of CDMSE three months after the group training, showing the efficiency of this group training. The reasons why the group training could achieve success were due to the following aspects.

4.1. Effective Application of Group Training

According to the requirement of group training on the number of group members, 17 students were included in each group, in order to guarantee the interaction among the members and the satisfaction of the different needs of group members. With an atmosphere of ease and safety, group training is a process in which group members can explore and exchange ideas on the problems they encounter. The group training in this study allowed the group members to discuss problems relevant to career choosing freely and openly. The group members could get instant reactions, suggestions and directions from both the other group members and the counselors, which enabled them to understand, analyze, explore and evaluate themselves more effectively and accurately and to open up their career development according to their own situation [16]. The counselors were aware of the importance of interaction among group members, which guaranteed a two-way communication. The counselors and group members together drafted rules and goals for the group, which were required to be obeyed by all group members. In addition, the counselors noticed the different requirements of different members. When the group members got together, their feeling of loneliness and alienation could be eliminated. Members in this atmosphere would effectively understand themselves and others and establish their self-confidence. With the interaction context and the ease and safety atmosphere, group members were likely to take part in group activities actively, experience the outcomes resulting from their behavior changes, acquire timely feedback from other members and counselors, and then correct their behaviors. Group members learned skills on dealing with interpersonal relations and skills on problem solution, obtained related experience, and improved their interpersonal communication abilities. Besides, group training on career planning caused the group members realize

the significance of individual career counseling.

4.2. Intervention Based on Bandura's Self-efficacy Theory Has Guaranteed the Effectiveness

As Bandura [15] has pointed out, individuals' self-efficacy is affected by such four factors as personal experience, modeling, verbal persuasion and emotion arousing. In the present group training, Bandura's self-efficacy theory was effectively incorporated into the intervention. Counselors allowed group members to recall their past success experience, provided group members with instant and positive feedback and encouragement, set modeling for them, applied breath relaxation, muscle relaxation, imagination relaxation techniques to help group members overcome the anxiety they experience in group activities.

4.3. The Comprehensive Application of Various Theories on Career Choosing and Career Development

The group training in present study was designed under the direction of relevant theories on career development and career choosing, including Holland's career theory, Ginzberg and Super's career development theory, social learning theory by Krumboltz, and social cognitive development theory [16]. In his career theory, Holland has assumed that most people can be divided into the following six types of personalities: realistic type, research type, artistic type, social type, management type and conventional type. He proposes that individuals' career choice should be in consistent with their personality styles. The career development theory by Ginzberg and Super has divided individuals' career development into five stages: growing up stage, exploration stage, establishment stage, maintenance stage, and declining stage. Ginzberg holds that each stage has its own task and individuals should be well aware which stage they are at, which reflects individuals' career maturity degree. Super believes that career choosing is actually individuals' choosing the way to realize their self-conceptions. Individuals are answering the question "who am I" in choosing their careers. The social learning theory by Krumboltz holds that individuals' career choice is influenced by individuals' genetic characteristic, environment condition, experience learned in the past, skills acquired in resolving new tasks and new problems, performance standard, and individuals' career values. Social development theory focuses on individuals' decision-making process, during which five kinds of information processing skills are used, including communication, analyzing, synthesizing, evaluation and execution. When the group training was designed, the theories above were taken into consideration. For instance, in the unit "understand your interests and abilities", Holland's career-personality matching theory was applied to help group members understand their career personalities and interests correctly. Ginzberg and Super's theory was employed in this unit to allow group members to answer "Who am I" through means of group discussion and brain storm, in order that group members have an accurate and through understanding on themselves.

4.4. The Combination of Obtaining Career Decision-making Skills and Attributional Training

In previous studies, researchers either focused on the acquirement of career decision-making skills or attributional training to improve individuals' CDMSE [5-8,17]. Self-efficacy is individuals' perceived abilities to accomplish certain tasks and is related with individuals' cognition on themselves. Therefore, as a means to change individuals' locus of control, forming positive attribution style through attrubitional training is an important means to help individuals know about their abilities properly. However, as a complicated process, career decision- making is affected by multi-aspect factors. Therefore, if only the lack of effort is attributed as the causes of failure in career decision-making and the skills that are needed during career decision-making are neglected, it is very likely that even individuals with positive attribution style are not able to reach a suitable career choice, which will discourage them and cause them depressed. Based on this consideration, Borkowski [9] proposed that only stress on hard working is not enough under many occasions; individuals who are lack of correct cognitive strategies will hardly make progress and then frustrate themselves. Therefore, in this study we combined the skills training with the attribution training, which made our intervention effective.

5. LIMITATIONS AND DIRECTIONS FOR FUTURE STUDY

In spite of the effective function of group training on improving college students' CDMSE in this study, limitations still exist. Only students whose scores were below 27% point of the total students were selected for the group training, which had limited the significance of this study. Students with high scores of CDMSE should be included in the future study. In addition, the small number of subjects would great reduce the reliability of the results based on objective interview and questionnaire. We recommend that naturalistic observation method can be used in the future study to further validate the effectiveness of group training.

6. CONCLUSIONS

Group training can help college students form positive attribution style and improve their CDMSE. Group training on CDMSE has a sustained effect and can be applied to the career counseling in reality.

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REFERENCES

[1] Taylor, K.M. and Betz, N.E. (1998) Applications of self-efficacy theory to the understanding and treatment of career indecision. *Journal of Vocational Behavior*, **22(1)**, 63-81.

[2] Luzzo, D.A. (1993) Value of career-decision-making self-efficacy in predicting career-decision-making attitudes and skills. *Journal of Counseling Psychology*, **40(2)**, 194-197.

[3] Betz, N.E. and Hackett, G. (1981) The relationship of career-related self-efficacy expectations to perceived career options in college women and men. *Journal of Counseling Psychology*, **28(5)**, 399-410.

[4] Clement, S. (1987) The self-efficacy expectations and occupational preference of females and males. *Journal of Occupational Psychology*, **60(3)**, 257-265.

[5] Krass, L.J. and Hughey, K.F. (1992) The impact of an intervention on career decision-making self-efficacy and career indecision. *Professional School Counseling*, **2(5)**, 384-391.

[6] Sullivan, K.B. and Mahalik, J.R. (2000) Increasing career self-efficacy for women: Evaluating a group intervention. *Journal of Counseling and Development*, 2000, **78(1)**, 54-62.

[7] Chartrand, M. J. and Rose, M. L. (1996) Career interventions for at-risk populations: Incorporating social cognitive influences. *The Career Development Quarterly*, **44 (4)**, 341-353.

[8] Luzzo, D.A. (1996) Attribution retraining increases career decision-making self-efficacy. *The Career Development Quarterly*, **44(3)**, 378-385.

[9] Borkowski, J.G., Weyhing, R. and Carr, M. (1988) Effect of attributional retraining on strategy-based reading comprehension in learning-disabled student. *Journal of Educational Psychology*, **80(1)**, 46-53.

[10] Peng, Y.X. (2000) Development of career decision-making self-efficacy scale. Published Postgraduate Dissertation, Central China Normal University, Wuhan.

[11] Taylor, K.M. and Popma, J. (1990) An examination of the relationships among career decision-making self-efficacy, career salience, locus of control, and vocational indecision. *Journal of Vocational Behavior*, **37(1)**, 347- 359.

[12] Rodriguez, M. and Blocher, D. (1988) A comparison of two approaches to enhancing career maturity in Puerto Rican college women. *Journal of Counseling Psychology*, **35(3)**, 275-280.

[13] Luzzo, D.A. (1994) Effects of verbal persuasion on the career self-efficacy of college freshmen. *California Association for Counseling and Development Journal*, **14 (1)**, 31-34.

[14] Rotter, J.B. (1966) Generalized expectancies of internal versus external control of reinforcements. *Psychological Monographs*, **80(1)**, 609-609.

[15] Bandura, A. (1977) Self-efficacy: Towards a unifying theory of behavioral change. *Psychological Review*, **84 (2)**, 191-215.

[16] Shen, Z.F. (2000) Psychological counseling on career. Shanghai Educational Press, Shanghai.

[17] Li, B. and Wang, X. (2006) Group training on improving women college students' career decision-making self-efficacy. *China Mental Health Journal*, **20(11)**, 765-767.

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Health, lifestyle and health care utilization among health professionals

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ABSTRACT

Health care workers are responsible for the execution of the health policy of a nation, yet little if any empirical evidence is there on health, lifestyle, health choices, and health conditions of health care workers in the rural parish of Hanover, Jamaica. The current study examines health, lifestyle and health behaviour among health professional in Hanover. The current study has a sample of 212 respondents. A 26-item questionnaire was used to collect the data. Data from the questionnaires were coded and entered into a micro-computer and analysis done using SPSS for Widows Version 15.0 software. The Chi-square test was used to test association between non-metric variables. A p-value < 0.05 (two-tailed) was selected to indicate statistical significance. It was found that 16.0% of respondents had diabetes mellitus (2.8% of males compared to 19.8% females); 22.6% had hypertension (25.5% of female and 12.8% of males); 0.5% breast cancer; 0.5% stomach cancer; 1.9% enlarged heart; and 0.5% ischemic heart disease. Forty-three percentage points of the sample was overweight, 33.5% obese and 24.1% had a normal weight. Over 15% of nurses and doctors were obese compared to 38% of ancillary staffers. Twenty percentage points of respondents consume alcohol on a regular basis; 15.6% do no regular physical exercise, 42.4% add sweetening to their hot beverages, and 4.7% were smokers. There is a need for public health practitioners to formulate a health intervention programme that will target people in Hanover, but also specific groups such as doctors, nurses, administrative, ancillary staffers and technical staffers.

Keywords: Health Care Workers; Health; Lifestyle; Health Choices; Health Behaviour; Hanover; Jamaica

1. INTRODUCTION

Empirically, it is well established that poverty and illness are positive associated with each other and that 80% of all chronic illnesses were in low-to-middle income countries [1-3]. Sen [1] encapsulated this well when he stated that low levels of unemployment in the economy is associated with higher levels of capabilities, suggesting that poverty predisposed people to illnesses. The World health organization (WHO) [2] reported that 60% of global mortality is caused by chronic illness and four-fifths of chronic dysfunctions are in low-to-middle income countries. This concurs with Sen's finding that poverty does not only predisposed people to illnesses but that it accounts for premature mortality.

In many developing countries, the living standards of low income households could be improved by improving health services [4]. The lack of resources available for use by the government of Jamaica to address poverty in a significant way negatively impacts health care. There is a significant statistical correlation between poverty and illnesses in Jamaica [5,6]. A study by Bourne [5] found: 1) a positive correlation between not seeking medical care and poverty ($r^2 = 0.58$), 2) a positive correlation between poverty and unemployment ($r^2 = 0.48$), and 3) an inverse correlation between mortality and poverty ($r^2 = 0.51$). These findings do not substantiate the findings in the literature of the correlation between premature mortality and poverty. Despite Bourne's findings, the level of poverty in Jamaica was 9.9% in 2007. This was 15.3% in rural areas compared to 6.2% in urban and 4.0% in peri-urban area [7]. Another important finding is that rural residents indicated the highest percentage points of illness (17.3%) compared to urban (14.1%) and

peri-urban residents (13.9%).

Although premature mortality was empirically not found using the data for Jamaica, the positive correlation between poverty and illness is present and cannot be overlooked as there are public health challenges owing to this reality. Jamaica an English-speaking Caribbean nation is a developing country. In 2007, it had a population of 2,682,120 people (49.3% males); 75% black and 13% mixed; a growth rate of 0.47; 10.9% elderly population (*i.e.*, 60+ year old); a crude death rate of 6.4 per 1,000; crude birth rate of 17.0 per 1,000 [8]; income inequality of 0.4 (Gini coefficient); and 71.3% of the poor lived in rural areas [7]. The country is geographical divided into 14 parishes and three counties (Cornwall, Middlesex and Surrey). Cornwall covers the Western belt which includes parishes such as Westmoreland, Hanover, St. James and St. Elizabeth. Middlesex constitutes the middle proportions of the island with parishes such as Clarendon and St. Catherine. Surrey comprises the Eastern region with parishes such as Kingston, St. Thomas and Portland. Cities accounts for 27.3% of the population, peri-urban 30.2% and rural areas, 42.5% in 2007.

With 43 out of every 100 Jamaicans resided in rural areas and those areas have 15.3% of the poverty, public health policy makers are concerned about health care and behaviour among rural residents. Hanover has the smallest percent of the nation's population (2.6%—69,660, in 2007), with one urban centre (*i.e.*, Lucea) [9]. Lucea, the capital of Hanover is home to about 5,951 people. The parish of Hanover therefore is substantially rural, and the people rely on tourism, agriculture and seasonal employment for their economic livelihood. Although Hanover is rural and shares many of the economic challenges of rural zones, little if no information is available about health, lifestyle practices and health care seeking behaviour of the residents. Since public health agencies relies on research information to make inform decision that can effectively aid in improving the health of a population, then it follows that pertinent information is needed on residents of Hanover in order to enhance public health capability on the parish. Most if not all the health information on Hanover is from the Ministry of Health (MOH) which only produce standard curative statistics (*i.e.*, health service utilization; mortality; health care expenditure; health care resources; morbidity) [10-12]. The findings from this study will add value to the existing literature by examining health information on persons in the parish. This was done by examining health, lifestyle and health behaviour among health professional in Hanover in order to understanding choices, decision and health among its residents, with the purpose of aiding policy formulation and health intervention programmes for the parish.

2. MATERIALS AND METHODS

2.1. Sample, Sampling Methods and Setting

We selected a representative sample of people from Hanover's health institutions, which had sufficient numbers to represent the people of the parish. The MOH in Jamaica sub-divided the country into 4 regional administrative authorities (RHAs): the South-East (SERHA); South (SRHA); North-East (NERHA), and Western (WRHA). The NERHA covers four parishes—Hanover, Westmoreland, St. James and Trelawny. Another classification of the island is statistical one based on Enumeration Districts (EDs). The Planning Institute of Jamaica (PIOJ) and Statistical Institute of Jamaica (STATIN) used Primary Sampling Units (PSUs) as its sampling frame from which it design surveys of the national population [7]. A PSU is an ED or a composition of EDs, usually consisting of 100 dwellings in a rural area and 150 dwellings in urban areas [7]. STATIN further refined required dwellings by stating that up to 400 households constituted a PSU [9]. The EDs are independent geographical units which share common boun- daries with contiguous EDs. In keeping with a sampling error of ± 3% and a confidence interval of 95%, the calculated population for selection was 280 respondents. In another survey, the researchers used 36 persons per ED to calculate a representative sample of the nation [13,14]. Hanover has 4 PSUs, which means that using 36 persons per ED the sample should be 144. Hence, based on previous surveys, the current study is sufficient to generalize on the parish because it is has representative sample size [13,14]. The current sample of 212 respondents represents 0.3% of the population of the parish of Hanover (in 2007; n = 69,660). For this study, the sample was stratified by area of work, area of residence, and a Kish Random Selection Method of sub-sampling was used to select the actual respondents thereby facilitating independence of response [15]. On occasions when an individual was selected and could not participate, no other person was used to replace the individual. In cases where the selected person was not available a minimum of three call-back visits would be made to that person's place of work. The response rate was 75.7%, of which 1.3% of the data were lost during data cleaning. This is in keeping with surveys conducted by PIOJ and STATIN [7], and Wilks *et al*. [14]. For the survey study 77.8% of the sample was female, which is similar to that reported by Wilks *et al*. [14] in which the female sample was 75.9%.

2.2. Questionnaire Reliability

Test-retest reliability of the questionnaire was conducted for a month (*i.e.*, February 2008) prior to the main study. The instrument was vetted by academics from the University of the West Indies, Mona, Jamaica. Then 20 respondents who were non-participants (*i.e.*, health professional in Westmoreland Health Services) in the main study were interviewed on two separate occasions approximately 7 days apart. The reliabilities were determined by the percentage of agreement. Modifications were made to the final instrument based on the recommendations, queries and issues raised by the participants in order to attain clarity and conciseness of questions.

A 26-item questionnaire was used to collect the data. The instrument was sub-divided into general demographic profile of the sample; family history; health seeking behaviour; chronic illnesses, perception on prostate examination and choice of method in prostate examination.

2.3. Measure

Regional Health Authorities: Decentralization of public health care the shifted the central government (*i.e.*, MOH) into four semi-autonomous regional bodies: South-East, North-East, Western, and Southern.

Standardized instruments were used to record participant's weight (in kilograms) and height (in squared metres). The body mass index (BMI) is the weight in kg divided by height in m^2. The classification of the World Health Organization was used in this study. The BMI was classified as normal, overweight and obese.

Normal BMI is defined as 18.5 kg/m^2 to 24.99 kg/m^2. Overweight BMI is defined as 25.00 kg/m^2 to 29.00 kg/m^2 and obese BMI is defined as $\geq$ 30.00 kg/m^2. Risky behaviour denotes unhealthy health choices such as smoking, alcohol consumption, infrequent exercise, poor dietary habit and food choices. The participants' health status was measured using BMI categorization.

Technical staffers include trained personnel such as dental nurses, health educators, nutritionists and public health inspectors, contact investigators, pharmacists, and lab technicians.

The technical support staff comprises community health-aides, psychiatric aides, ward assistants, porters, mosquito spray men and community peer educators. Administrative staffers constitute administrator, parish manager, personnel officer, and matron. The administrative support staff comprises accountants, security personnel, medical records officers, secretaries, drivers, telephone operators; cashiers and clerks. The ancillary staffers are cleaners, cooks and gardeners.

2.4. Data Analysis

The data were double entered using SPSS, verified and cleaned. Data was stored, retrieved and analyzed, using SPSS for Windows (16.0). Percentages were used to provide background information on demographic characteristics on sample, knowledge of prostate and self-reported information on prostate. Chi-square tests were utilized to examine whether statistical associations existed between non-metric dependent and independent variables. A p-value of 5% (*i.e.*, 95% confidence interval) will be used to determine statistical associations between variables.

2.5. Ethics

This study sought and was granted ethical approval by the University of the West Indies, Mona, Ethics Committee. All participants gave written consent, and they were informed of procedures and the choice of withdrawal at any time convenient to them if they so desire. The data received from the participants is reported below.

3. RESULTS

A sample of 212 respondents was interviewed for this study: females, 77.8%; blacks, 90%; single, 46.7%; tertiary level education, 39.8%; full-time employed, 86.8%; religious, 97.6%; nurses and doctors comprised of 22.3% of the sample (**Table 1**). Forty-seven percentage points of the sample were Seventh Day Adventist and Pentecostal members; 42.5% were overweight, 33.5% obese and 24.1% had a normal weight.

In **Table 2** which reports information on particular self-reported diagnosed health conditions, 16.0% of respondents had diabetes mellitus; 22.6% had hypertension; 0.5% breast cancer; 0.5% stomach cancer; 1.9% enlarged heart and 0.5% ischemic heart disease.

In **Table 3** which reports information on the lifestyle behaviour of respondents, 20.3% of respondents consumed alcohol on a regular basis; 15.6% do no regular physical exercise, 42.4% add sweetening to their hot beverages, and 4.7% were smokers.

A significant statistical relationship exists between BMI categorisation and occupation of the persons in the study ($P < 0.01$). Just over 15% of nurses and medical doctors were obese compared to 38.2% of ancillary staffers (**Table 4**). In **Table 5**, of the 178 respondents who indicated that they do some form of physical activity per week over the survey period, 52.3% spent at least one hour on the activity. Of the different typology of occupation, technical support staff had the lowest percentage points of engagement for at least one hour (20.0%); with administrative support staff recorded the greatest engagement of 1 hour or more in physical activity (63.5%).

On disaggregating the aforementioned demographic,

Table 1. Demographic characteristics of sample.

Characteristics	n	%
Gender		
Male	47	22.2
Female	165	77.8
Ethnicity		
Black	191	90.0
Burmese	1	0.5
Indian	5	2.4
Mixed	15	7.1
Marital status		
Single	99	46.7
Married	80	37.7
Common-law	14	6.6
Widowed	4	1.9
Divorced or separated	5	7.1
Education		
Primary or below	11	5.2
Secondary	116	55.0
Tertiary	84	39.8
Employment status		
Employed	184	86.8
Unemployed	25	11.8
Not stated	3	1.4
Religious		
Yes	207	97.6
No	5	2.4
BMI categorization		
Normal	71	33.5
Overweight	90	42.5
Obese	51	24.0
Occupation		
Nurses and doctors	45	22.3
Other technical staffers	28	13.9
Technical support staff	56	27.7
Administrative staffers	10	5.0
Administrative support staff	29	14.4
Ancillary	34	16.8
Age Mean (SD)	41.0 years (11.8)	

Table 2. Self-reported diagnosed chronic health conditions.

Characteristics	n	%
Diabetes mellitus		
Yes	34	16.0
No	178	84.0
Hypertension		
Yes	48	22.6
No	164	77.4
Cancer		
Breast		
Yes	1	0.5
No	211	99.5
Stomach		
Yes	1	0.5
No	211	99.5
Enlarged heart		
Yes	4	1.9
No	208	98.1
Ischemic heart disease		
Yes	1	0.5
No	211	99.5

health and lifestyle characteristic of the sample, 2.8% of those with diabetes mellitus were males compared to 19.8% females. Of the diagnosed diabetics, the majority were ancillary staffers (36.4%); 45.5% were 40 to 49 years old; and 36.4% were 31 to 45 years old (χ^2 = 10.577, P < 0.005).

Of the 22.6% of the sample who had hypertension, 25.5% were female and 12.8% were males. The highest percentage points of the sample that had hypertension were 31 to 45 years old (47.9%), 27.1% were at least 45 years old, and 6.3% were unable to recall the age when they were first diagnosed with hypertension. When occupation of respondents was disaggregated by diagnosed hypertensive cases, technical staffers recorded the high percentage points of cases (33.9%) followed by ancillary staffers (32.4%); nurses and medical doctors (22.2%); administrative staffers (20.0%) and administrative support staff (13.8%) (χ^2 = 15.375, P < 0.0001). Concurrently, a statistical relationship existed between overweight respondents and hypertensive respondents (P < 0.0001).

No significant statistical association was found between BMI categorisation and gender of respondents (χ^2 = 3.793, P = 0.150). However, a significant relationship

Table 3. Lifestyle behaviour.

Characteristics	n	%
Smoking behaviour		
Smoke	10	4.7
Do not smoke	202	95.3
Regular alcohol consumption		
Yes	43	20.3
No	169	79.7
Physical activity (*i.e.*, exercise)		
None	33	15.6
1 – 2 times a week	100	46.7
4 – 6 time a week	61	29.0
7 times a week	18	8.7
Dietary habits		
Special dieting	49	23.0
Eat anything	163	77.0
Adding sweetening to hot beverage		
Yes	90	42.4
No	122	57.6
Breast examination		
Monthly	69	42.0
Rarely	31	18.4
Never	65	39.6
Leisure time activity		
Sitting watching TV/reading	73	34.2
Cycling	30	14.2
Gardening or farming	56	26.4
Playing indoor games (chess, scrabble, domino, etc)	35	16.5
Regular physical activity (*i.e.*, exercise)	18	8.7
Rectal examination		
Yes	10	4.7
No	30	14.2
Did not answer	172	81.1
How do you prepare or eat meat		
Eat no meat	29	13.7
Fried	71	33.3
Stewed	55	26.0
Baked	12	5.9
Jerked	45	21.1

Table 4. BMI categorisation by occupation.

Occupation	BMI categorisation		
	Normal	Overweight	Obese
	%	%	%
Nurses/doctors	33.3	57.1	15.6
Other technical staff	46.4	42.9	10.7
Technical support staff	28.6	37.5	33.9
Administrative staff	50.0	40.0	10.0
Administrative support staff	41.4	44.8	13.8
Ancillary staff	20.6	41.2	38.2

$P < 0.01$

existed between BMI categorisation and self-reported diagnosed health condition ($P < 0.0001$). Disaggregated the smoker cohort revealed that 57.1% consumed between 1 to 9 cigarettes per day, and that males were more likely to be smokers (57.1%) than females (42.9%).

Significant more males regularly consume alcohol (12.6%) than females (9.1%) ($P < 0.0001$). However, more females (47.9%) than males (13.3%) indicated that they were regularly engaged in physical activities (or exercise). The age cohort that indicated the most engagement in physical activity was 60+ year olds (67.1%). Of the 60+ year olds who are engaged in regular physical activities, 13.7% indicated that they do so every day over the survey period. The percentage of other age cohorts and their engagement in physical activities were 50 to 59 years (59.1%); 30 to 39 years (56.5%) and 40 to 49 years (54.5%).

Of the 34.2% who indicated sitting watching television or reading as their leisure activities, 65% did this on a daily basis and 18% between 4 to 6 times per week. Furthermore, 43% of overweight respondents were engaged in sitting and watching television or reading as their leisure activities compared to 32% of those who were obese. Concurrently, 17% of females were engaged in sitting and watching television or reading as their leisure activities compared to 8.3% of males. Forty-three percent of overweight respondents were 40- 49 years old and 24.1% of the obese were in this age cohort.

Of the respondents who indicated being on a special dietary programme (23%), 35.4% were on low salt; 25.2% vegetarians; 16.8% weight loss; 12.4% low fat; 4.0% weight gain and 6.2% were on low cholesterol programme. Health care workers in Hanover prefer to consume fried meats, and this was mostly higher among those younger than 20 years (50.0%) followed by those 50-59 years (49.0%) and those 40-49 years (31.3%) as

Table 5. Physical activity (in duration of time per day) by occupation.

Characteristic	Physical activity (in duration of time per day)					Total
	< 15 minutes	15 – 29 minutes	30 – 44 minutes	45– 59 minutes	> 1hr	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Occupation						
Nurses/doctors	6 (16.2)	7 (18.9)	3 (8.1)	1 (2.7)	20 (54.1)	37 (20.8)
Other technical staff	3 (13.0)	6 (26.1)	3 (13.0)	1 (4.3)	6 (43.5)	19 (10.7)
Technical support staff	4 (40.0)	3 (30.0)	1 (10.0)	0 (0.0)	2 (20.0)	10 (5.6)
Administrative staff	5 (18.5)	6 (22.2)	2 (7.4)	0 (0.0)	14 (51.9)	27 (15.2)
Administrative support staff	5 (9.6)	7 (13.5)	2 (3.8)	5 (9.6)	33 (63.5)	52 (29.2)
Ancillary staff	2 (6.1)	2 (6.1)	9 (27.3)	2 (6.1)	18 (54.5)	33 (18.5)
Total, n	25	31	20	9	93	178

well as the those 20-29 years (31.3%). Ancillary workers were most likely to consume fried meats (68%) compared to any other occupational group. Twenty five percent of the same had fruit juice (17.5% had it 2-3 times daily; 11.3% had it occasionally), and 49.5% had soda (57.1% had it occasionally; 14.3% daily and 1.9% 6 days per week). Twenty nine percent had vegetables daily, 23% 2-3 times per week and 0.5% never had vegetables.

On general health care-seeking behaviour, 28% of female respondents indicated having visited a health care provider in the last 6 months for breast examination. There was no significant statistical association between breast examination and occupation ($P > 0.05$); BMI ($P > 0.05$) and health conditions ($P > 0.05$). The majority of the females had done a pap smear (75%). Of those who indicated that they had not done a pap smear, the highest were among administrative staff (42.9%) followed by other technical staff (35.7%); administrative support staff (31.2%) and the least by technical support staff (12.2%). Forty percent of female have not done a breast examination compared to 62.9% of males who had never had a rectal examination. A significant relationship existed between rectal examination and occupational type ($P < 0.0001$). The percentage points of males who had never done a rectal examination by occupational type can be disaggregated as technical support staff, 87.5%; administrative support staff, 60.1%; other technical staff, 55.6%. Furthermore, the highest number of males who had not done a rectal examination was among those 50 to 59 years old (69.2%).

4. DISCUSSIONS

This study examined the lifestyle, health and the use of health care services of some health care workers in the parish of Hanover, Jamaica. Generally, the health status of people who are employed to health institutions in Hanover is good, but when this was disaggregated into occupational types more information was revealed that indicated worrying signs for health care in the future. Using BMI categorisation to measure health status, the findings revealed that 34% of employees were classified as having normal weight, 43% overweight and 24% obese. Apart from the afore-mentioned findings, 16.0% had diabetes mellitus, 22.6% hypertension, 0.5% breast cancer, 0.5% stomach cancer, 1.9% enlarged heart and 0.5% ischemic heart disease. Concurrently, 22% of men had done a rectal examination for prostate cancer, 60% of women had done a breast examination, 77% indicated that they eat every and/or anything, 42% added sweetening to their hot beverage, 5% were smokers and 16% do no physical activities and 34% indicated that their leisure time was spent sitting watching television and/or reading. The disaggregation of BMI by occupation revealed that most doctors and nurses were at least overweight (73%); other technical staff (54%); technical support staff (71%) and those in the ancillary categorization were most likely to be in the overweight category (79%).

In 2007, statistics from the PIOJ and STATIN [7] revealed that 12% of Jamaicans had diabetes mellitus and 22% had hypertension. On disaggregating the figures, 8% of males had diabetes mellitus compared to 14% of females, and 16% of males had hypertension compared to 27% of females. Although 1.3 times more people in the current study had diabetes mellitus compared to the

population, disaggregating the figures by sexes revealed a remarkable difference. In the current study diabetes mellitus disparity between the sexes was 7.1 times (males, 2.8%; females, 19.8%) compared to 1.8 times in the national survey. With respect to hypertension, there was no difference between the percentage of those with diabetes in the country and health workers in Hanover. There are no available statistics on diabetes mellitus and hypertension by occupational type in the literature, and therefore the findings from this study provides this valuable information. The findings showed that hypertension was not greater among females than males; but it was also highest among those 31-45 years and among technical support as well as ancillary staffers. Both technical support and ancillary staffers are among the poor, which concurs with the literature that poverty is associated with more illness as concur by the findings of this study [2,15-17].

This study highlighted that there was no statistical association between gender and BMI categorization, however one existed for BMI and self-reported diagnosed health condition. Low socioeconomic status is empirically established as having more people with illness, but the current study further shows that they were more likely to be obese than those who are more likely to be in the middle-to-upper class. The study also revealed that 34% of those in the technical support staff and 38% of those in ancillary staff category were obese and these persons are in the low socioeconomic status compared to 16% of medical doctors and nurse who are middle-to-upper class individuals. Despite this finding, it can be inferred that the schedules of medical doctors and nurses in addition to their and lifestyle may account for a significant percent of them being at least overweight. This has implications for the future of the health sectors as overweight and obesity are associated with increased risk of morbidities and mortalities.

The sedentary lifestyle of health care professionals in Hanover may lead to a public health problem which may become worse in the future if not addressed. The health behaviour of the persons in the study is also a cause for concern and although their lifestyle is a sedentary one, they make more unhealthy lifestyle choices than healthy ones. It is clear from the findings that education, knowledge of health and health care are not influencing the decision of health care providers in Hanover. The findings concur with a study which showed that non-communicable diseases are largely apart of the lifestyle of Jamaicans, and that 50% of deaths were owing to non-communicable diseases such as heart, stroke, diabetes mellitus, cancers and obesity [18]. A study in by O'Connell and Gray [19] found that four-fifths of those with stroke had high blood pressure when they were taken to hospital for treatment post-stroke, and that two-thirds of them had a history of hypertension. A later study by Woo *et al*. [8] found that untreated hypertension was a significant risk of hemorrhagic stroke (*i.e.*, OR = 3.5, 95% CI = 2.3, 5.2; P < 0.0001), and that treated hypertension was significantly lower in causing hemorrhagic (OR = 1.4, 95% CI = 1.0 to 1.9; *P* = 0.03). The WHO revealed that obesity was associated with health problems such as respiratory difficulties, chronic musculoskeletal problems, skin problems and infertility [20], indicating the pending public health challenge in the health sectors in Hanover.

In 2000, the Jamaica Lifestyle Survey revealed that 8% of Jamaican had diabetes mellitus (96.1% of males and 9.1% of females) and that the most cases were among the elderly (*i.e.*, 60 + years) [21], which reiterate the health problem challenge that Hanover faces and speaks to the role of culture and low socioeconomic status influencing the healthy lifestyle choices of residents in Hanover. Morrison [22] in an article entitled 'Diabetes and hypertension: Twin Trouble' showed that diabetes mellitus and hypertension have now become the two major chronic non-communicable diseases thus health problems for Jamaicans and in the wider Caribbean. This finding was also corroborated by Callender [23] who found that there is a positive association between diabetic and hypertensive patients (*i.e.*, 50% of individuals with diabetes had a history of hypertension), which emphasizes the public health problem of unhealthy health workers in Hanover.

When the sedentary lifestyle, unhealthy lifestyle choices, and low socioeconomic status are coupled with the fact that the sample is relatively middle-aged to old (*i.e.*, mean age was 41.0 years), it was observed that with the increased risk of morbidities and disabilities associated with ageing, the health of individuals in this study becomes exacerbated by the unhealthy diet, alcohol consumption, lack of exercise and sedentary lifestyle. While the prevalence of smokers among residents and health workers in Hanover (4.7%) are lower than the national figures (17.7%), the percentage points of male smokers in this was 2.3 times more than the prevalence in the national (24.8%) and this was 5.9 times more females in this sample compared to the national figures (7.1%).

The fact that the majority in each category of health care workers are obese is a worrying finding. Instructing patients to take care of their health in an environment where healthcare workers are overweight including nurses and medical doctors may cause patients to ignore the information they receive from health care staff. There is an area for future research. Another area for future research should be an examination of the reasons

why some health care workers particularly the professionals who have years of education, knowledge, experience and training become engaged in unhealthy lifestyle practices. Once the reasons for the poor lifestyle choices of health care workers that affect their health are understood, further research is necessary on the content and procedures that are required for a strategic and effective national health literacy communication programme. This programme should be cognizant of the fact that education and knowledge about health does not automatically influence the educated knowledge holders' behavior in a positive way. There is also need for research on how training and wellness programme for health care workers on the job including medical doctors and nurses would influence the choices they make about their health.

5. CONCLUSIONS

The current study has revealed pertinent information on the perception of health care workers about healthy lifestyle, health choices and general perception of residents in Hanover on their health. Smoking, obesity, overweight, high cholesterol, sedentary lifestyle, unhealthy lifestyle practices and low socioeconomic status increased the risk of cardiovascular diseases in health workers in Hanover and this is further complicated by hypertension, diabetes mellitus and unhealthy choices. The level of health education is greater among health workers than non-health care workers which indicate that the pending health problems in Hanover would have been understated by the current study. There is a need for public health practitioners to formulate a health intervention programme that will target people in Hanover including specific groups such as medical doctors, nurses, administrative, ancillary staffers and technical staffers in health care institution in the parish of Hanover. Clearly education and knowledge of health do not lead to better and healthier choices by health care workers in Hanover, and this could be a general social dilemma as the general populace may be left to use home remedy if premature mortality were to befall those high risk health workers in Hanover. Then, there is reality of an increase burden of health care workers in Hanover on the health care services in the future, which would increase health care expenditure for the country.

REFERENCES

[1] Sen, A. (1979) Poverty: An ordinal approach to measurement. *Econometricia*, **44(2)**, 219-231.

[2] World Health Organization (2005) Preventing chronic diseases a vital investment. Geneva.

[3] Emerson, E. (2009) Relative child poverty, income inequality, wealth, and health. *Journal of the American Medical Association*, **301(4)**, 425-426.

[4] Mitra, A. and Tsujita, Y. (2009) Health-Poverty nexus: A study of low income households in India. *International Journal of Child and Adolescent Health*, **2(1)**, 99-107.

[5] Bourne, P.A. (2009) Impact of poverty, not seeking medical care, unemployment, inflation, self-reported illness, health insurance on mortality in Jamaica. *North American Journal of Medical Sciences*, **1(3)**, 99-109.

[6] Levy, H. (1996) They cry 'Respect': Urban violence and poverty in Jamaica. The Centre for Population, Community and Social Change, University of the West Indies, Kingston.

[7] Planning Institute of Jamaica and Statistical Institute of Jamaica (2007) Jamaica survey of living conditions. Kingston.

[8] Woo, D., Haverbusch, M., Sekar, P., Kissela, B., Khoury, J., Schneider, A., Kleindorfer, D., Szaflarski, J., Pancioli, A., Jauch, E., Moomaw, C., Sauerbeck, L., Gebel, J. and Broderick J. (2004) Effect of untreated hypertension on hemorrhagic stroke. *Stroke*, **35(7)**, 1703-1708.

[9] Statistical Institute of Jamaica (2008) Demographic statistics, 2001, 2007. Kingston.

[10] Ministry of Health (2005-2008) Annual report, 2004-2007. Policy, Planning and Development Division, Kingston.

[11] Ward, E. and Grant, A. (2005) Epidemiological prolife of selected health conditions and services in Jamaica, 1990-2002. Health Promotion and Protection Division, Ministry of Health, Kingston.

[12] Pan American Health Organization (2007) Health in the Americas. *Countries*, **2**, Washington DC.

[13] Ataman, S.L., Cooper, R., Rotimi, C., McGee, D., Osotimehin, B., Kadiri, S., Kingue, S., Muna, W., Fraser, H., Forrester, T. and Wilks, R. (1996) Standardization of blood pressure measurement in a international comparative study. *Journal of Clinical Epidemiology*, **49(8)**, 869-877.

[14] Wilks, R., Younger, N., Mullings, J., Zohoori, N., Figueroa, P., Tulloch-Reid, M., Ferguson, T., Walters, C., Bennett, F., Forrester, T., Ward, E. and Ashley, D. (2007) Factors affecting study efficiency and item non-response in health surveys in developing countries: The Jamaica national healthy lifestyle survey. *BMC Med Research Methodology*, **7(13)**, 1-14.

[15] List, J. (2006) Illness, poverty and the invisible patient. *American Medical Association Journal of Ethics, Virtual Mentor*, **8(11)**, 705-708.

[16] Link, B.G. and Phelan, J.C. (1995) Social conditions as fundamental cause of disease. *Journal of Health and Social Behavior*, **36(Special Issue)**, 80-94.

[17] Foster, A. (1994) Poverty and illness in low-income rural areas. *American Economic Review*, **84(2)**, 216-220.

[18] Ministry of Health (2004) National policy for the promotion of healthy lifestyle in Jamaica. Health Promotion and Protection Division, Kingston.

[19] O'Connell, J.E. and Gray, C.S. (1994) Treating hypertension after stroke. *British Medical Journal*, **308(6943)**, 1523-1524.

[20] World Health Organization (2003) Obesity and overweight. Geneva. http://www.who.int/dietphysicalactivity/publications/facts/obesity/en/.
[21] Ministry of Health (2004) National policy for the promotion of healthy lifestyle in Jamaica. Health Promotion and Protection Division, Kingston.
[22] Morrison, E. (2000) Diabetes and hypertension: Twin trouble. *Cajanus*, **33(2)**, 61-63.
[23] Callender, J. (2000) Lifestyle management in the hypertensive diabetic. *Cajanus*, **33(2)**, 67-70.

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Birth outcomes and pregnancy complications of women with uterine leiomyoma—a population-based case-control study

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ABSTRACT

Objective Uterine leiomyoma is not a rare pathological condition in pregnant women; thus the aim of the study was to evaluate the recent progress in the treatment of these pregnant women on the basis of the association of leiomyoma in pregnancy (LP) with pregnancy complications and birth outcomes including structural birth defects, *i.e.* congenital abnormalities (CA) in the offspring. Design Cases with CA and matched controls without CA in the population-based Hungarian Case-Control Surveillance System of Congenital Abnormalities (HCCSCA) were evaluated. Only women with prospectively and medically recorded LP in prenatal maternity logbook and medically recorded birth outcomes (gestational age, birth weight, CA) were included to the study. Setting the HCCSCA, 1980-1996 contained 22,843 cases with CA and 38,151 matched controls without CA. Population Hungarian pregnant women and their informative offspring: live births, stillbirths and prenatally diagnosed malformed fetuses. Methods Comparison of birth outcomes of cases with matched controls and pregnancy complications of pregnant women with or without LP. Main outcome measures Pregnancy complications, mean gestational age at delivery and birth weight, rate of preterm birth, low birthweight, CA. Results A total of 34 (0.15%) cases had mothers with LP compared to 71 (0.19%) controls. There was a higher incidence of threatened abortion, placental disorders, mainly abruption placentae and anaemia in mothers with LP. There was no significantly higher rate of preterm birth in the newborns of women with LP but their mean birth weight was higher and it associated with a higher rate of large birthweight newborns. A higher risk of total CA was not found in cases born to mothers with LP (adjusted OR with 95% CI = 0.7, 0.5-1.1), the specified groups of CAs were also assessed versus controls, but a higher occurrence of women with LP was not revealed in any CA group. Conclusions Women with LP have a higher risk of threatened abortion, placental disorders and anaemia, but a higher rate of adverse birth outcomes including CAs was not found in their offspring.

Keywords: Uterine Leiomyoma in Pregnant Women; Pregnancy Complications; Preterm Birth; Large Birth Weight; Congenital Abnormalities; Population-Based Case-Control Study

1. INTRODUCTION

Uterine leiomyoma (fibroid) is benign, smooth muscle tumour and most common non cancerous neoplasm in women of child-bearing age. Though the onset of uterine leiomyoma is increasing with advanced maternal age, this pathological condition occurs in pregnant women as well and because leiomyoma tends to grow under the influence of estrogens, 15-30% of leiomyoma may enlarge during the first trimester of pregnancy [1]. Compressive effect of leiomyoma may distort the intrauterine cavity and alter the endometrium thus after conception may interfere implantation, placental development and the growth of the conceptus mechanically [2]. In addition there is an increased uterine irritability and contractility secondary to rapid fibroid growth. Thus the direct mechanical effect and indirect alteration in oxytocinase activity may disrupt the normal progression of uterus and development of the fetus, therefore uterine leio-

myoma is a cause of pregnancy loss, fetal malpresentation, intrauterine growth retardation and premature labour [3].

However, most studies of pregnancy complications and birth outcomes in uterine leiomyoma patients were composed of participants from only one hospital or clinic [4-7] and the results of population-based studies have been published only recently [8-9]. The objective of our study was the evaluation the possible association between maternal uterine *leiomyoma in pregnancy* (LP) and pregnancy complications, in addition adverse birth outcomes, particularly structural birth defects, *i.e.* congenital abnormalities (CAs) in the population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA) [10].

2. MATERIALS

The protocol of the HCCSCA included five steps. The first step was the selection of *cases* from the data set of the Hungarian Congenital Abnormality Registry (HCAR), 1980-1996 [11] for the HCCSCA. Notification of CAs is compulsory for physicians from the birth until the end of first postnatal year to the HCAR. Most cases with CA are reported by obstetricians and paediatricians. In Hungary practically all deliveries take place in inpatient obstetric clinics and the birth attendants are obstetricians. Paediatricians are working in the neonatal units of inpatient obstetric clinics, or in various inpatient and outpatient paediatric clinics. Autopsy was mandatory for all infant deaths and common in stillborn fetuses during the study period. Pathologists sent a copy of the autopsy report to the HCAR if defects were identified in stillbirths and infant deaths. Since 1984 fetal defects diagnosed in prenatal diagnostic centres with or without termination of pregnancy have also been included into the HCAR. Isolated minor anomalies (e.g., umbilical hernia, small haemangioma, hydrocele) were recorded but not evaluated in the HCAR. The total (birth + fetal) prevalence of cases with CA diagnosed from the second trimester of pregnancy through the age of one year was 35 per 1000 *informative offspring* (liveborn infants, stillborn fetuses and electively terminated malformed fetuses) in the HCAR, 1980-1996, and about 90% of major CAs were recorded in the HCAR during the 17 years of the study period [12].

There were three exclusion criteria at the selection of cases with CAs from the HCAR for the data set of the HCCSCA. 1) Cases reported after three months of birth or pregnancy termination were excluded. The longer time between birth or pregnancy termination and data collection decreases the accuracy of information about pregnancy history. This group of excluding cases involved 33% of cases and most had mild CAs. 2) Three mild CAs (such as congenital dislocation of hip based on Ortolani click, congenital inguinal hernia, and large haemangioma), and 3) CA-syndromes caused by major mutant genes or chromosomal aberrations with preconceptional (*i.e.* non teratogenic) origin were also excluded.

The second step was to ascertain appropriate *controls* from the National Birth Registry of the Central Statistical Office for the HCCSCA. Controls were defined as newborn infants without CA. In most years two controls were matched to every case according to sex, birth week, and district of parents' residence.

3. DATA COLLECTION AND EVALUATION

The third step was to obtain the necessary *maternal*, particularly *exposure* data from three sources:

3.1. Prospective Medically Recorded Data

Mothers were asked in an explanatory letter to send us the *prenatal maternity logbook* and other *medical records* particularly discharge summaries concerning their diseases during the study pregnancy and their child's CA. Prenatal care was mandatory for pregnant women in Hungary (if somebody did not visit prenatal care clinic, she did not receive a maternity grant and leave), thus nearly 100% of pregnant women visited prenatal care clinics, on average 7 times in their pregnancies. The first visit was between the 6th and 12th gestational week. The task of obstetricians was to record all pregnancy complications, maternal diseases and related drug prescriptions in the prenatal maternity logbook.

3.2. Retrospective Self-Reported Maternal Information

A structured *questionnaire* along with a list of medicinal products (drugs and pregnancy supplements) and diseases, plus a printed informed consent form were also mailed to the mothers immediately after the selection of cases and controls. The questionnaire requested information on pregnancy complications and maternal diseases, on medicinal products taken during pregnancy according to gestational months, and on family history of CAs. To standardize the answers, mothers were asked to read the enclosed lists of medicinal products and diseases as a memory aid before they filled in the questionnaire. We also asked mothers to give a signature for informed consent form which permitted us to record the name and address of cases both in the HCCSCA and in the HCAR.

The mean ± S.D. time elapsed between the birth or pregnancy termination and the return of the "information

package" (questionnaire, logbook, discharge summary, and informed consent form) in our prepaid envelope was 3.5 ± 1.2 and 5.2 ± 2.9 months in the case and control groups, respectively

3.3. Supplementary Data Collection

Regional nurses were asked to visit all non-respondent case mothers at home and to help mothers to fill-in the same questionnaire used in the HCCSCA, to evaluate the available medical records, to obtain data regarding lifestyle (smoking, drinking, illicit drug use) through personal interview of mothers and her close relatives living together and to ask mothers to sign informed consent. Regional nurses visited only 200 non-respondent control and 600 other control mothers as part of two validation studies [13,14] using the same methods as in non-respondent case mothers because the committee on ethics considered this follow-up to be disturbing to the parents of all healthy children.

Overall, the necessary information was available on 96.3% of cases (84.4% from reply to the mailing, 11.9% from the nurse visit) and 83.0% of the controls (81.3% from reply, 1.7% from visit). Informed consent form was signed by 98% of mothers, names and addresses were deleted in the rest 2%.

The procedure of data collection in the HCCSCA was changed in 1997 such that regional nurses visited and questioned all cases and controls, however, these data have not been validated until now, thus only the data set of 17 years between 1980 and 1996 is evaluated here.

The fourth step at the evaluation of cases and controls in the HCCSCA is the definition of exposure and to determine its diagnostic criteria. The diagnosis of LP was based on the personal manual and ultrasound examination of pregnant women by obstetrician. In general the size of uterine leiomyoma and their types (intramural, subserosal, submucosal, pedunculated, etc) was given in the prenatal maternity logbook but unfortunately these data were not copied out. Pregnant women with dysfunctional uterine bleeding, endometrial polyp, endometriosis, etc were excluded from the study.

Gestational time was calculated from the first day of the last menstrual period. Beyond birth weight (g) and gestational age at delivery (wk), the rate of low birthweight (< 2500 g) and large birth weight (4000 or more g) newborns, in addition the rate of preterm births (< 37 weeks) and postterm birth (42 or more weeks) were analyzed on the basis of discharge summaries of inpatient obstetric clinics. The critical period of most major CAs is in the second and/or third gestational month.

Drug treatments and folic acid/multivitamin supplements were also evaluated. The latter may indicate the level of pregnancy care, and indirectly may show the socio-economic status and the motivation of mothers to prepare and/or to achieve a healthy baby. In addition it is necessary to consider folic acid and folic acid-containing multivitamins in the evaluation of preventable CAs [15-18]. Other potential confounding factors included maternal age, birth order, marital and employment status which had a good correlation with the level of education and income, thus was regarded at the indicator of socioeconomic status [19], and high fever related diseases such as influenza.

We used SAS version 8.02 (SAS Institute Ins., Cary, North Carolina, USA) for statistical analyses as the fifth step of the HCCSCA. The occurrence of LP was compared in the two study groups and the crude odds ratios (OR) with 95% confidence intervals (CI) were calculated. Contingency tables were prepared for the main study variables. The prevalence of other maternal diseases, drug intakes and pregnancy supplements used during pregnancy were compared between the group of case and control mothers with LP. We compared the prevalence of LP during the study pregnancy in specific CA groups including at least 2 cases with the frequency of LP in their all matched control pairs. Crude and adjusted OR with 95% CI were evaluated in conditional logistic regression models. We examined confounding variables by comparing the OR for LP in the models with and without inclusion of the potential confounding variables. Finally, maternal age (< 20 yr, 20-29 yr, and 30 yr or more), birth order (first delivery or one or more previous deliveries), employment status, influenza-common cold (yes/no), and use of folic acid supplement (yes/no) were included in the models as potential confounders.

4. RESULTS

As it appeared at the preliminary evaluation of LP, two groups could be differentiated: 1) prospectively and medically recorded LP in the prenatal maternity logbook, and 2) LP based on retrospective maternal information in the questionnaire. However, the diagnosis of leiomyoma can be frequently questioned without medical record in the latter group and in general it was not possible to differentiate the leiomyoma with myomectomy before the study pregnancy. Thus, only the first group, *i.e.* medically recorded LP was evaluated.

The case group consisted of 22,843 malformed newborns or fetuses ("informative offspring"), of whom 39 (0.15%) had mothers with medically recorded leiomyoma during the study pregnancy. However, of these 39 pregnant women, 5 (12.8%) had previous myomectomy due to leiomyoma. The total number of births in Hungary was 2,146,574 during the study period between

1980 and 1996. Thus the 38,151 controls represented 1.8% of all Hungarian births, and among those controls, 82 were born to mothers with medically recorded leiomyoma. Of these 82 control mothers, 11 (13.4%) had previous myomectomy. Our objective was to evaluate the possible association of leiomyoma during the study pregnancy with pregnancy complications and adverse birth outcomes, therefore 34 case mothers (0.15%) and 71 control mothers (0.19%) with leiomyoma, *i.e.* LP were evaluated. Surgical intervention due to LP during the study pregnancy was not recorded in the prenatal maternity logbook and in the discharge summaries of these 105 pregnant women with LP. The number of pregnant women with previous myomectomy of leiomyoma was too small, thus these pregnant women were excluded from this analysis.

Of 34 case mothers, 30 (88.2%), while of 71 control mothers, 60 (84.5%) had diagnosed LP in the first visit of prenatal maternity clinic, thus the onset of this pathological condition was before conception. The so-called new-onset LP occurred in 4 case mothers and 11 control mothers diagnosed after the fourth gestational month.

Table 1 summarizes the characteristics of mothers with and without LP as reference. This comparison indicates a much higher mean maternal age (due to the larger proportion of women with the age group of 30 and more years) in women with LP. However, the mean birth order was only higher in control mothers with LP, but somewhat lower in case mothers with LP than case mothers without LP. Mean pregnancy order (previous birth + recorded miscarriages) was also evaluated, and the difference between birth and pregnancy order was somewhat higher in pregnant women with LP in both case mothers and control mothers and these data may indicate a higher rate of miscarriages in previous pregnancies. The proportion of unmarried pregnant women was larger in the groups of LP, while LP was more frequently recorded in the prenatal maternity logbooks of professional pregnant women. In the group of case mothers, the proportion of managerial women was also larger.

Among pregnancy supplements (**Table 1**), the use of folic acid and iron was similar between mothers with or without LP, but these supplements were used more frequently by control mothers. However, medicinal products containing calcium were used more frequently by mothers with LP, and a much higher rate of case mothers were treated with vitamin E.

Of 2,640 case mothers visited at home, only 4 had LP and one was smoker during the study pregnancy. Of 2,636 mothers without LP, 576 (21.9%) smoked. Of 800 control mothers visited at home, 152 (19.0%) smoked during the study pregnancy.

Acute maternal diseases (e.g. influenza) did not occur more frequently in mothers with LP. Among chronic diseases, the prevalence of diabetes mellitus and epilepsy was similar in the study groups, but essential hypertension (19.0% vs. 7.0%, OR with 95% CI: 3.1, 1.9-5.1), haemorrhoids (18.1% vs. 3.9%, OR with 95% CI: 5.4, 3.3-8.8) and constipation (7.6% vs. 2.1%, OR with 95% CI: 3.9, 1.9-8.1) were more frequent in 105 women with LP than in 60,889 mothers without LP.

The incidences of pregnancy complications are shown in **Table 2**, because they were different in case and control mothers with LP. Threatened abortion, placental disorders (mainly abruption placentae) and anaemia occurred more frequently in case mothers with LP than in case mothers without LP. However, LP did not associate with a higher rate of threatened abortion, placental disorders and anaemia in control mothers. Thus LP and fetal defects may have some causal association with the higher risk of certain pregnancy complications, such as placental disorders. Unexpectedly the incidence of threatened preterm delivery was not significantly higher in case and control mothers with LP.

There was some difference in the distribution and frequency of drugs used by mothers with LP explained by the higher use of antihypertensive (methyldopa, metoprolol, nifedipine) drugs (12.4% vs. 2.6%) and the usual treatment of threatened abortion with allylestrenol (21.0% vs. 14.5%) and diazepam (25.7% vs. 11.3%) in Hungary. In addition the use of hydroxyprogesterone (5.7% vs. 1.2%) and human chorionic gonadotropin (2.9% vs. 0.3%) was more frequent in 105 pregnant women with LP than in 60,889 pregnant women without LP.

Birth outcomes are shown in case and control newborns (**Table 3**) but statistical testing was used only in controls because CAs may have a more drastic effect for these variables in cases than LP itself. (There was no difference in the sex ratio of the study groups, and twin did not occur among newborns of mothers with LP.) The mean gestational age at delivery was somewhat (0.1 wk in cases and 0.2 wk in controls) longer, the rate of preterm birth was higher in controls but lower in cases. There was no difference in the rate of postterm births among study groups. However, these differences were not significant. The mean birth weight was 159 and 95 g larger in cases and controls of mothers with LP and these differences were significant. However, these differences were not reflected in the rate of low birthweight newborns because there was no difference in their rates between cases and controls born to mother with or without LP. There was a higher proportion of large birthweight newborns of both cases and controls but this difference was significant only in cases (OR with 95% CI: 3.0, 1.9-7.2).

Table 1. Maternal characteristics of women with or without leiomyoma in pregnancy (LP).

Variables	Case mothers				Control mothers			
	without		with		without		with	
Quantitative	LP				LP			
	(N = 22,809)		(N = 34)		(N = 38,080)		(N = 71)	
Maternal age, yr.	No.	%	No.	%	No.	%	No.	%
– 19	2,506	11.0	0	0.0	3,277	8.6	0	0.0
20 – 29	15,580	68.3	13	38.2	25,777	72.4	25	35.2
30 –	4,723	20.7	21	61.8	7,226	19.0	46	64.8
Mean, S.D.	25.5 ± 5.3		32.1 ± 6.0		25.4 ± 4.9		31.9 ± 5.7	
Birth order (parity)								
1	10,691	46.9	17	50.0	18,175	47.7	34	47.9
2 or more	12,118	53.1	17	50.0	19,905	52.3	37	52.1
Mean, S.D.	1.9 ± 1.1		1.8 ± 1.0		1.7 ± 0.9		1.9 ± 1.1	
Pregnancy order								
1	9,493	41.6	14	41.2	16,296	42.8	24	33.8
2 or more	13,316	58.4	50	58.8	21,784	57.2	47	66.2
Mean, S.D.	2.1 ± 1.4		2.1 ± 1.2		1.9 ± 1.2		2.2 ± 1.2	
Categorical	No.	%	No.	%	No.	%	No.	%
Unmarried	1,265	5.5	4	11.8	1,467	3.9	5	7.0
Employment status								
Professional	1,969	8.6	8	23.5	4,399	11.6	24	33.8
Managerial	5,083	22.3	14	41.2	10,249	26.9	16	22.5
Skilled worker	6,493	28.5	8	23.5	11,886	31.2	22	31.0
Semiskilled worker	4,196	18.4	1	2.9	6,159	16.2	2	2.8
Unskilled worker	1,775	7.8	1	2.9	2,187	5.7	0	0.0
Housewife	2,404	10.5	2	5.9	2,351	6.2	3	4.2
Others	889	3.9	0	0.0	849	2.2	4	5.6
Pregnancy supplements								
Iron	14,721	64.5	21	61.8	26,722	70.2	49	69.0
Calcium	1,798	7.9	5	14.7	3,570	9.4	13	18.3
Folic acid	11,263	49.4	16	47.1	20,736	54.5	39	54.9
Vitamin B6	2,010	8.8	3	8.8	4,080	10.7	6	8.5
Vitamin D	6,093	26.7	8	23.5	10,131	26.6	19	26.8
Vitamin C	908	4.0	4	11.8	1,681	4.4	4	5.6
Vitamin E	1,410	6.2	8	23.5	2,281	6.0	6	8.5
Multivitamin	1,328	5.8	2	5.9	2,501	6.6	8	11.3

Table 2. Incidence of pregnancy complications in women with or without leiomyoma in pregnancy (LP).

Pregnancy complications	Case mothers				Comparison of cases with or without LP OR with 95% CI	Control mothers				Comparison of controls with or without LP OR with 95% CI	Comparison between case and control mothers with LP
	without LP		with LP			without LP		with LP			
	(N = 22,809)		(N = 34)			(N = 38,080)		(N = 71)			
	No.	%	No.	%		No.	%	No.	%		
Threatened abortion	3,483	15.3	14	41.2	**3.9 (2.0 – 7.7)**	6,494	17.1	16	22.5	1.4 (0.8 – 2.5)	2.4 (0.9 – 5.8)
Nausea-vomiting, severe	1,739	7.6	3	8.8	1.2 (0.4 – 3.8)	3,849	10.1	6	8.5	0.8 (0.4 – 1.9)	1.0 (0.2 – 4.5)
Preeclampsia–eclampsia	667	2.9	3	8.8	3.2 (0.9 – 10.5)	1,156	3.0	2	2.8	0.9 (0.2 – 3.8)	3.3 (0.5 – 21.0)
Pregnancy related renal diseases	337	1.5	1	2.9	2.0 (0.3 – 14.8)	490	1.3	2	2.8	2.2 (0.5 – 9.1)	1.0 (0.1 – 11.9)
Placental disorders*	294	1.3	2	5.9	**4.8 (1.1 – 20.1)**	592	1.6	1	1.4	0.9 (0.1 – 6.5)	4.4 (0.4 – 50.0)
Polyhydramnios	211	0.9	0	0.0	0.0	190	0.5	1	1.4	2.8 (0.4 – 20.6)	–
Threatened preterm delivery**	2,601	11.4	5	14.7	1.3 (0.5 – 3.5)	5,437	14.3	10	14.1	1.0 (0.5 – 1.9)	1.1 (0.3 – 3.3)
Anemia	3,233	14.2	9	26.5	**2.2 (1.0 – 4.7)**	6,345	16.7	13	18.3	1.1 (0.6 – 2.0)	1.6 (0.6 – 4.2)
Others***	288	1.3	1	2.9	2.4 (0.3 – 17.4)	674	1.8	1	1.4	0.8 (0.1 – 5.7)	2.1 (0.1 – 35.0)

*incl. placenta praevia, premature separation of

**incl. cervical incompetence as well

***e.g. trauma, poisoning, blood isoimmunisation

Bold numbers show significant associations

Table 3. Birth outcomes of cases and controls born to mothers with or without leiomyoma in pregnancy (LP).

Variables	Case mothers				Control mothers				Comparison	
	without LP		with LP		without LP		with LP			
	(N = 22,809)		(N = 71)		(N = 38,080)		(N = 71)			
Quantitative	Mean	S.D.	Mean	S.D	Mean	S.D.	Mean	S.D.	t =	p =
Gestational age, wk**	38.6	3.2	38.7	2.4	39.4	2.0	39.6	2.2	0.9	0.37
Birth weight, g*	2,977	705	3,136	752	3,275	511	3,370	575	**2.1**	**0.03**
Categorical	No.	%	No.	%	No.	%.	No.	%	OR	with 95% CI
Preterm birth*	3,760	16.5	5	14.7	3,487	9.2	9	12.7	1.6	0.8 – 3.2
Postterm birth*	573	2.5	1	2.9	3,854	10.1	8	11.3	1.1	0.6 – 2.4
Low birthweight**	4,622	20.3	7	20.6	2,163	5.7	4	5.6	0.8	0.2 – 2.5
Large birthweight**	1,169	5.1	5	14.7	2,865	7.5	7	9.9	1.4	0.7 – 3.0

*adjusted for maternal age, birth order and maternal socio-economic status

**adjusted for maternal age, birth order, maternal socio-economic status and gestational age

Bold numbers show significant association

At the estimation of possible higher risk for CAs, the occurrence of LP during the entire pregnancy of mothers who had cases with different CAs was compared with the occurrence of LP in the mothers of *all matched controls* (**Table 4**). (We supposed that 4 case and 11 control mothers with the diagnosis of LP after the fourth gestational month might have some effect for the uterus during the critical period of most major CAs, *i.e.* during the second and third gestational month.) There was no higher risk for total CA and any CA group, including

Table 4. Estimate the association between women with leimyoma in pregnancy (LP) and different CAs in their offspring using all matched controls as reference.

Study groups	Grand total No.	Entire pregnancy		Crude		Adjusted	
		No.	%	OR	95% CI	OR	95% CI
Controls	38,151	71	0.2	reference		reference	
Isolated CAs							
Neural-tube defects	1,203	2	0.2	0.9	0.2 – 3.6	0.6	0.1 – 3.3
Cleft lip ± palate	1,374	3	0.2	1.2	0.4 – 3.7	0.8	0.2 – 3.4
Cleft palate only	601	2	0.3	1.8	0.4 – 7.3	3.5	0.3 – 39.1
Hypospadias	3,038	9	0.3	1.6	0.8 – 3.2	1.3	0.5 – 3.3
Undescended testis	2,051	3	0.1	0.8	0.2 – 2.5	1.1	0.3 – 4.8
Cardiovascular CAs	4,479	6	0.1	0.7	0.3 – 1.7	0.9	0.3 – 2.4
Clubfoot	2,424	4	0.2	0.9	0.3 – 2.4	0.6	0.2 – 2.1
Limb deficiencies	548	3	0.5	3.0	0.9 – 9.4	1.4	0.3 – 6.0
Other isolated CAs	5,776	2**	0.0	0.2	0.0 – 0.8	0.2	0.0 – 0.8
Multiple CAs	1,349	0	0.0	0.0	0.0 – 0.0	–	–
Total	22,843	34	0.1	0.8	0.5 – 1.2	0.7	0.5 – 1.1

*ORs adjusted for maternal age and employment status, use of folic acid during pregnancy, and birth order

**torticollis, branchial cyst

clubfoot (*i.e.* typical manifestation of postural deformation due to fetal malposition).

5. DISCUSSION

We examined the possible association between LP and pregnancy complications, in addition birth outcomes. The previously found higher risk of threatened abortion and placental disorders particularly abruption placentae was found [4-6,8,20], but this risk was significant only in the mothers of cases with CA, but not in the mothers of controls without CA in our study. Birth outcomes showed controversial pattern: the previously reported somewhat higher rate of preterm birth was confirmed (e.g. [9]), but only in controls without CA and this increase was not significant. On the other hand, there was a larger mean birth weight of cases and controls born to mothers with LP, but it does not associate with a lower risk of low birthweight. In fact cases had a higher rate of large birthweight. There was no higher risk of total and any CA group.

The secondary findings of the study confirmed the well-known fact that LP is more frequent in elder pregnant women (e.g. [8]). The advance maternal age may explain the higher prevalence of essential hypertension, haemorrhoids and constipation. Previously the higher rate of anaemia (mostly iron deficiency) in women with LP due to abnormal menstruation and haemorrhoids (frequently with bleeding) was not frequently mentioned. However, it is worth mentioning that these associations achieved the significant level only in case mothers thus this study suggests that case mothers with LP (*i.e.* having a malformed fetus) had a higher risk of pregnancy complications.

The higher mean maternal age did not associate with a higher mean birth order, and the difference between birth and pregnancy order was somewhat larger in women with LP likely due to the higher rate of previous miscarriages [21].

The high use of vitamin E (particularly in case mothers), human chorionic gonadotropin and hydroxyprogesterone may indicate some infertility problem in women affected with leiomyoma.

The prevalence of LP ranged from 0.1-3.9% in previous epidemiological studies [4,6,20,22,23], while this prevalence was 0.15-0.19% in our study, *i.e.* near to the lower level of this range. A similar lower prevalence (0.37%) was found in another population-based material [8]. Of course, the prevalence is determined by the age distribution of women and the diagnostic criteria, in addition underreporting might occur if a woman did not have a sonographic examination, or the diagnosis was

not reported in the prenatal maternity logbook.

The unexpected finding of the study is the larger mean birth weight, and a somewhat higher rate of large birthweight newborns of pregnant women with LP. Coronado *et al.* [8] reported a higher rate of low birthweight newborns and prolonged labor. Our data were not appropriate to evaluate the latter, but newborns had larger birth weight. Women with LP had a better socioeconomic status but this confounder was considered at the calculation of adjusted mean birth weight. Obviously these pregnant women at high risk had also a special prenatal medical management but it did not associate with a higher level of folic acid supplementation. Thus further studies are needed to check the efficacy of recent medical management of women with LP and to explain some unexpected findings in this study.

The strengths of the HCCSCA are that is a population-based and large data set including 105 women with prospectively and medically recorded LP in prenatal maternity logbook, furthermore medically recorded gestational age at delivery and birth weight in an ethnically homogeneous Hungarian (Caucasian) population. Additional strengths include the matching of cases to controls without CAs; available data for potential confounders, and finally that the diagnosis of medically reported CAs was checked in the HCAR [11] and later modified, if necessary, on the basis of recent medical examination within the HCCSCA [10].

However, this data set also has limitations. 1) There is underreporting of LP in our data set. 2) The occurrence of previous surgical and other medical management in women with leiomyoma was not checked in validation studies, only the medically recorded data in the prenatal maternity logbook were evaluated 3) The size of LP was not recorded thus there was no chance to estimate the dose-effect relation. 4) The occurrence of previous miscarriages could be estimated only on the basis of difference of birth and pregnancy order in the data set of the HCCSCA, in addition the higher risk of women with LP was supported by the higher rate of vitamin E and hydroxyprogesterone treatment. 5) The lifestyle data were known only in the subsamples of pregnant women visited at home because previous validation study indicated the unreliability of maternal information regarding their smoking and drinking habit [24].

In conclusion, a higher occurrence of threatened abortion and placental disorders was found in the mothers of cases with CA, but not in the mothers of controls without CA. There was larger mean birth weight of babies born to mothers with LP and it associated with a higher rate of large birthweight in cases. A higher risk of CAs was not found among the offspring of pregnant women with LP. Thus the pregnancy of women with uterine leiomyoma does not to be discouraged if they wish to have babies, but they need specific and high medical care.

REFERENCES

[1] Stewart, E.A. (2001) Uterine fibroids. *Lancet*, **357(9752),** 293-298.

[2] Ouyang, D. and Hill, J.A. (2002) Leiomyoma, pregnancy and pregnancy loss. *Infertility and Reproductive Medicine Clinics of North America*, **13**, 325.

[3] Agdi, M. and Tulandi, T. (2008) Endoscopic management of uterine fibroids. *Best Practice and Research Clinical Obstetrics and Gynacology*, **22(4)**, 569-760.

[4] Katz, V.L., Dotters, D.J. and Droegemueller, W. (1989) Complications of uterine leiomyomas in pregnancy. *Obstetrics and Gynecology*, **73(4)**, 593-596.

[5] Davis, J.L., Ray-Mazumder, S., Hobel, C.J., *et al.* (1990) Uterine leiomyomas in pregnancy: A prospective study. *Obstetrics and Gynecology*, **75(1),** 41-44.

[6] Exacoustos, C. and Rosati, P. (1993) Ultrasound diagnosis of uterine myomas and complications in pregnancy. *Obstet- rics and Gynecology*, **82(1)**, 97-101.

[7] Vergani, P., Ghidini, A., Strobelt, N., *et al.* (1994) Do uterine leiomyomas influence pregnancy outcomes? *American Journal of Perinatology*, **11(5)**, 356-358.

[8] Coronado, G.D., Marshall, L.M. and Schwartz, S.M. (2000) Complications in pregnancy, labour, and delivery with uterine leiomyomas: a population-base study. *Obstetrics and Gynecology*, **95(5)**, 767-769.

[9] Chen, Y-H., Lin, H-C., Chen, S.-F. and Lin, H.-C. (2009) Increased risk of preterm births among women with uterine leiomyoma: a nationwide population-based study. *Human Reproduction*, **24(12)**, 3049-3056.

[10] Czeizel, A.E., Rockenbauer, M., Siffel, C. and Varga, E. (2001) Description and mission evaluation of the Hungarian Case-Control Surveillance of congenital Abnormalities, 1980-1996. *Teratology*, **63(5)**, 176-185.

[11] Czeizel, A.E. (1997) The first 25 years of the Hungarian Congenital Abnormality Registry. *Teratology*, **55(5)**, 299-305.

[12] Czeizel, A.E., Intődy, Z. and Modell, B. (1993) What proportion of congenital abnormalities can be prevented? *British Medical Journal*, **306(6880)**, 499-503.

[13] Czeizel, A.E., Petik, D. and Vargha, P. (2003) Validation studies of drug exposures in pregnant women. *Pharmacoepidemiology and Drug Safety*, **12(5)**, 409-416.

[14] Czeizel, A.E. and Vargha, P. (2004) Periconceptional folic acid/multivitamin supplementation and twin pregnancies. *American Journal of Obstetrics and Gynecology*, **191(3)**, 790-794.

[15] Czeizel, A.E. and Dudas, I. (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *New England Journal of Medicine*, **327(26)**, 1832-1835.

[16] Czeizel, A.E. (1996) Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. *American Journal of Medical Genetics*, **62(2)**, 179-183.

[17] Czeizel, A.E., Dobo, M. and Vargha, P. (2004) Hungarian

cohort-controlled trial of periconceptional multivitamin supplementation shows reduction in certain congenital abnormalities. *Birth Defects Research*, **70(11),** Part A, 853-861.

[18] Botto, L.D., Olney, R.S. and Erickson, J.D. (2004) Vitamin supplements and the risk for congenital anomalies other than neural-tube defects. *American Journal of Medical Genetics*, **125C(1)**, 12-21.

[19] Puho, H.E., Métneki, J. and Czeizel, A.E. (2004) Maternal employment status and isolated orofacial clefts in Hungary. *Cental European Journal of Public Health*, **13(3)**, 144-148.

[20] Rice, J.P., Kay, H.H. and Mahony, B.S. (1989) The clinical significance of uterine leiomyoma in pregnancy. *American Journal of Obstetrics and Gynecology*, **160 (5 Pt 1)**, 1212-1216.

[21] Probst, A.M. and Hill, J.A. (2000) Anatomic factors associated with recurrent pregnancy loss. *Seminars in Reproductive Medicine*, **18(4)**, 341-350.

[22] Burton, C.A., Grimes, D.A. and March, C.M. (1989) Surgical management of leiomyomata in pregnancy. *Obstetrics and Gynecology*, **74(5)**, 707-709.

[23] Hasan, F., Arumugam, K. and Sivanesaratman, V. (1991) Uterine leiomyomata in pregnancy. *International Journal of Gynaecology and Obstetrics*, **34(1)**, 45-48.

[24] Czeizel, A.E., Petik, D. and Puho, E. (2004) Smoking and alcohol drinking during pregnancy. The reliability of retrospective maternal self-reported information. *Central European Journal of Public Health*, **12(4)**, 179-183.

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Knowledge and health seeking behavior for malaria among the local inhabitants in an endemic area of Ethiopia: implications for control

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ABSTRACT

This cross-sectional study was conducted to assess the knowledge and health seeking behavior for malaria among the local inhabitants in an endemic area of Ethiopia: Implications for control. 98.6% and 80.7% of respondents had awareness about malaria and the cause ('mosquito bite') of malaria, respectively. 186 (81.6%) respondents seek treatment for a febrile disease from health care facilities. Chi-square analysis revealed a strong association between the educational status of respondents and the measures they take to prevent malaria ($X^2 = 58.7$; $df = 16$; $p < 0.001$). The findings clearly suggest that the majority of the respondents had adequate knowledge and enviable health seeking behaviour. However, still a sizable faction had misconception and undesirable health seeking behaviour. It's a major barrier to implement effective malaria control strategies in the resource-limited settings particularly in country like Ethiopia. In this context, appropriate communication strategies apparently inevitable. Therefore, appropriate communication strategies should be designed to promote the knowledge and health seeking behaviour of vulnerable section of the society in this vicinity.

Keywords: Malaria; Knowledge; Health seeking behaviour; Ethiopia

1. INTRODUCTION

Malaria remains a major cause of morbidity and mortality in tropical and subtropical regions of the world, despite decades of malaria control efforts. There are approximately 300-500 million clinical cases and about one million deaths due to malaria globally, and Africa south of the Sahara accounts for over 90% of the disease burden [1]. Most of the infections and deaths in highly endemic areas occur in children and pregnant women, who have little access to health systems [2-4].

109 countries were endemic for malaria in 2008, 45 within the WHO African region. Ethiopia had approximately 6% of malaria cases in the African Region in 2006. Malaria is present everywhere except in the central highlands. A total of 1.2 million cases were reported in 2007, the lowest number in the period 2001-2007 [5]. Over the past years, the disease has been consistently reported as the leading cause of outpatient visits, hospitalization and death in health facilities across the country. The diverse eco-climatic condition in the country makes the malaria transmission pattern seasonal and unstable usually characterized by frequent focal and cyclic widespread epidemics [6].

Except for southern Africa, many countries in the continent do not have successful malaria control programmes due to the magnitude of the problem compounded by lack of adequate health infrastructure, as well as financial and human resources [7]. Vector-borne disease control programs mostly rely on controlling the parasite and/or vector and have often overlooked the importance of the target population's knowledge, beliefs and behavior in the transmission and control of disease [8]. Malaria control programs must consider the broad, complex and interrelated factors that influence human behavior, especially now that malaria control is theoretically within reach of even the poorest countries through the availability of insecticide treated bednets and highly effective antimalarial drug combinations [9].

Malaria protective measures are related to knowledge and beliefs of people; when they think malaria risk is low, it is more difficult to implement protective meas-

ures [10]. The poor and vulnerable populations are disproportionately affected by malaria and the severe consequences of malaria are borne more by the poorest [11]. Studies on knowledge, attitudes and practices are becoming more important to design and improve malaria control activities, to establish epidemiological and behavioral baselines and to identify indicators for monitoring programs [12].

Poor knowledge about malaria was significant factor for death from malaria among the household members in Sudan [13]. Thus, there is an urgent call for updated information on key sociocultural, socio-economic indictors and human understanding about malaria to apply appropriate control strategies. Therefore, the purpose of this study was to assess the knowledge and health seeking behavior for malaria among the local inhabitants in an endemic area of Ethiopia: Implications for control. The present study findings could provide baseline information to design effective and sustainable malaria control strategies suited to local conditions in the near future.

2. MATERIALS AND METHODS

2.1. Study Settings

The study was conducted in Serbo town, which is located 345km south-west of the capital Addis Ababa in Oromia Regional State, south-western Ethiopia. It's located between latitudes 7º35-8º00 N, and between longitudes 36º46-37º14 E, at altitudes between 1,740-2,660 m above sea level and has a mean annual temperature of 19°C. According to the 2005 census, the study area had a total population of 6,115 and 511 households. Malaria is the major health problem in the Serbo town. As the six consecutive years data (2002-2007) from Serbo Health Center showed, the number of malaria cases ranged between 3,925 and 22,938, with the peak being during 2004/5. The prevalence seems decreasing although the number of cases per year is still high [14]. The main socio-economic activities of the local communities are small business, subsistence mixed farming involving the cultivation of staple crops (maize, teff and sorghum), and cattle and small stock raising.

2.2. Study Design

The study was a descriptive cross-sectional survey. A structured questionnaire was designed and administered by trained field workers. The first part of the questionnaire included sociodemographic characteristics, whereas the second part had questions on, adult residents' knowledge and perceptions about malaria transmission, cause, treatment seeking patterns, preventive measures and practices. To improve the quality of the data, pre-testing of the questionnaire was carried out prior to the actual data collection. The questionnaire was tested on ten respondents by the enumerators, in an area different from the study area, but with a similar socio-demographic pattern.

2.3. Data Collection

The questionnaire was administered to 228 randomly selected households between January and March 2009. The head of household or a responsible adult was interviewed. Only one person per household was interviewed. To minimize bias information and variables the questionnaire prepared in English language was translated into native local language Amharic to make it easy to understand and to administer by interviewers and interviewees.

2.4. Ethical Considerations

The study was approved by the ethical clearance committee of the Jimma University, Jimma, Ethiopia. Before the commencement of the survey, meetings with community health workers, community leaders and members of the neighborhood associations were held in which the objectives of the survey were clearly explained. Written consent was obtained from each study participant. Every participant was assured to withdraw the interview at any phase if they wish to do so. However, all the informants actively involved and no one declined to finish the interview.

2.5. Statistical Analysis

Statistical analysis was carried out using SPSS, version 9.0. Range and mean were analysed and appropriate tables, graphs and percentage were displayed. Level of significance also determined by using 95% of confidence intervals and *p*-value.

3. RESULTS

3.1. Characteristics of Study Population

The socio-demographic characteristics of respondents are presented in **Table 1**. The study participants consisted of 46.5% males and 53.5% females. Majority of the respondents (44.3%) were in between 20-29 years old. 33.5% of the study population had no formal education. About 34.7% of the participants monthly income was 20-30 USD **(Table 1)**.

3.2. Knowledge and Perceptions of Respondents about Malaria Cause, Transmission and Mosquitoes Breeding Sites

Tables 2 presents respondents awareness about malaria

Table 1. Socio-demographic characteristics of study population.

Socio-demographic characteristics	n	%
Sex		
Male	106	46.5
Female	122	53.5
Age		
15-19	37	16.2
20-29	101	44.3
30-39	54	23.7
40-49	21	9.2
≥50	15	6.6
Ethnicity		
Oromo	136	59.6
Amhara	34	14.9
Gurage	13	4.9
Tigray	8	3.5
Kaffa	16	7.1
Dawuro	23	10.0
Educational status		
Illiterates	81	35.5
Can read & write	26	11.4
1-4th grade	32	13.6
5-8th grade	51	22.4
9-12th grade	23	10.1
>12th grade	16	7.0
Occupational status		
Civil servants	32	14
Merchants	84	36.8
Housewives	41	18.0
Farmers	53	23.3
Private sector worker	12	5.3
NGO worker	6	2.6
Monthly income (Ethiopian Birr)*		
< 100	31	13.6
101-200	53	23.3
201-300	79	34.7
301-400	33	14.5
401-500	20	8.8
> 500	12	5.1

Note*: 1$ = 12.4 Ethiopian Birr.

transmission, cause, and mosquito breeding sites. In general, 98.6% of respondents had awareness about malaria. Majority of respondents (80.7%) were aware about the cause ('mosquito bite') of malaria irrespective of sex, age, monthly income and occupation. During the survey, 80.7% of the respondents knew that mosquitoes are transmitting the malaria. A total of 178 (78.1%) people knew that stagnant water bodies are serving as mosquito's breeding sites **(Table 2)**. About 40.4% of respondents had known about malaria through mass media **(Figure 1)**.

3.3. Knowledge and Perceptions of Respondents Regarding Malaria Prevention and Control

Table 2. Respondents knowledge and perception about malaria causes, transmission and mosquito breeding sites.

Variables	n	%
Awareness about malaria		
Yes	224	98.6
No	4	1.4
Causes of malaria*		
Mosquito bites	184	80.7
Chill climate	167	73.2
Malnutrition	96	42.1
Eating raw vegetable	71	31.1
Drinking dirty water	123	54
I don't know	46	20.2
Malaria transmission*		
Cold weather	101	44.3
Mosquitoes bites	184	80.7
Heat/Sun shine	23	10.1
Dirty stagnant water /swamp	106	46.5
Due to poor personal hygiene	102	44.7
Starvation	46	20.2
Mosquitoes breeding sites*		
Stagnant water	178	78.1
Tree holes	29	12.7
Waste/polluted water	43	18.9
Stream/River	26	11.4
Dirty places/Dustbin	97	42.5
I don't know	22	9.6

Note*: Percentages do not add up to 100 due to multiple responses.

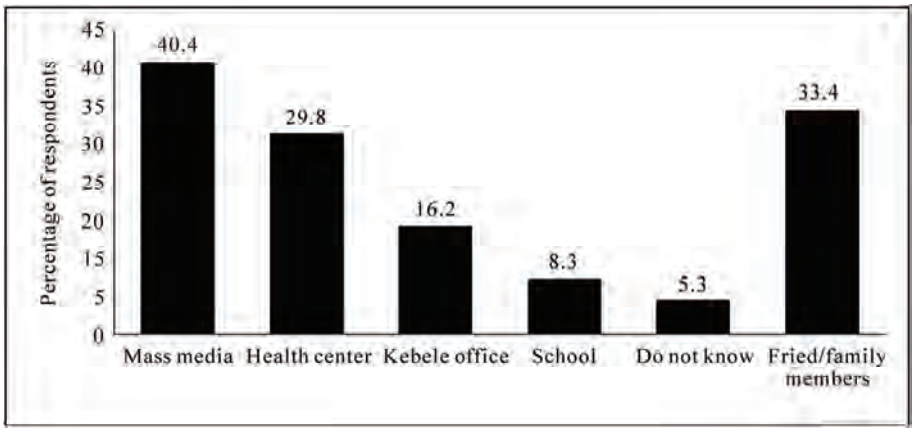

Figure 1. Sources of information about malaria related information as reported by respondents.

95.6% and 77.2% of respondents believe that regular deployment of bednets and DDT indoor residual spray (IRS) could prevent malaria, respectively **(Table 3)**. Chi-square analysis revealed a strong association between the educational status of individual households and the measures they take to prevent malaria (X^2 =58.7; *df* =16; *p* <0.001) **(Table 4)**.

3.4. Febrile Disease Treatment Seeking Behavior of Respondents

As shown in **Figure 2**, 186 (81.6%) respondents seek treatment for a febrile disease from the health care facilities. However, few respondents cited such as self medication, approaching traditional healers and eating health foods.

4. DISCUSSIONS

This study sheds light on a group of adults in a malaria endemic area of Ethiopia regarding the level of under standing community knowledge about malaria and health seeking behavior. It provides information for educators and policy makers that are necessary for guidance towards malaria preventive campaigns. In the present survey, majority of the study participants (98.6) demonstrated general awareness about malaria, which is relatively higher than a recent study, which was conducted in Swaziland showed that of 320 households surveyed 298 (93.1%) of the respondents had heard about malaria [15]. This discrepancy could be because of the fact that usually the population in malaria endemic settings has higher awareness than the residences of en-

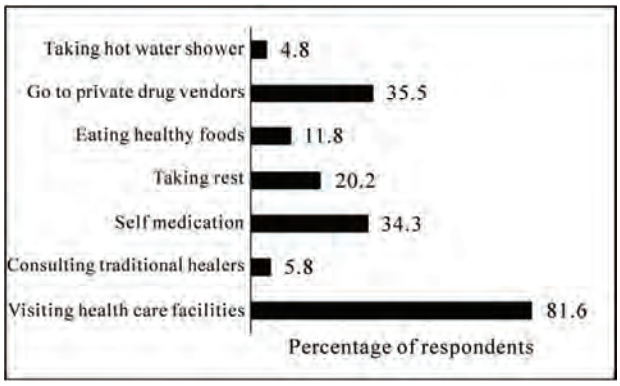

Figure 2 Treatment-seeking behaviors for febrile disease as reported by respondents.

Table 3. Respondents knowledge and perception about malaria prevention and control.

Variables	n	%
Possible options to prevent/control malaria*		
Residual house spraying with DDT	176	77.2
Environmental management	112	49.1
Regular deployment of bednets	218	95.6
Early diagnosis and treatment	22	9.6
Personal hygiene	79	34.6
Healthy food/Nutrition	92	40.3
Benefits of IRS		
To prevent from malaria /mosquito bite	126	55.3
To avoid bites from other insects	84	36.8
I don't know	18	7.9
Benefits of ITNs/Bednets*		
To avoid insects bites	221	96.9
To prevent malaria and other diseases	213	93.4
I don't know	3	1.4
To kill domestic insects	5	2.2

Note*: Percentages do not add up to 100 due to multiple responses.

Table 4. Association between mosquito preventive measures and educational status of the respondents.

Types of prevention measures	Total respondents	Educational level of the respondents					*p*-value
		Illiterates	Can read & write	1-8th grade	9-12th grade	>12th grade	
Mosquito net	57	16	9	13	10	9	
DDT spraying	52	3	12	22	8	7	$X^2 = 58.7$
Draining stagnant water	93	41	19	28	5	0	$df = 16$
Don't use	13	13	0	0	0	0	$p < 0.001$*
Burning repellent plants	13	8	4	1	0	0	
Total	**228**	**81**	**44**	**64**	**23**	**16**	

Note*: $p<0.05$ statistically significant

demic/nonendemic area. In addition this study was conducted in urban area too.

80.7% of the respondents knew the role of mosquitoes in malaria transmission ('mosquito bite'). The respondents' level of awareness about mode of malaria transmission was very low when compared to the findings in previous studies carried out in Ethiopia which reported awareness levels of up to 93% [16,17]. However, it's relatively higher than that reported in other studies 55% of the surveyed population in a nationwide study in Malawi (Ziba *et al*., 1994) [18], 67% in Turkey [19], and 17.3% in Ethiopia [20,21].

However, many people had not known the real cause of malaria. Such misconceptions have also been reported from other studies in Ethiopia and other countries [22-24]. The present study findings were comparable with previous studies in India [25] and in Ethiopia [17] although the association of malaria with mosquitoes is widespread in these communities, other causal factors of malaria such as traditional beliefs like eating maize stalks, contact with malaria patients, exposure to rains and cold weather, bad smell and dirty water were frequently suggested. The correction of such misconceptions about the relationship between mosquito bite and malaria through health education messages is very critical for the success of malaria prevention and control using ITNs [26].

78.1% of respondents indicated that stagnant water bodies serving as potential mosquito breeding sites. Previous studies in Ethiopia have also confirmed similar findings [17,27]. However, the level of awareness regarding mosquito breeding site was relatively lower than earlier studies. Most of the respondents knew about malaria related information through mass media and friends/family members. Findings were consistent with a study in Ethiopia [17]. The most common source of information about malaria was from relatives. Radio was ranked third after medical personnel as a major information source [28].

The great majority of the respondents believe that regular deployment of bednets and DDT indoor residual spray (IRS) could prevent insect's bites and malaria. The findings comparable with an earlier study in Mozambique demonstrated that the majority of respondents associate malaria with mosquitoes and are aware of various methods to prevent illness, including IRS and bed nets [29]. **Table 4** Chi-square analysis suggest an association between the educational status of individual households and the measures they take to prevent malaria (X^2 =58.7; df =16; p <0.001). Results consistent with an earlier study, which was conducted in Swaziland, found that most respondents believed that malaria is preventable, and mentioned clinic, spraying and the use of bed nets as key malaria preventive measures. Despite these positive responses a substantial number of them (43.4%) did not take any personal protective measures against malaria infection [15]. Indeed, several studies across the globe particularly in Africa evidently suggest that Bednets/Insecticide-treated nets are regarded as one of the most effective prevention methods and sleeping under the protection of bednets could substantially reduce the malaria burden.

Another interesting finding was that the majority of the respondents preferred to seek treatment in the health facilities rather than approaching traditional healers and self medication. This may be due to the fact that the Serbo health center is located within the study area as result accessibility is extremely high among the local inhabitants. In addition, it's providing services free of charge. The present study findings are comparable with few earlier studies. In Ethiopia, 98% respondents had their first visit to health care facilities including public and private health services as well as malaria control laboratories, drug venders/pharmacy and CHWs seeking treatment for malaria [17]. Another study in Swaziland found that almost 90% of the respondents seek treatment in the health facilities [15].

The scope of malaria control is changing worldwide. With less emphasis being placed on insecticide use, in-

creased community participation in malaria control and prevention measures will be of higher importance. With greater emphasis being placed on community control and prevention, health education based on understanding community and individual behaviors, attitudes and knowledge pertaining to malaria is moving to the forefront as a measure necessary for malaria control [30].

The present study findings clearly suggest that the majority of the study participants had adequate knowledge and ample enviable health seeking behavior. However, still a sizable proportion had misconception and undesirable health seeking behavior. Indeed, it's a major barrier to implement effective as well sustainable malaria control strategies in the resource-limited and ethnically-diverse settings particularly in country like Ethiopia. Therefore, appropriate communication strategies should be designed and implemented in the study area to bring the constructive outcome in the near future.

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REFERENCES

[1] Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y. and Hay, S.I. (2005) The global distribution of clinical episodes of Plasmodium falciparum malaria. *Nature*, **434 (7030)**, 214-217.

[2] Luxemburger, C., Mc Gready, R., Kham, A., Morison, L., Cho, T., Chongsuphajaisiddi, T., White, N.J. and Nosten, F. (2001) Effects of malaria during pregnancy on infant mortality in an area of low malaria transmission. *American Journal of Epidemiology*, **154(5)**, 459-465.

[3] World Health Organization (1996) Fighting disease, fostering development. Geneva.

[4] Snow, R.W., Craig, M., Diechmann, U. and Marsh, K. (1999) Estimating mortality, morbidity and disability due to malaria among Africa's nonpregnant population. *Bulletin of the World Health Organization*, **77(8)**, 624-640.

[5] World Health Organization (2008) World malaria report. Geneva.

[6] Federal Ministry of Health of Ethiopia (2006) National five years strategic plan for malaria control in Ethiopia: 2006-2010. Malaria and Other Vector-borne Diseases Prevention and Control Team, Diseases Prevention and Control Department, Ministry of Health, Addis Ababa.

[7] Mabaso, M.L.H., Sharp, B. and Lengeler, C. (2004) Historical review of malarial control in Southern Africa with emphasis on the use of indoor residual house-spraying. *Tropical Medicine and International Health*, **9(8)**, 846-856.

[8] Ruebush, T.K., Weller, S.C. and Klein, R.E. (1992) Knowledge and beliefs about malaria on the pacific coastal plain of Guatemala. *American Journal of Tropical Medicine and Hygiene*, **46(4)**, 451-459.

[9] White, N.J., Nosten, F., Looareesuwan, S., *et al.* (1999) Averting a malaria disaster. *Lancet*, **353(9168)**, 1965-1967.

[10] Winch, P.J., Makemba, A.M., Kamazina, S.R., Lwihula, G.K., Lubega, P., Minjas, J.N. and Shiff, C.J. (1994) Seasonal variation in the perceived risk of malaria: Implication for the promotion of insecticide impregnated bednets. *Social Science Medicine*, **39(1)**, 63-75.

[11] Barat, L.M., Palmer, N., Basu, S., Worrall, E., Hanson, K. and Mills, A. (2004) Do malaria control interventions reach the poor? A view through the equity lens. *American Journal of Tropical Medicine and Hygiene*, **71 (Suppl 2)**, 174-178.

[12] Schultz, L.J., Ettling, M., Chitsulo, L., Steketee, R.W., Nyasulu, Y., Macheso, A. and Nwanyanmu, O.C. (1994) A nation-wide malaria knowledge, attitudes and practices survey in Malawi: Objectives and methodology. *Tropical Medicine and Parasitology*, **45(1)**, 54-56.

[13] Saeed, I.E. and Ahmed, E.S. (2003) Determinants of malaria mortality among displaced people in Khartoum state, Sudan. *Eastern Mediterranean Health Journal*, **9(4)**, 593-599.

[14] Federal Ministry of Health of Ethiopia (2007) Entomological profile of malaria in Ethiopia. Addis Ababa.

[15] Hlongwana, K.W., Mabaso, M.L.H., Kunene, S., Govender, D. and Maharaj, R. (2009) Community knowledge, attitudes and practices (KAP) on malaria in Swaziland: A country earmarked for malaria elimination. *Malaria Journal*, **8(1)**, 29-37.

[16] Jima, D., Tesfaye, G., Deressa, W., Woyessa, A., Kebede, D., Ali, A. and Enquoselassie, F. (2003) Knowledge, attitude and practice about malaria, the mosquito and antimalarial drugs in a rural community. *Ethiopian Journal of Health Development*, **17(2)**, 99-104.

[17] Jima, D., Tesfaye, G., Deressa, W., Woyessa, A., Kebede, D. and Alameraw, D. (2005) Baseline survey for the implementation of insecticide–treated mosquito nets in malaria control in Ethiopia. *Ethiopian Journal of Health Development*, **19 (1)**, 16-23.

[18] Ziba, C., Slutsker, L., Chitsulo, L. and Steketee, R.W. (1994) Use of malaria prevention measures in Malawian households. *Trop Med Parasitol*, **45(1)**, 70-73.

[19] Simsek, Z. and Kurcer, M.A. (2005) Malaria: knowledge and behaviour in an endemic rural area of Turkey. *Public Health*, **119(3)**, 202-208.

[20] Yeneneh, H., Gyorkos, T.W., Joseph, L., Pickering, J. and Tedla, S. (1993) Antimalarial drug utilization by women in Ethiopia: a knowledge-attitudes-practice study. *Bulletin of the World Health Organization*, **71(6)**, 763-772.

[21] Deressa, W., Ali, A. and Hailemariam, D. (2008) Malaria-related health seeking behaviour and challenges for care providers in rural Ethiopia: Implications for control. *Journal of Biosocial Science*, **40(1)**, 115-135.

[22] Hamel, M.J., Odhacha, A., Roberts, J.M. and Michael, S.D. (2001) Malaria control in Bangoma district, Kenya: A survey of home treatment of fever, bed net use and attendance at antenatal clinics. *Bulletin of the World Health Organization*, **79(11)**, 1014-1123.

[23] Legesse, Y., Tegegn, A., Belachew, T. and Tushune, K. (2007) Knowledge, attitude and practice about malaria transmission and its preventive measures among households in urban areas of Assosa Zone, western Ethiopia. *Ethiopian Journal of Health Development*, **21(2)**, 157-165.

[24] Adongo, P.B., Kirkwood, B. and Kendall, C. (2005) How local community knowledge about malaria affects insecticide treated net use in northern Ghana. *Tropical Medicine and International Health*, **10(4)**, 366-78.

[25] Vijayakumar, K.N., Gunasekaran, K., Sahu, S.S. and Jambulingam, P. (2009) Knowledge, attitude and practice on malaria: a study in a tribal belt of Orissa State, India with reference to use of long lasting treated mosquito nets. *Acta Tropica*, **112(2)**, 149-155.

[26] Belay, M. and Deressa, W. (2008) Use of insecticide treated nets by pregnant women and associated factors in a pre-dominantly rural population in northern Ethiopia. *Tropical Medicine and International Health*, **13(10)**, 1303-1313.

[27] Deressa, W., Ali, A. and Enquosellassie, F. (2003) Knowledge, attitude and practice about malaria, the mosquito and antimalarial drugs in a rural community. *Ethiopian Journal of Health Development*, **17(2)**, 99-104.

[28] Alilio, M.S., Eversole, H. and Bammek, J. (1998) A KAP study on malaria in Zanzibar: implications for prevention and control a study conducted for UNICEF Sub-office Zanzibar. *Evaluation and Program Planning*, **21(4)**, 409-413.

[29] Pool, R., Munguambe, K., Macete, E., Aide, P., Juma, G., Alonso, P. and Menendez, C. (2006) Community response to intermittent preventive treatment delivered to infants (IPTi) through the EPI system in Manhica, Mozambique. *Tropical Medicine and International Health*, **11(11)**, 1670-1678.

[30] Cropley, L. (2004) The effect of health education interventions on child malaria treatment seeking practices among mothers in rural refugee villages in Belize, Central America. *Health Promotion International*, **19(4)**, 445-452.

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Enumeration of microbial contaminants in sachet water: a public health challenge

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ABSTRACT

Accessibility and availability of fresh clean water is a key to sustainable development and essential element in health, food production and poverty reduction. In the present study, we have collected water sachet containing CM/L number and they were analysed for physical and bacteriological nature. The organisms isolated in this study were *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas vesicularis* and *Pseudomonas aeruginosae*. The harmful effects of these isolates were evidenced by antibiotic resistance, heavy metal tolerance and antibacterial activity. They were resistant to the antibiotics like amoxiclav, methicillin, chloramphenicol and streptomycin. They showed tolerance to the heavy metals at 5 mM conc. except for lead. For antibacterial activity, they were tested against human pathogens *Klebsiella pnemoniae*, *Proteus mirabilis, Micrococcus leuteus* and *Salmonella paratyphium*. But at the same time these organisms could be exploited for the industrial production of amylase, protease and cellulase.

Keywords: Sachet Water; Bacterial Contaminants; Pathogens; Industrially Useful Bacteria

1. INTRODUCTION

Bottled water is defined as water that is intended for human consumption and this is sealed in bottles or other container with no added ingredients except that it may contain safe and suitable fluorides. Its price is within the reach of tautology. Small scale entrepreneurs introduced small nylon sachets which are electrically heated and sealed at both ends to the market and is popularly called pure water. It finds patronage from members of low socio-economic class.

Besides, majority of the water sachets do not carry the National Food and Drug Administration Control (NAFD AC) approval number. This means that they are either not registered or the procedures have not completed the registration of their products with NAFDAC. It was therefore considered implication of sachet water. Accessibility and availability of fresh clean water is a key to sustainable development and essential element in health, food production and poverty reduction. 1.2 billion People around the world lack access to safe water and 2.5 billion are not provided with adequate sanitation (Third World water forum on water, 2003). In metropolis as a whole pipe born water is inadequate both in quality and quantity.

Consequently water born diseases such as cholera and typhoid often have their epidemic during the dry season. Typhoid remains a great socio-economic problem in developing countries. Proliferation of intestine is associated with high mortality with wound infection occurring in 50-75% of surviours [1]. Controlling wound sepsis or wound infection also affected mortality [2,3].

Since this health problem was largely traceable to unhygienic water supply, an alternative to the seemingly inadequate water supply was found in bottled water.

Drinking water including bottled water, may reasonably to expected to contain at least small amounts of some contaminants includes microbial pathogens, organic pollutants like heavy metals. The presence of contaminants does not necessarily indicated that water poses a health risk. Environmental Protection Agency (EPA) sets standards for approximately 90 contaminants in drinking water. EPA standards, along with each contaminant's likely source and health effects, are available at www.epa.gov/safewater/mcl.html.

Microbiological contamination of water has long been a concern to the public. There is a concern in increasing due to outbreak of coliform bacteria, and protozoans like Giardiasis, Cryptosporidiosis.

Coliforms are not a single type of bacteria but a group of bacteria that includes *Klebsiella*, *Proteus*, *E. coli*, and *Salmonella*. Coliform organisms are not necessarily

pathogens and are rarely found in bottled water, they serve as an indicator of insanitation or possible contamination.

Microbial potability of bottled and packaged drinking water hawked in Ilorin metropolis was done by selecting 81 samples containing 11 brands of drinking water packaged and hawked in cellophane bags, did not met drinking water standards. *Pseudomonas* was frequently recovered as a contaminant of packaged water [4]. Bottled mineral water consumption has significantly increased in Brazil. Public health determines the parasitological and microbiological status of some brands and found occurrence of Cryptosporidial oocysts and Guardia cyst in bottled mineral water [5]. An assessment of the health and social economic implantations of sachet water in Ibadam Nigeria [6] selected 78 samples from 20 brands of sachet water from hawkers/vendors. Bacteria obtained include: *Klebsiella* sp., *Streptococcus faecalis* and *Pseudomonas aeruginosae*. By sterile filtration of water, broad diversity of viable bacteria was isolated by using 0.2 µ filter for the removal of microorganisms and is commonly referred as 'sterile filtration'. 19 bacterial taxa were isolated by the acclimatization method from 0.2 micron filtered fresh water samples. *Cryptosporidium parvum* infection in Bergen and Norway was found during the large water borne Giardiasis outbreak [7].

The enforcement of the regulation guiding water quality before the National Agency for Food and Drug Administration Control (NAFDAC) to employ with the checking water qualities guideline values as recommended by World Health Organization (W.H.O) becomes urgent.

The water sachets are the products of middle class entrepreneurs and some small scale business ventures. The objective of the present study was to find out the quality of sachet water. We have collected seven different brands of water sachet containing CM/L number and the samples were subjected to physical and bacteriological analysis.

2. MATERIALS AND METHODS

2.1. Media Used

LB (Luria–Bertani) Agar, King's B medium, Nutrient Agar and EMB (Eosine Methylene Blue) Agar were used to screen the sachet water sample for bacterial contamination.

2.2. Collection of Samples

Seven sachet water samples supplied in and around Pallavaram were collected. All of them contained CM/L number along with ISI–14543, Ozonized and UV treated. Some of them were Reverse Osmosis processed.

3. PHYSICAL PARAMETERS

pH: pH was checked for all the water samples immediately after opened.

4. BACTERIOLOGICAL ANALYSIS ISOLATION AND IDENTIFICATION OF BACTERIA

4.1. Isolation of Bacteria

Seven different sachet water samples were taken up for the present study. The samples include Freeze, VSP, VPZ, Aqua fresh, Jai, Hi-tech and Sakthi. The water samples were serially diluted and spread on EMB medium, Nutrient agar, King's B medium and kept for 24 hours incubation at 37˚C. The isolated bacterial colonies were purified to homogeneity by quadrant streaking, store in LBA, NA and KBA slants periodically subcultured.

4.2. Identification of Organism

The bacteria isolated were identified based on the biochemical tests outlined in the Bergey's Manual of determinative bacteriology [8].

4.3. Antibiotic Resistance/Susceptibility Screening

The sensitivity/resistance of the isolates to various antibiotics such as Chloramphenicol, Ceffriaxone, Amoxiclav, methicillin, Nalidixic acid and Streptomycin was studied by inoculating a loopful of the overnight grown cultures on Nutrient Agar plates amended with 30µg/ml concentrations of the appropriate antibiotics and incubated at 37˚C. After 24 hours of incubation, the plates were observed for growth. Nutrient agar plates without antibiotics served as control. The minimum concentration at which no growth was taken as the MIC (Minimum Inhibitory Concentration).

4.4. Heavy Metal Tolerance Spectrum

The tolerance of the bacterial isolates to various heavy metals such as zinc (zinc sulphate), lead (lead acetate), copper (copper sulphate), chromium (potassium chromate) was studied by inoculating loopful of overnight grown cultures on Nutrient Agar plates amended with 1, 3 and 5mM concentrations of heavy metals and incubated at 37˚C. After 24 hours of incubation, the plates were observed for growth. Nutrient agar plates without heavy metal served as control. The minimum concentration at which there was no growth was taken as the MIC

value.

4.5. Screening for Extra Cellular Enzyme Production

Four industrially important extra cellular enzymes were selected and screened for primary extra cellular enzyme production.

4.6. Protease

Nutrient agar along with 1% Gelatin (substrate) and 1% casein (substrate) was taken in separate conical flasks and autoclaved. They were poured into respective petriplates (triplicates). After solidifying, isolates were streaked on them and kept for incubation at 37˚C. After 24 hours of incubation, the plates were stained with 15% $HgCl_2$ (indicator) and observed for zone of inhibition.

4.7. Amylase

Nutrient agar along with 1% soluble starch (substrate) was taken in a conical flask and then autoclaved. They were poured into respective petriplates (triplicates). After solidifying, isolates were streaked on them and kept for incubation at 37˚C. After 24 hours of incubation, the plates were stained with Gram's Iodine (indicator) and observed for zone of inhibition.

4.8. Cellulase

Nutrient agar along with 1% cellulose (substrate) was taken in a conical flask and then autoclaved. They were poured into respective petriplates (triplicates). After solidifying, isolates were streaked on them and kept for incubation at 37˚C. After 24 hours of incubation, the plates were stained with 0.3% of Congo red (indicator); plates were kept in orbital shaker (mild shaking) for 15 minutes. Congo red was discarded and then 1 N NaCl was added to the plate and kept in shaker for 10 minutes and observed for zone of inhibition.

4.9. Antibacterial Activity of Pure Bacterial Isolates

Four bacterial isolates (*Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas vesicularis* and *Pseudomonas aeruginosae*) from sachet water were selected for antibacterial activity against the four human pathogenic bacteria (*Micrococcus luteus*, *Salmonella paratyphium*, *Proteus mirabilis* and *Klebsiella pneumoniae*) obtained from CAS Botany, University of Madras. Isolated bacteria were grown in King's B Broth and kept for incubation at 37˚C in orbital shaking incubator for 48 hours. After 48 hours incubation, samples were transferred to sterile centrifuge tubes and centrifuged at 8000 rpm for 15 minutes at 4˚C. Then supernatant was collected and it is filtered using 0.45 µ membrane filter. Filtered supernatant was stored in refrigerator for further study.

Human pathogens as mentioned above were grown in Nutrient Broth and incubated for 24 hours in orbital shaking incubator at 37˚C. After 24 hours pathogens were stored in refrigerator for further study.

Nutrient agar plates were prepared and pathogens were swabbed on it and well were made on the plates with the help of borer. 100 µl of filtered supernatant of bacterial isolates was added into the wells and kept for incubation for 48 hours. After 48 hours, plates were observed for antibacterial activity.

4.10. Antibacterial Activity of Isolates with Organic Solvent

The supernatant of the bacterial isolates as mentioned above was taken. To that equal amount of Ethyl Acetate (EA), an universally proved polar organic solvent that could dissolve many compounds was added and kept in orbital shaker for one hour or more preferably overnight. Then transfer the organic layer and distribute it equally in to different conical flasks and cover the flask with cheese cloth to prevent contamination and after complete drying, add EA. To the residue presenting the flask, the supernatant was used for further study.

Human pathogens as mentioned above were grown in Nutrient Broth and incubated for 24 hours in orbital shaking incubator at 37˚C. After 24 hours, pathogens were stored in refrigerator for further study.

Nutrient agar plates were prepared and pathogens were swabbed on it and well were made on the plates with the help of borer. 100 µl of filtered EA supernatant was added into the wells and keep it for incubation for 48hours. After 48 hours, the plates were observed for antibacterial activity.

5. RESULTS AND DISCUSSION

The objective of the present study was to find out the quality of sachet water. For this, we randomly selected seven different brands of sachet water and they were collected from in and around pallavaram, Chennai. The samples were subjected to physical and bacteriological analysis. The water samples were assessed for coliform and other bacteria using Nutrient agar, KB agar and EMB agar. The results for this study support an earlier observation that the sachet water being produced is of questionable quality [9,10].

As useful as sachet water is to the society, the result of the analysis raised doubts as to its quality. The pH of water sachets has an upper range of 9.26 (**Table 1**), a value higher than the upper limit of pH 8.5, as recommended by W.H.O. Even though pH has no direct effect on health, its indirect action on physiological processes

Table 1. pH of the sachet water samples.

Name of the sachet	pH
Freeze	9.20
VSP	9.26
VPZ	8.30
Aqua fresh	8.45
Jai	9.10
Hi-tech	8.38
Sakthi	8.20

cannot be over emphasized [6] got the upper pH unit of 9.7 among the 78 samples from 20 brands of sachet water tested.

Bacteriological analyses results (**Table 2**) showed that all the seven different brands of sachet water produced growth after 24 hours of incubation. The organisms isolated in this study are *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas vesicularis*, and *Pseudomonas aeruginosae*. [11] reported that pure water vending machine may not be so pure, after all, because investigations found bacteria like *E. coli* in the machine. Isolation of bacteria from pure water samples especially *Streptococcus facealis* indicates possible contamination from human excreta.

The procedure most often than not are those people who may not know and are very little about the quality of water sachets they produced. Some even imitate other good products. For the price, pure water sachets are affordable, to the middle class society and some of the lower class also. Thus this type of packaged water is now popularly and freely served at the common people parties and social functions.

Most of the small scale producers of pure water may not be able to afford the price or space for a bore hole in their premises, hence they still depend on the already condemned public water supply and water from doubtful environmental sources, for the sources of the water they use in packaging their products, some of them under very poor environmental conditions. It should be noted that the seven brands of sachet water selected for the present study carried the CM/L number and ISI certified but even they showed bacterial contamination. In the present study, *Pseudomonas* was isolated from all the samples and we are suggesting that the *Pseudomonas* also be included as an added indicator for determining their safety standard. Like us, Olayemi (1999) was frequently isolated *Pseudomonas* from packaged waters.

Table 2. Biochemical characterization of the isolates from the sachet water.

Biochemical test	Bacterial isolates			
	Proteus mirabilis	***Klebsiella pneumoniae***	***Pseudomonas vesicularis***	***Pseudomonas aeruginosa***
Colony morphology	Large, transparent, circular, raised, entire moist colony	Small, non-pigmented irregular lobate, raised moist colonies	Small, pigmented, circular, flat, entire dry colonies	Small, pigmented, circular, flat, entire dry colonies
Gram's staining	Gram negative rods	Gram negative rods	Gram negative rods	Gram negative rods
Motility	Motile	Non-motile	Active motile	Active motile
Catalase	+	+	+	+
Oxidase	–	–	–	+
Indole	–	–	–	+
Methyl red	+	–	–	–
VP test	–	+	–	–
Citrate test	+	+	–	+
Nitrate test	+	–	–	+
Starch hydrolysis	–	–	–	–
TSI	Alkaline slant, acid butt, H_2S positive, No gas production	Acid butt, alkaline slant, No gas and H_2S production	Alkaline butt, acid slant, No gas and H_2S production	Alkaline butt, acid slant, No gas and H_2S production

The isolates of the present study (*Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas vesicularis* and *Pseudomonas aeruginosae*) were tested for their antibiotic resistance against ceffriaxone, nalidixic acid, amoxiclav, methicillin, chloramphenicol and streptomycin (**Table 3**). All of the isolates are sensitive to ceffriaxone and nalidixic acid and are resistant to amoxiclav, methicillin, chloramphenicol and streptomycin. That is, their presence in the water sachet pose a danger for those who are taking. The consuming public also must be informed of the consequences of consuming packaged water. Even though the water sachets are UV-treated and ozonized, it could be also recommended that produced packaged water should endeavor to disinfect their products with solar radiation, which is simple to construct and easy to maintain.

Proteus mirabilis, *Klebsiella pneumoniae*, *Pseudomonas vesicularis* and *Pseudomonas aeruginosae* were tested for their heavy metal tolerance. For this study, we selected only four heavy metals namely, copper (copper sulphate), chromium (potassium dichromate), zinc (zinc sulphate) and lead (lead acetate) at a concentration of 1 mM, 3 mM and 5 mM. All of the four isolates showed growth at 1 mM and 3 mM concentration. But at 5 mM concentration, the heavy metal except lead seems to be toxic to the isolates and no growth was observed. This result indicates that these organisms are resistant to heavy metals at 1 mM and 3 mM concentration. For lead, they are showing tolerance even at 5 mM concentration (**Table 4**).

We screened three of the industrially important extra cellular enzymes (protease, amylase and cellulase) production by our test organisms. For protease enzyme, two different substrates were used as nitrogen source *i.e.*, Gelatin and casein. All the test organisms showed protease production by plate assay method. For amylase production, starch served as a sole carbon source and the *Proteus mirabilis* and *Klebsiella pneumoniae* is the producer of the sole carbon source but this was not produced by any of the organisms tested. The isolates of sachet water shows some harmful (antibiotic resistance, heavy metal resistance) and useful properties. Useful property includes industrial enzyme production. So we can exploit these organisms for the mass production of industrial enzymes like protease and amylase (**Table 5**).

Among four bacteria *Klebsiella pneumoniae* and *Proteus mirabilis* were considered as human pathogens. To know its antibacterial property, we selected four human pathogens like *Klebsiella pneumoniae*, *Proteus mirabilis*, *Micrococcus luteus* and *Salmonella paratyphium*. *Proteus mirabilis* and *Klebsiella pneumoniae* showed antibacterial activity against *Micrococcus luteus* and *Salmonella paratyphium*. *Pseudomonas vesicularis* showed no antibacterial activity against *Proteus mirabilis*, *Micrococcus luteus* and *Salmonella paratyphium* but it formed zone of clearance against *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* showed antibacterial activity against *Proteus mirabilis* and *Salmonella paratyphium* but no activity for *Klebsiella pneumoniae* and *Micrococcus luteus* (**Table 6**). The same result was observed for ethyl acetate fractions of bacterial isolates except *Proteus mirabilis* showed antibacterial activity against *Klebsiella pneumoniae* (**Table 7**).

Since *Proteus mirabilis* and *Klebsiella pneumoniae* itself was a human pathogen, it does not showed antibacterial activity against the same human pathogen brought from outside. But we could not exploit this to control *Micrococcus luteus* and *Salmonella paratyphium*. *Pseudomonas vesicularis* showed activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed activity against *Proteus mirabilis* and *Salmonella paratyphium*. We could also exploit these two isolates against

Table 3. Antibiotic resistance/sensitivity spectrum of isolates from sachet water.

Antibiotic	Concentration µg/ml	Bacterial isolates			
		Proteus mirabilis	***Klebsiella pneumoniae***	***Pseudomonas vesicularis***	***Pseudomonas aeruginosa***
Ceffriaxone	30	+	+	+	+
Chloramphenicol	30	–	–	–	–
Amoxiclav	30	–	–	–	–
Methicillin	30	–	–	–	–
Nalidixic acid	30	+	+	+	+
Streptomycin	30	–	–	–	–

+ ➔ sensitive

– ➔ resistant

Table 4. Heavy metal tolerance spectrum of isolated strains from sachet water.

Heavy metals	Concentration µg/ml	Bacterial isolates			
		Proteus mirabilis	***Klebsiella pneumoniae***	***Pseudomonas vesicularis***	***Pseudomonas aeruginosa***
Copper (copper sulphate)	1	+	+	+	+
	3	+	+	+	+
	5	+	+	–	–
Chromium (potassium chromate)	1	+	+	+	+
	3	+	+	+	+
	5	+	–	–	+
Zinc (Zinc sulphate)	1	+	+	+	+
	3	+	+	+	+
	5	+	–	–	+
Lead (Lead acetate)	1	+	+	+	+
	3	+	+	+	+
	5	+	+	+	+

+ ➔ sensitive
– ➔ resistant

Table 5. Screening for extra cellular enzyme production.

Name of the enzyme	Bacterial isolates			
	Proteus mirabilis	***Klebsiella pneumoniae***	***Pseudomonas vesicularis***	***Pseudomonas aeruginosa***
Protease (Gelatin)	+	+	+	+
Protease (Casein)	+	+	+	+
Amylase (Starch)	+	+	+	+
Cellulase (Cellulose)	–	–	–	–

+ ➔ presence of activity
– ➔ absence of activity

Table 6. Antibacterial activity of pure bacterial isolates.

Name of the isolates	Pathogens			
	Proteus mirabilis	***Klebsiella pneumoniae***	***Micrococcus luteus***	***Salmonella paratyphium***
Proteus mirabilis	–	–	+	+
Klebsiella pneumoniae	–	–	+	+
Pseudomonas vesicularis	–	–	+	+
Pseudomonas aeruginosa	+	+	+	+

+ ➔ Presence of activity
– ➔ Absence of activity

Table 7. Antibacterial activity of isolates with organic solvent (Ethyl Acetate).

Name of the isolates	Pathogens			
	Proteus mirabilis	***Klebsiella pneumoniae***	***Micrococcus luteus***	***Salmonella paratyphium***
Proteus mirabilis	–	–	+	+
Klebsiella pneumoniae	+	–	+	+
Pseudomonas vesicularis	–	–	+	+
Pseudomonas aeruginosa	–	+	+	+

+ ➔ Presence of activity

– ➔ Absence of activity

Klebsiella pneumoniae, *Proteus mirabilis* and *Salmonella paratyphium*. The same happened when the isolates when dissolved in Ethyl acetate, an organic solvent.

But the presence of pathogens in drinking water is serious health risks. Here the isolates like *Proteus mirabilis*, was a causative agent of urinary tract infection and *Klebsiella pneumoniae* was a causative of Pneumonia. The Environmental officers in the local Government employment owe it a duty to educate about the health risk in taking this kind of contaminant sachet water and to create awareness to the public when drinking water standards are violated.

6. CONCLUSIONS

People are increasingly concerned about the safety of their drinking water. As improvements in analytical methods allow detecting impurities at very low concentrations in water, water supplies once consider pure are found to have contaminants. The enforcement of the regulation guiding water quality before the national agency for Food and Drug Administration Control (NAFDAC) to comply with the drinking water qualities guidelines values as recommended by W.H.O. becomes urgent.

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REFERENCES

[1] Archimpong, E.Q. (1976) Typhoid ideal perforation: Why such Mortalities. *British Journal of Surgery*, **63**, 317-321.

[2] Ajao, O.G. (1984) Typhoid perforation: Factors influencing mortality and morbidity. *International Surgery*, **67(4)**, 317-319.

[3] Badejo, O.O. and Arigbabu, A.O. (1980) Treatment of Typhoid perforation and peritoneal irrigation and comparative study. *Gut*, **21(2)**, 141-145.

[4] Olayemi, A.B. (1999) Microbial potability of bottled and packaged drinking water hawked in Ilorin metropolis. *International journal of Environmental Health Research*, **9(3)**, 245-248.

[5] Franco, R.M.B. and Cantusio, N.R. (2000) Occurrence of cryptosporidial oocytes and Giardia cysts in Bottled mineral water commercialized in the city of Campinas, State of Sao Paulo, Brazil. *Memórias do Instituto Oswaldo Cruz*, Riode Janero, **97(2)**, 205-207.

[6] Adekunle, L.V., Sridhar, M.K.C., Ajayi, A.A., Oluwade, P.A. and Olawuyi, J.F. (2004) An assessment of the health and social economic implication of sachet water in Ibadan Nigeria. *African journal of Biomedical Research*, **7(1)**, 5-8.

[7] Robertson, L.J., Forbeeg, T. Hermansen, L. Gjerde, B.K. Alsvag, J.O. and Langeland, N. (2006) Cryptosporidium parvum infection in Bergen, Norway, during an extensive out break of water borne Giardiasis. *Applied and Environmental Microbiology*, **72(3)**, 2218-2220.

[8] Holt, J.G. (1994) Bergey's manual of determinative bacteriology. 9th Edition, Lippincott Williams and Wilkins, Baltimore.

[9] Ademoroti, C.M.A. (1996) Standard method for water and effluents analysis. Folludex Press Ltd., Ibadan, Nigeria.

[10] Aganda, O.A. (1998) Result of baseline studies on water supply & sanitation conducted by federal Ministry of water resources & rural development, Nigeria, UNICEF assisted National water supply & sanitation monitoring programme–an overview. *FGN–UNICEF WEB Review meeting*, Ibadan, 1-14.

[11] Rutz, D. (1996) Pure water vending machines may not be so pure. Food & Health, Cable News Network.

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Crithidia deanei infection in normal and dexamethasone–immunosuppressed Balb/c mice

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ABSTRACT

Monoxenous trypanossomatids protozoa are not believed to cause *in vivo* infection in vertebrate hosts throughout their life cycle. However, there are reports mentioning some cases of HIV-positive patients who have presented opportunistic infections caused by these protozoa. Recently, we have demonstrated the *in vitro* infection of mouse dermal fibroblasts by these protozoa. The aim of the present work is to investigate the possibility of *Crithidia deanei*, a endosymbiont-bearing monoxenous trypanossomatid, infect BALB/c mice under or not Dexamethasone treatment. To attend it, distinct gro- ups of adult BALB/c mice were immunosuppressed with 50 mg/kg of Dexamethasone. This immunosuppressor was administered 24 hours before infection and daily, for 15 days after *C. deanei* inoculation. Control groups: *C. deanei*–inoculated animals but non-immunosuppressed and non-inoculated animals but immunosuppressed were also used. Light Microscopy analysis revealed an infection process characterized by the presence of the trypanossomatid inside dermal cells in the groups studied. The experimental inoculation resulted in a non-lethal infection characterized by the presence of the trypanossomatid inside dermal cells in the normal BALB/c mice, but notably, in the *C. deanei*–inoculated immunosuppressed group. These preliminary results lead to the following conclusions: 1) *C. deanei* is able to infect normal BALB/c mice; 2) the immunosupressed mice seemed to be more susceptible to the *C. deanei* infection compared to the control group. Besides *C. deanei* in dexamethasone-immunosuppressed mice provides a useful model for studies of monoxenous trypanosomatids 'in vivo' infection, resembling that one presumably occurring in imunodeficient individuals with AIDS.

Keywords: Monoxenous Trypanossomatid; 'In Vivo' Infection; Immunosuppression

1. INTRODUCTION

Trypanossomatids parasitize a diverse range of hosts including animals, plants and protists [1]. Some of them, such as Trypanosoma and Leishmania, are heteroxenous and are ethiological agents of serious diseases in humans and experimental animals. Others are a monoxenous and are mostly found in insects [2]. Monoxenous trypanosomatids had never been confirmed as pathogenic in vertebrate host. However, there is one report of trypanosomatid, other than Trypanosoma and Leishmania, in some opportunistic cutaneous infections in immunocompromised individuals [3] or those without any previous history of immunodepression [4]. In addition, our group was pioneer in proving the infection of mouse dermal fibroblasts by two different monoxenous trypanosomatid species—*Crithidia deanei* and *Herpetomonas roitmani* [5]. Although some of these trypanosomatids were classified as a divergent member of the Leishmania genus [6], a visceral leishmaniasis—like infection was described in an HIV-positive patient as caused by Leptomonas pulexsimulantis, a monoxenous trypanosomatid found in dog's flea [3], suggesting that monoxenous protozoa can be considered opportunistic agents in immunocompromised individuals. Therefore,

we investigated the ability of *C. deanei* to infect vertebrate host. For that purpose, we have used BALB/c mice under or not Dexamethasone treatment as an experimenttal model, based on a previous report of mouse dermal fibroblasts infection by *C. deanei* and *H. roitmani* [5].

2. MATERIALS AND METHODS

Parasite culture. *Crithidia deanei* was kindly provided by Dr. M. Auxiliadora de Souza (Trypanosomatids Collletion of the Oswaldo Cruz Institute, Rio de Janeiro, Brasil). The monoxenous were kept at 28°C with serial passages at 48 h intervals in Warrens' medium [7] containing 10% fetal calf serum.

Experimental animal infection. Female 8-week old BALB/c mice (Nau, Instituto de Biologia /UFF) were used. Animals housed in standard conditions were treated with Dexamethasone (Azium^{e}) [8] 24 hours before infection with *C. deanei*. After infection with 10^{7} 2-day-old promastigotes *C. deanei* by subcutaneous route (hind foot pad) —day 0, dexameth-asone 50 mg/kg was administered daily, for 15 days. Four BALB/c mice group were used: control without dexamethasone; control with dexamethasone; *C. deanei*–inoculated with dexamethasone and *C. deanei*–inoculated without dexamethasone (**Table 1**). A determined number of mice from each group were euthanasiated at 6 h, 1 d, 2 d, 3 d, d 7 and d 15 after *C. deanei* inoculation. At each control point, mice were weighted and parasite burdens were determined in foot pad by histological analysis.

Histological analysis. Specimens of foot pad were fixed in 10% buffered formalin. After dehydration in graded ethanol, the tissues were embedded in paraffin and, then, processed routinely as previously reported [9]. 5 μm thick sections were obtained with a Leica microtome. After that, they were collected on glass slides for Hematoxilin-Eosin (HE) staining. The tissues samples infected or not were observed at least 400 randomly selected cells at 1000 × magnification, using a Zeiss photomicroscope.

3. RESULTS

Clinical finding's. No mortality, weight loss or clinical signs were observed in mice infected with either dexamethasone or not.

Macroscopy findings. Both groups *C.deanei*—inoculated immunosuppressed mice and not inoculated immunosuppressed mice displayed splenomegaly and heaptomegaly.

Histological analysis. Through light microscopy the morphological analysis just of the foot pad was done. At necropsy, parasites were found in the foot pad from the mice inoculated with *C. deanei*, regardless immunosuppressed or not. In the Dexamethasone treated-controls groups (in the absence of *C. deanei* inoculation), no histological and inflammatory reations of the foot pad were observed until d15 (**Figure 2(e1)**).

Surprisingly, in both experimental design-in the presence or not of dexamethasone, *C. deanei* was infective to BALB/c mice (**Figure 1** and **2**), but, notably, in the immunosupressed BALB/c mice (**Figure 2**).

Using light microscopy, it observed *C. deanei*–infected mouse dermal cells after 24 h infection (**Figures 1(a1)** and **(a2)**). On the 2nd post infection day, *C. deanei* was also observed within mice dermal cells (**Figures 1(b1)** and **(b2)**). and, between whiles, extracellular parasites were seen (**Figure 1(b1)**). A large numbers of parasites were clearly present in the dermal cells after the third post-infection day (**Figures 1(c1)** and **(c2)**). Although it was possible to observe *C. deanei* within the dermal cells, their mechanism of entrance is still not clear as it can involve phagocytosis, penetration in the cell or inducing membrane invagination. Anyway, one mechanism of the *C. deanei*–infection might be through sincicious formation from the host cells as the image of the **Figures 1(c1)** and **(c2)** suggest. After 7 days of infection it still can observe parasites present in dermal cells *C. deanei*–infected mice (**Figures 1(d1)** and **(d2)**). At this time, some extracellular parasites were still seen (**Figures 1(d1)** and **(d2)**).

After 15 days of infection, the light microscopy still revealed intracellular forms of *C. deanei* as well as some extracellular forms of this parasite attached to the dermal cells surface (**Figures 1(e1)** and **(e2)**).

In the controls groups (in the absence of *C. deanei* inoculation and presence of dexamethasone) no histologycal and inflammatory reactions of the foot pat were observed until day 15 (**Figure 2(e1)**).

Interestingly, the kinetics of infection in foot pad from *C. deanei*–inoculated Dexamethasone immunosuppressed mice showed parasites as early as 6h in the subcutaneous tissues (**Figures 2(a1)** and **(a2)**). Notably, the most exuberant *C. deanei*–infection was observed in the presence of Dexamethasone on the first day of infection (**Figures 2(b1)** and **(b2)**. In the meanwhile, it can clearly observe a *C. deanei* within a vacuole (**Figure 2(b2)**). On the following day, it can still observe a large numbers of *C. deanei* inside the cells (**Figures 2(c1)** and **(c2)**). In this time of infection, similar to the findings on the previous day, it can see that each parasite occupies its own vacuole (**Figures 2(c1)** and **(c2)**). The image of *C. deanei* inside

Table 1. Distribution of the experimental groups according to the animals number e the respective data of necropsy.

Groups	Animals number / time of necropsy					
	6 h	24 h	48 h	72 h	day 7	day 15
I (*C. deanei*–inoculated DMT treated mice)	2	2	2	2	2	2
II (*C. deanei*–inoculated mice)	2	2	2	2	2	2
III (DMT treated mice)	1	1	1	1	1	1
IV (not DMT treated and not *C. deanei* inoculated mice)–Control Groups	1	1	1	1	1	1
Total	6	6	6	6	6	6

- DMT-Dexamethasone 50 mg/kg.
- In a total 36 animals were used.
- The animals were euthanazed according to the rules of ethical comitê (Comissão de ética no uso de animais (CEUA- FIOCRUZ) P8317 ação: 1201 no. P024705).

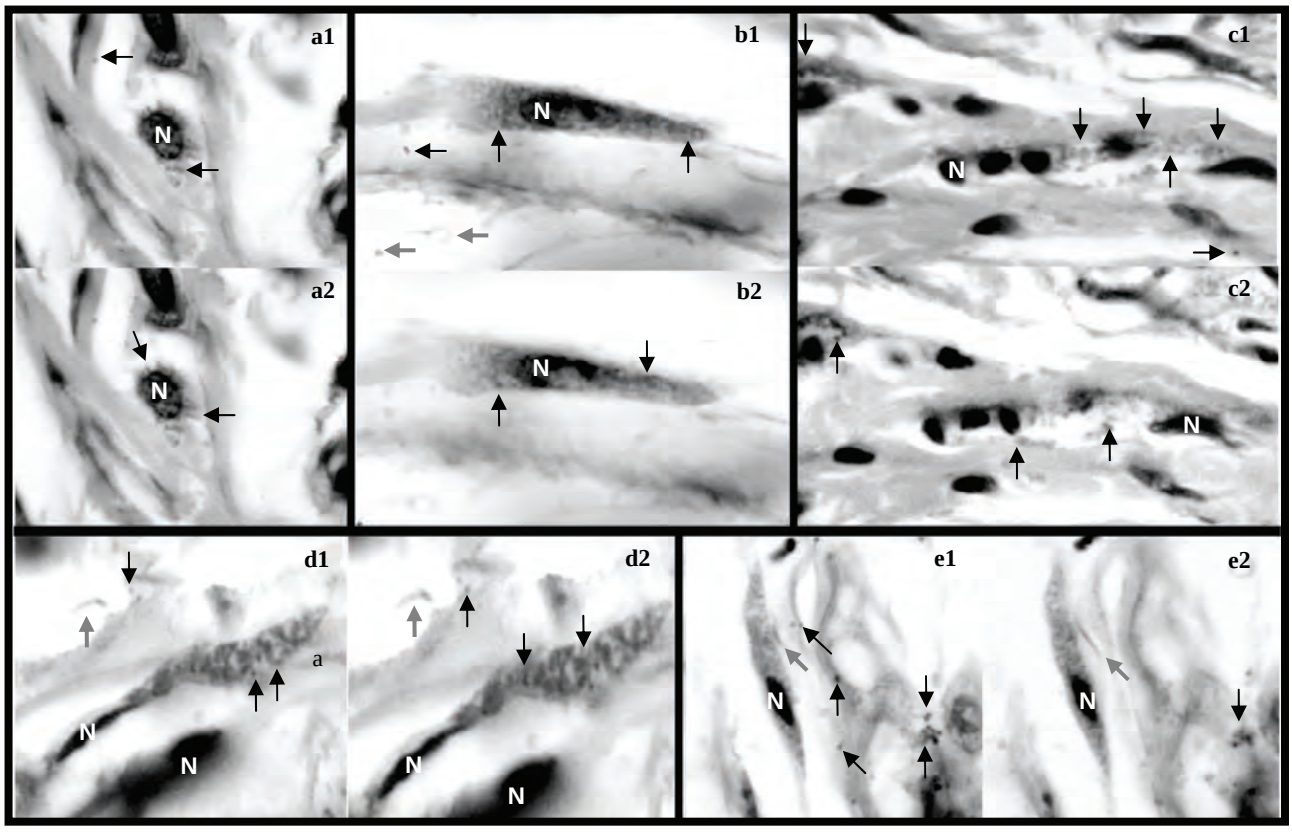

Figure 1. Analysis by light microscopy showing *C. deanei* interaction with Balb/c mouse. Pictures were taken in a two different plans from the same field (e.g. a1 and a2, etc…) in order to show whole extension of cell infection. Representative sections from skin samples of 24h (a1 and a2); 48h (b1 and b2); 72h (c1 and c2); 7 days (d1 and d2) and 15 days (e1 and e2) *C. deanei* post-infection (original magnification x 100). Grey Arrow shows some free *C. deanei* extracellular forms (b1; d1 and d2) as well as *C. deanei* extracellular forms attached to the dermal cells surface (e1 and e2). Dark arrow show multiple *C. deanei*-infected dermal cells. Note the presence of sincicious formation in c1 and c2. N = Dermal cells nucleous.

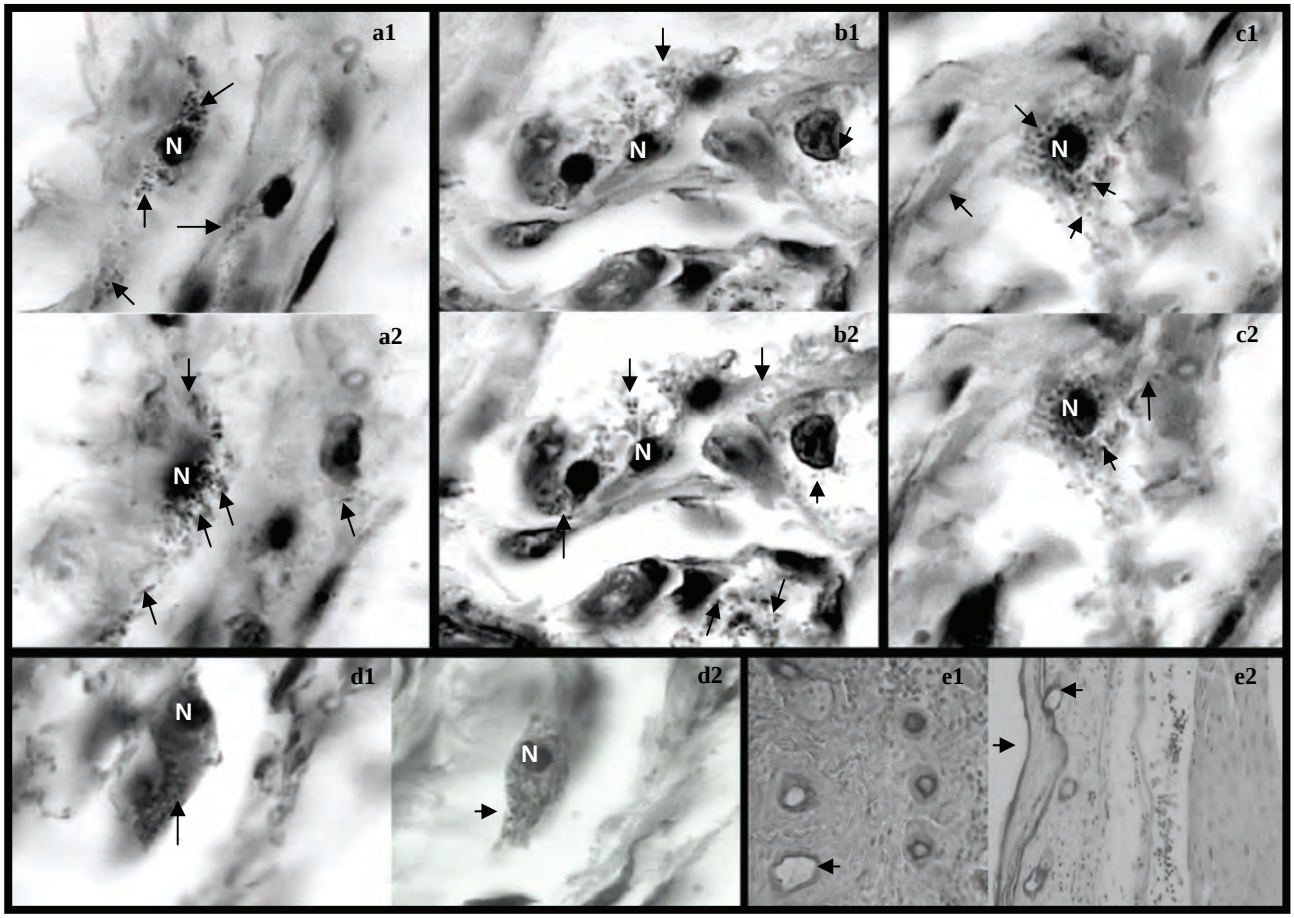

Figure 2. Analysis by light microscopy showing *C. deanei* interaction with dexamethasone immunosuppressed Balb/c mouse. Pictures were taken in a two different plans from the same field (e.g. a1 and a2, etc…) in order to show whole extension of cell infection. Representative sections from skin samples of 6h (a1 and a2); 24h (b1 and b2); 48h (c1 and c2); 72 h(d1 and d2) *C. deanei* post-infection (original magnification x 100). Dark arrow show multiple strongly *C. deanei*–infected dermal cells. Occasionaly, some parasites seemed to be clearly envolved by a vacuole (a2, b2, c1, c2 and d2). Controls groups 15 days after dexamethasone treatment or not (e1: dexamethasone-immunosuppressed Balb/c mouse; and e2: normal Balb/c mouse; original magnification (e1) × 50 and (e2) × 10). N = Dermal cell nucleous.

vacuoles continues to be seen in the third day of infection (**Figures 2(d1)** and **(d2)**). Here some tissue degradation was also observed (**Figures 2(d1)** and **(d2)**). Interestingly, at the last days of infection time (7 and 15 days) in immunosupressed BALB/c mice, no parasites were found contrasting to some tissue alterations which were observed (data not shown).

4. DISCUSSION

Several clinical cases suggesting that monoxenous trypanosomatids could be implicated in human infections have been described in the last years. They have been emerging as possible opportunistic pathogens in imunocompromised individuals. An unusual Leishmania-like parasite was found in a HIV-positive patient with symptoms of Leishmania infection [10]. Despite the previous-mentioned data, genotypic and phenotypic characterization showed that a flagellate parasite, found in the bone marrow of a Brazilian HIV-positive patient presenting a visceral leishamniasis-like reaction, was indeed a monoxenous trypanosomatid, although no tissue invasion could be detected [3]. Surprinsingly, a new case of cutaneous infection by a presumed monoxenous trypanosomatid was reported in the island of Martinique; however, the individual had no history of immunosuppression, particularly HIV infection [4].

As stated earlier, Santos *et al.* (2004) first reported that endosymbiont-bearing trypanosomatid *C. deanei* and *Herpetomonas roitmani* are able to infect mouse dermal-derived fibroblasts while *Crithidia fasciculate* and *Herpetomonas samuelpessoai* (trypanossomatid

endosimbiont free) did not infect. It is also of interest to observe that both *C. deanei* and *H. roitmani* can be resistant to lysis mediated by the complement system. In contrast, *H. samuelpessoai* and *C. fasciculate* displayed 100% of lysis after incubation with the complement system [5]. The symbionts of *C. deanei* can influence the phagocytosis of these parasites by macrophages as have been presented by [11]. And, most recently, [12], reported the infection of HIV-1-infected prim- ary human macrophages by *Blastocrithidia culicis* (another endosymbiont-bearing monoxenous trypanosomatid). Our present data further emphasize the large capacity of *C. deanei* to infect vertebrate host and reinforce the idea that monoxenous trypanosomatids present low host specifity [2,13,14]. As demonstrated by our work, *C. deanei* can readily infect normal BALB/c mice by subcutaneous route and infection persist in the dermal cells for 15 days. These are very interestingly results, since we have previously reported the "in vitro" *C. deanei*–infection of dermal cells obtained from a different specie of mouse-the Swiss mouse [5]. Besides, as observed in our present work, extracellular forms of *C. deanei* are displayed in dermal tissue of the BALB/c mice (**Figures 1(b1)**, **(d1)** and **(d2)**). This fact is interesting to be mentioned since it might suggest that, after intracellular *C. deanei* cycle, these parasites leave the host cell and, after that, appear in the extracellular medium (in a flagellate form) to re-infect others dermal cells. Taken together, these evidences reinforce the idea that monoxenous trypanosomatids are able to infect and to survive once reaching the vertebrate host. Over and again, we demonstrated the infection of BALB/c mice, but, a much more pronounced *C. deanei*–infection in a different experimental design: in Dexamethasone-immunodepressed mice (**Figure 2**). Through its limphopenic activity, specially about T cell production [15], the dexamethasone can reduce the mechanisms of anti-parasite effect of immune system and it might explain the increase of susceptibily to *C. deanei* infection observed in all immunosuppressed animals. The important survival of the parasite in the murine experimental host contrast strikingly with the weak clinical-pathological effects observed with absence of lymphocytic infiltrates in parasitized foot pad. This can be paralleled to that observed during human visceral leishmaniasis where patent infections with parasite dissemination are frequently associated with T cell unresponsiveness to Leishmania antigen [16], while cure is accompanied with restoration of the cellular response [17,18]. Although monoxenous trypanosomatids in humans are more correlated to opportunistic parasites, our work is pioneer in demonstrating that *C. deanei* is able to infect normal mice (whithout dexamethasone treatment). Our findings corroborate to the reports of [4], who also found monoxenous tripanosomatids in a non-immunocompromised individual though in a localized skin lesion. Besides, our previous report demonstrated the monoxenous trypanosomatid infection by dermal cells isolated from skin of normal Swiss mice [5]. Neverthless, our data shows that the infection of *C. deanei* by dexamethasone-treated mice, although earlier proeminent at the beginning of the time of infection (**Figures 2(a),(b)**), could not be followed longer, since the dermal cells seemed to be degenerated (data not shown). These results suggest that *C. deanei* might induce dermal cells degeneration. Most recently, [19] reported that *C. deanei* was able to induce fibroblasts lysis.

Besides the interaction of monoxenous trypanossomatids with vertebrate cells, the literature have also mentioned some results obtained from the interaction of these trypanossomatids with invertebrate cells. Then, [20,21], reported the colonization of *Aedes aegypti* midgut by the endosymbiont-bearing trypanosomatid *Blastocrithidia culicis* and *C. deanei* respectively.

Considering the colonization of hematophagous insects by monoxenous trypanosomatids and their low host specificity, human cases of infection with lower trypanosomatids could have been largely underestimated until now due to their morphological similarity with *Leishmania* species. This emphasizes the relevance of enzymatic characterization, whenever possible, of all *Leishmania*-like parasites isolated from skin or visceral lesions of patients with or not immunosuppression history. Taken together, these reports reinforce the idea of the urgent need of elucidating the epidemiology of these lower trypanosomatids that so far remains poorly known.

5. ACKNOWLEDGEMENTS

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REFERENCES

[1] Vickermank, K. (1994) The evolutionary expansion of the trypanossomatid flagellates. *International Journal for Parasitology*, **24(8)**, 1317-1331.

[2] Wallace, F. G. (1966) The trypanosomatid parasites of insects and arachnids. *Experimental Parasitology*, **18(1)**, 124-193.

[3] Pacheco, R.S., Marzochi, M. and Pires, M. (1998) Parasite genotypically related to a monoxenous trypnosoamtid of dog's flea causing opportunistic infection in HIV-positive patient. *Memórias do Instituto Oswaldo Cruz*, **93 (4)**, 531-537.

[4] Boisseau–Garsaud, A.M., Cales-Quist, D. and Desbois, N. (2000) A new case of cutaneous infection by a presumed monoxenous trypanosomatid in the island of Mar-

tinique (French WEst Indies). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94(1)**, 51-52.

[5] Santos, D.O., Bourguignon, S.C. and Castro, H. C. (2004) Infection of mouse dermal fibroblasts by the monoxenous trypanosomatid Protozoa Crithidia deanei and Her- petomonas roitmani. *Journal of Eukaryotic Microbiology*, **51**, 570-574.

[6] Noyes, P., Pratlong, F. and Chance, M. (2002) A previously unclassified trypnosomatid responsible for human cutaneous lesions in Martinique (French West Indies) is the most divergent member of the genus Leishmania ss. *Parasitology*, **124(Pt 1)**, 17-24.

[7] Warren, L.G. (1960) Metabolism of schizotrypanumcruzi Chagas. 1. Effect of culture age and substrate concentration on respiratory rate. *Journal of Parasitology*, **46**, 529-539.

[8] Lallo, M.A., Santos, M.J. and Bondam, E.F. (2002) Experimenatal encephalitozoon cuniculi infection in dexamethasone-immunosuppressed mice. *Revista de Saúde Pública*. **36(5)**, 621-626.

[9] Santos, D.O, Castro, H.C. and Bourguignon, S.C. (2007) Expression of B7-1 costimulatory molecule in patients with multibacillary-leprosy and reactional states. *Clinical and Experimental Dermatology*, **32(1)**, 75-80.

[10] Jiménez, M.L., lópez-Vélez, R. and Molina, R. (1996) HIV coinfection with a currently non-pathogenic flagellate. *Lancet*, **347(8996)**, 264-265.

[11] Rozenthal, S., De Carvalho, T.U. and De Souza, W. (1987) Influence of the endosymbiont on the interaction of Chrithidia deanei with macrophages. *Microscopy Electron Biology Cell*, **11**, 167-179.

[12] Barreto-de-Souza, V., Xavier Medeiros, T. and Machado Motta, M.C. (2008) HIV-1 infection and HIV-1 Tat protein permit the survival and replication of a non-pathogenic trypanosomatid in macrophages through TGF beta 1 production. *Microbes and Infection*, **10(6)**, 642-649.

[13] Podlipaev, S.A. (2001) The more insect trypanosomatids under study-the more diverse Trypanosomatidae appears. *International Journal for Parasitology*, **31(5-6)**, 648-652.

[14] Podlipaev, S.A., Strurm, N.R. and Fiala, I. (2004) Diversity of insect trypanossomatids assessed from the splice leader RNA and 5S rRNA genes and intergenic region. *Journal of Eukaryotic Microbiology*, **51(3)**, 283-290.

[15] Diasio, R.B. and LoBuglio, A.F. (1996) Immunomodulators: Immunosuppressive agents and immunostimulants the pharmacological basis of therapeutics. 9th Edition, Mcgraw Hill, London.

[16] Ghalib Ghalib, H.W., Piuvezam, M.R. and Skeiky, Y.A.W. (1993) Interleukin 10 production correlates with pathology in human Leishmania donovani infections. *Journal of Clinical Investigation*, **92(1)**, 324-329.

[17] Carvalho, E.M., Bacellar, O. and Brownell, C. (1994) Restoration of the IFN-g production and lymphocyte proliferation in visceral leishmaniasis. *Journal of Immunology*, **152(12)**, 5949-5956.

[18] Mary, C., Lamouroux, D. and Dunan, S. (1992) Western blot analysis of antibodies to Leishmania infantum antigens: potencial of the 14-KD and 16–KD antigens for diagnosis and epidemiologic purposes. *American Journal of Tropical Medicine and Hygiene*, **47(6)**, 764-771.

[19] Matteoli, F.P., D'Avila-Levy, C.M. and Santos, L.O. (2009) Roles of the endosymbiont and leishmanolysin-like molecules expressed by Crithidia deanei in the interaction with mammalian fibroblasts. *Experimental Parasitology*, **121(3)**, 246-253.

[20] Corrêa-da Silva, M., Fampa, P. and Lessa, L.P. (2006) Colonizatin of Aedes aegypti midgut by the endosymbiont-bearing trypanosomatid Blastocrithidia culicis. *Parasitology Research*, **99(4)**, 384-391.

[21] D'Avila-Levy, C.M., Santos, L.O., Marinho, F.A. and Matteoli, F.P. (2008) *Crithidia deanei*: Influence of parasite gp63 homologue on the interaction of endosymbiont–harbouring and aposymbiotic strains with *Aedes aegypty* midgut. *Experimental Parasitology*, **118(3)**, 345-353.

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Inhibition of H_2O_2-induced DNA damage in single cell gel electrophoresis assay (comet assay) by castasterone isolated from leaves of centella asiatica

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ABSTRACT

Brassinosteroids (BRs) are a large group of polyhydroxy steroids, which regulate numerous aspects of plant growth and development, including stem elongation, leaf bending, tracheary element differentiation, stress protection and photomorphogenesis. Recent studies indicate antigenotoxic and anticancerous activities of these compounds. The role of natural BRs in H_2O_2 (hydrogen peroxide) -induced DNA damage in human lymphocytes is still unknown. The present study reports the presence of Castasterone from leaves of *Centella asiatica*, an important medicinal herb commonly used as a memory enhancer and immunomodulator. CA50 fraction isolated from *Centella asiatica* was characterized as Castasterone by electrospray ionization mass spectral data with standard Castasterone. An attempt has been made to study antigenotoxic activity of the isolated Castasterone against H_2O_2 -induced DNA damage in human blood lymphocytes using Single cell gel electrophoresis assay (Comet Assay). Castasterone at 10^{-9} M concentration proved to be effective in diminishing the DNA damage by 89.42 %.

Keywords: Brassinosteroids; Castasterone; Comet Assay; Hydrogen Peroxide

1. INTRODUCTION

In living system, oxidative stress results in the production of reactive oxygen species (ROS) like superoxide radical (O_2^-), hydroxyl radical (HO^-) and hydrogen peroxide (H_2O_2). H_2O_2 in Fenton reaction is spontaneously converted to the highly reactive hydroxyl radicals ($HO^{\bullet-}$). These hydroxyl radicals oxidize proteins, lipids and nucleic acids leading to even mutations at the cellular level [1]. Several plant hormones are implicated in modulating the response to oxidative stress like ethylene [2], abscisic acid [3], auxins and plant steroids [4]. Brassinosteroids are a group of naturally occurring plant hormones, which are structurally similar to animal steroid hormones. They influence diverse physiological processes by regulating the expression of genes like their animal counterparts [5]. Recent studies indicate antiviral activeties of BRs against various viruses, like herpes simplex virus type I (HSVI), arena virus, measles virus and vesicular stomatitis virus [6-8]. The treatment of BRs to these viruses was 10-18 folds more effective than ribavirin towards HSV-I and arenavirus. It has further been reported that 24-epibrassinolide can increase the mitochondrial membrane potential, reduce intercellular antibody levels, increase the proportion of cells in G_0/G_1 phase, reduce the population of cells in s-phase and increase the population of viable hybridoma mouse cells at subnanomolar concentrations [9]. Anticancerous activeties of 28-homocastasterone and 24-epibrassinolide were studied in several normal and cancer cell lines. The anticancer and antiproliferative activities have been documented very recently [10]. The BRs used showed high cytotoxic activity in breast (MCF-7/MDA-MB-468) and prostate cancer cell lines (LNCaP/DU-145) [11].

Centella asiatica (L.) (Family Apiaceae) commonly known as urban herb regarded as rasayana or rejuvenating herb reputed to increase intelligence and memory in Ayurvedic medicines. The methanol extracts of whole plants of *Centella asiatica* showed a significant increase in the phagocytic index and total WBC count thereby showing immunomodulatory activity. [12] isolated a

water soluble arabinogalactan, HBN and traced remarkable immunoenhancing activities on T-and B-lymphocytes *in vitro* and *vivo* tests. The antioxidative properties of *Centella asiatica* were evaluated by [13,14]. The *C. asiatica* extract has a chemopreventive effect [15]. [16] studied the healing effects of *C. asiatica* when orally administered to rats with acetic acid induced gastric ulcers, It reduced the size of ulcers in dose-dependent manner. Chemical studies reveal that triterpene saponins Asiaticoside and Madecassoside are the main active constituents of *Centella asiatica*. The other saponins and triterpene acids present in this plant are brahmoside, brahmnoside, brahmic acid, isobrahmic acid, betulic acid, centelloside and cetillic etc. The presence and role of brassinosteroids in this plant is yet to be studied. The present study was therefore planned to study the presence of BRs and inhibition of H_2O_2-induced DNA damage by Castasterone isolated from *Centella asiatica* which is the first report in this direction.

2. MATERIALS AND METHODS

2.1. Extraction and Purification of Brassinosteroids

Study material for the present investigation included leaves of *Centella asiatica* procured from Dehradun (M/s Gautam globals, Dehradun, India). Fresh leaves of *Centella asiatica* (2 kg) were homogenized and percolated with 80% methanol (3 × 1000 ml). The combined methanol extract was dried under vacuum using rotary evaporator (Strike 202, Stereoglass, Italy). 80% methanol extract (449.6 g) was partitioned between chloroform and water. Chloroform extract was then partitioned between 80% methanol and hexane. The resulting 80% methanol extract (28.3 g) was partitioned between ethyl acetate and distilled water. The ethyl acetate fraction (20 g) was dried and subjected to silica gel (60-120 mesh) column chromatography with step-gradient elution from 0, 1, 2, 3, 4, 5, 6, 7, 10, 15, 20, 50, 100% (each 500-1000 ml). All the fractions were subjected to radish hypocotyl bioassay with the aim to find the bioactive fraction. Four fractions CA5, CA10, CA50 and CA60 were found to be active (**Figure 1**). CA50 fraction was directly subjected to ESI-MS and MS/MS analysis (**Figures 2(a), 3(a)**).

2.2. Radish Hypocotyl Bioassay

The bioactivity of isolated fractions was determined using intact plants of *Raphanus sativus* as described by [17]. 5 days-old seedlings were placed into the test solutions (0.03 ml of fraction diluted with distilled water to get the final volume 3 ml). 3 ml solution was poured in each petriplate containing radish seedlings and kept in the dark for 24 h at 25 ± 2˚C. After 24 h, the length of hypocotyls were measured and compared to control. Percent increase over control was calculated.

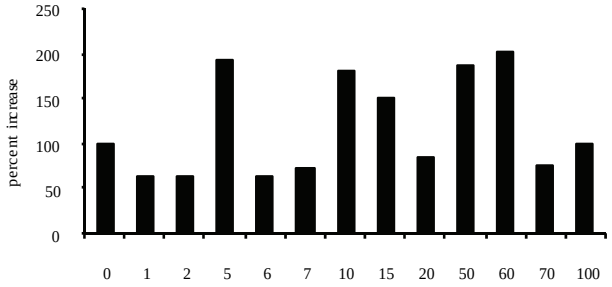


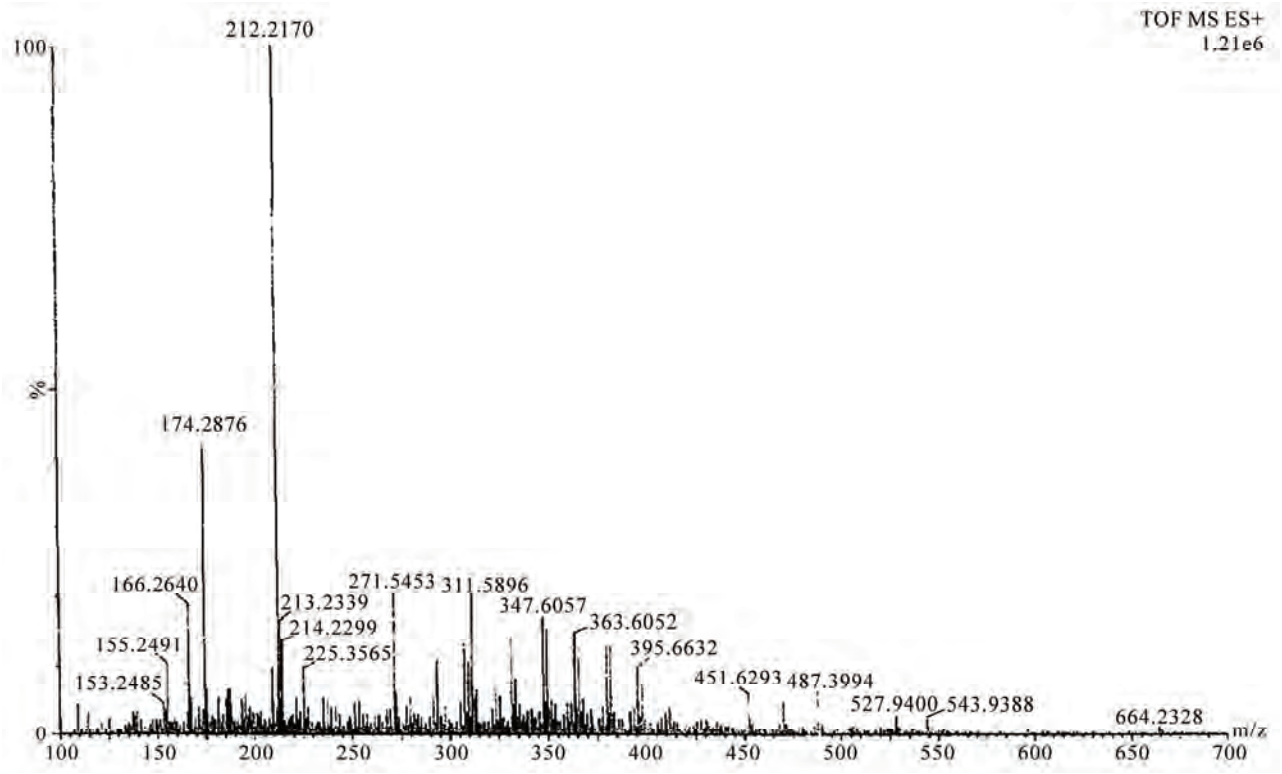
Figure 1. Biological activity of fractions of *Centella asiatica* in Radish hypocotyls bioassay after silica gel column chromatography.

2.3. Electrospray Ionization Mass Spectrometry of CA 50 Fraction

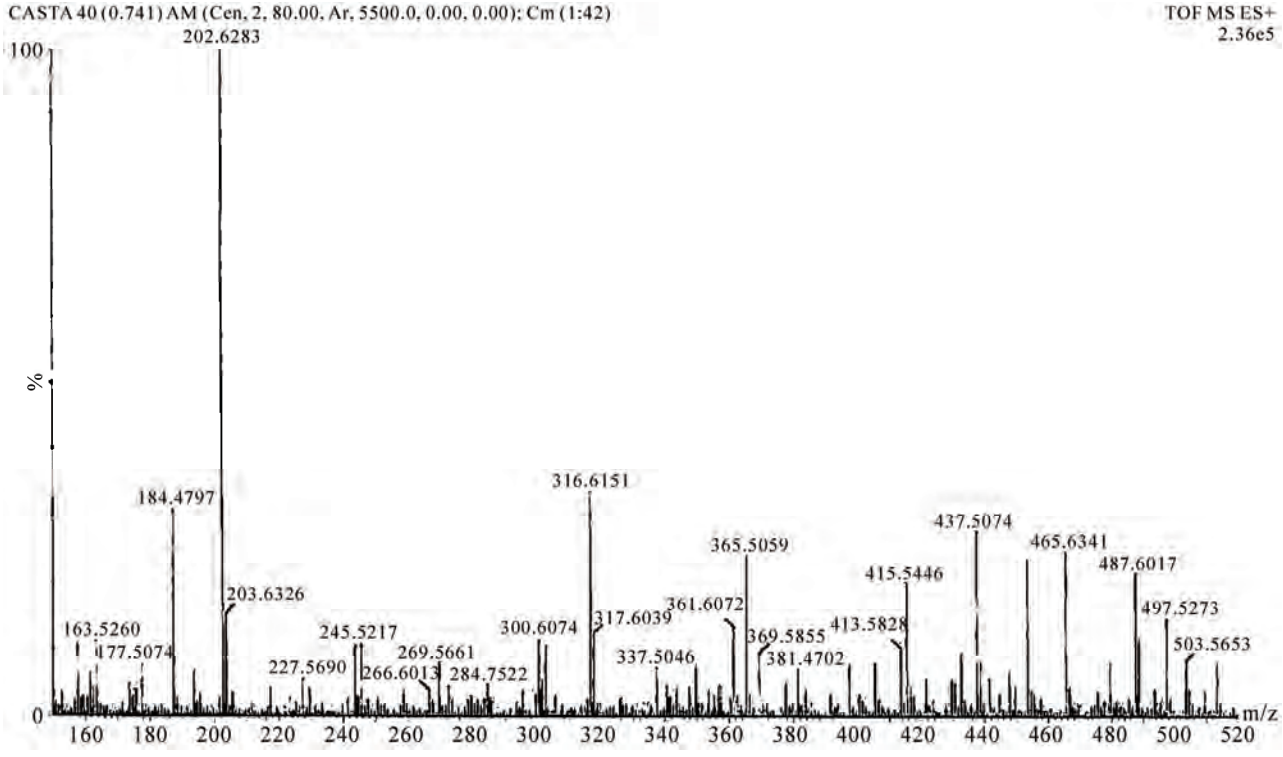
ESI-MS analysis of CA50 and standard Castasterone was carried out by the addition of 10 µl of concentrated aqueous formic acid solution to the sample mixture to a total volume of 1000 µl making 0.1% as final concentration. ESI-QTOF-MS was performed in positive ionization mode in QTOF Mass Spectrometer (Micromass, Manchester, UK). The general conditions were: Source temperature of 280˚C, capillary voltage of 2.1 kV and cone voltage of 23 V. ESI-MS was performed by direct infusion with a flow rate of 10 µl/min using a syringe pump and mass spectra were acquired and accumulated over 60 s. MassLynx 4.0 (Waters, Manchester, UK) was used for data analysis. Tandem mass spectrometry of single molecular ion in the mass spectra was performed by mass-selecting the ion of interest, which was in turn submitted to 15-35 eV collisions with argon in the collision quadrupole.

2.4. Comet Assay

DNA damage was determined by alkaline single cell microgel electrophoresis (comet assay) assay following the method proposed by [18] with minor modifications as suggested by [19]. Heparinized blood samples were obtained by venipuncture from a non-smoking, healthy male donor aged 30-40 years. Lymphocytes were isolated by the method of [20] and mixed with equal volume of Phosphate Buffer Saline (PBS) pH 7.2. This mixture was then overlayed to double volume of Histopaque 1077 and centrifuged at 1500 rpm for 20 minutes. The layer containing lymphocytes was aspirated very carefully with the help of pasture pipette. The lymphocytes were diluted in PBS and centrifuged at 2000 rpm for 15 min. The supernatant was discarded and pellet was again suspended in PBS and centrifuged at 2000 rpm

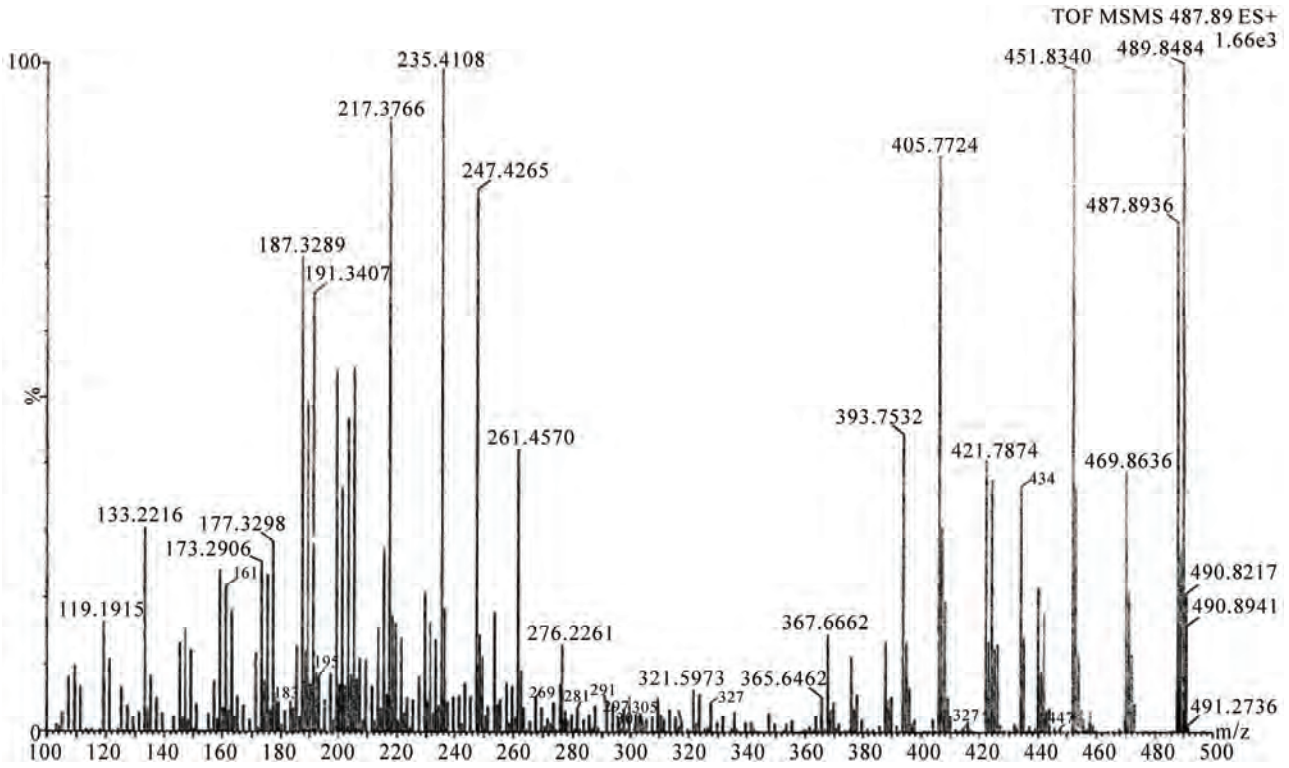


(a)

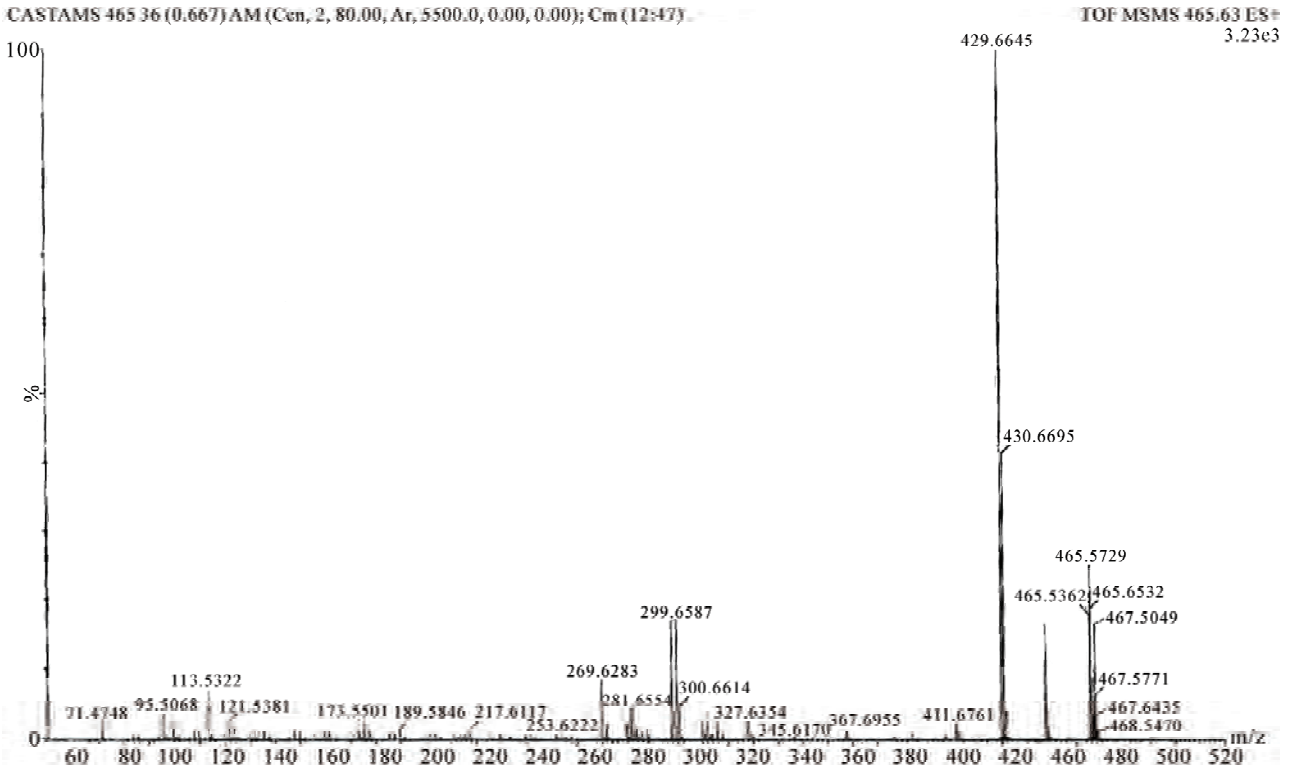


(b)

Figure 2. (a) ESI-QTOF-MS analysis of Castasterone fraction (CA50) isolated from *Centella asiatica*; (b) ESI-QTOF-MS of Standard Castasterone.



(a)



(b)

Figure 3. (a) ESI-QTOF-MSMS analysis of Castasterone fraction (CA50) isolated from *Centella asiatica*; (b) ESI-QTOF-MSMS analysis of Standard Castasterone.

Human blood lymphocytes suspended in 1 ml PBS, were incubated in a shaking water-bath for 30 minutes at 37˚C with 5×10^{-5} M H_2O_2 in the presence of different concentrations of CA50 fraction tested in duplicate. To evaluate the extent of DNA damage, 100 randomly selected cells were analysed from each sample by Nikon Epifluorescent Microscope (Nikon Eclipse E200) connected to a digital camera. Imaging was performed by using a computerized image analysis system (Lucia Comet Assay Software 4.8 of Laboratory Imaging Ltd.) which acquires images, computes the integrated intensity profile for each cell, estimates the comet cell components (head and tail) and evaluates a range of derived parameters. These include % Head DNA, % Tail DNA and tail moment (an index of DNA damage that considers both the tail length and fraction of DNA in comet tail).

The Antigenotoxic activity of CA50 was expressed by percent decrease of Tail moment:

Inhibition (%) = a – b/a – c × 100

a = Tail moment induced by H_2O_2 (positive control).

b = Tail moment of the fraction in the presence of H_2O_2.

c = Tail moment of the negative control.

3. STATISTICAL ANALYSIS

The results were obtained as the mean and standard error of three experiments. The data in all the experiments were analyzed for statistical significance using analysis of variance (one-way ANOVA). The difference among average values was compared by high-range statistical domain (HSD) using Tukey's Test [21].

3.1. Results and Discussion

CA50 fractions was isolated and characterized as castasterone by ESI-QTOF-MS/MS analysis. Electrospray ionization mass spectroscopy of standard castasterone and CA50 fraction (**Figures 2(a)** and **2(b)**) showed the pseudomolecular mass ion peaks at *m/z* 465 $[M + H]^+$ and 487 $[M + Na]^+$ corresponded to the molecular weight 464 $[M]^+$ and molecular formula as $C_{28}H_{48}O_5$. ESI-QTOF-MS/MS analysis of *m/z* 465/487 revealed similar kind of fragmentations for CA50/ standard castasterone (**Figures 3(a)** and **3(b)**). The fragments at *m/z* 447/469,429/451 were observed due to the sequential loss of two H_2O molecules. The fragments at *m/z* 393/415 and 305/327 were observed due to the C_{23}-C_{24} and C_{17}-C_{20} bond fissions. Other fragments were detected at *m/z* 411/433,297/319,281/303, 269/291,173/195 and 161/183. **Figure 4** shows the important fragmentation pattern of castasterone. We report for the first time, the presence of Castasterone in C*entella asiatica*. The

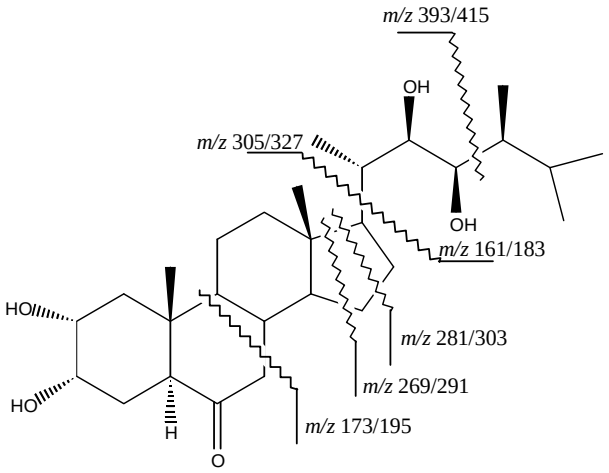


Figure 4. Structure of Castasterone and major mass fragmentations.

presence of brassinosteroids in medicinal plant like *Centella asiatica* suggests a possible medicinal application of these compounds. Brassinosteroids are found in gymnosperms, monocotyledonous and dicotyledonous plants, and in algae. Studies [22-24] confirmed that BRs are obligatory plant constituents, the highest concentration being found in the reproductive organisms and in growing tissues (pollen, immature seeds and shoots).

H_2O_2-induced DNA damage was studied using various parameters. These parameters were measured using Lucia Comet Assay Software. Percent inhibition was calculated on the basis of comet evaluation *i.e.* Tail moment. The observations made on various parameters of DNA indicated that 10^{-9}, 10^{-10} and 10^{-11} M concentrations of Castasterone were not toxic as no significant change was noticed when compared with negative control *i.e.* distilled water.

Percent head DNA in the treatments of Castasterone revealed no significant change when compared with negative control *i.e.* only distilled water. The H_2O_2 treatment however reduced %Head DNA. But supplementation of Castasterone with H_2O_2 to lymphocytes revealed an observable enhancement in % Head DNA. It increases from positive control (83.08%) to (93.66%) at 10^{-9} M concentration (**Table 1**). Similar observations were made for % Tail DNA and Tail moment. BRs treatments showed amelioration of toxicity. % Tail DNA decreased significantly with the increase in concentration of castasterone. A significant decrease in Tail moment was observed with the increase in the concentration of castasterone (**Figure 5**). It varied from 1.35 (10^{-11}M), 1.02 (10^{-10} M), 0.48 (10^{-9} M). Percent inhibition was maximum in 10^{-9} M concentration *i.e.* 89.42% (**Table 1**). The reports obtained on the toxicity of BRs suggested that they do not have negative influences in mammals,

Table 1. Inhibition of H_2O_2-induced DNA damage in Human blood lymphocytes by CA50 fraction isolated from *Centella asiatica* using Comet assay.

Treatment	Dose concentration	%Head DNA (Mean ± SE)	% Tail DNA (Mean ± SE)	Tail moment (Mean ± SE)	% Inhibition
Negative control	D.W	96 ± 0.32	4.0 ± 0.32	0.30 ± 0.02	
	10^{-11}M	92.28 ± 2.06	7.72 ± 0.40	0.24 ± 0.03	
Castasterone	10^{-10}M	96.09 ± 0.41	3.91 ± 0.41	0.26 ± 0.03	
	10^{-9}M	95.82 ± 0.56	4.22 ± 0.58	0.26 ± 0.03	
Positive control	H_2O_2 (50 µM)	83.02 ± 0.72	16.98 ± 0.72	2.34 ± 0.13	
	10^{-11}M	88.68 ± 1.06*	11.32 ± 1.06*	1.35 ± 0.13*	47.14%
Castasterone + H_2O_2 (50µM)	10^{-10}M	91.69 ± 0.8*	8.28 ± 0.81*	1.02 ± 0.11*	63.46%
	10^{-9}M	93.66 ± 0.98*	6.34 ± 0.98*	0.48 ± 0.07*	89.42%

*indicates significant values at $p \leq 0.05$.

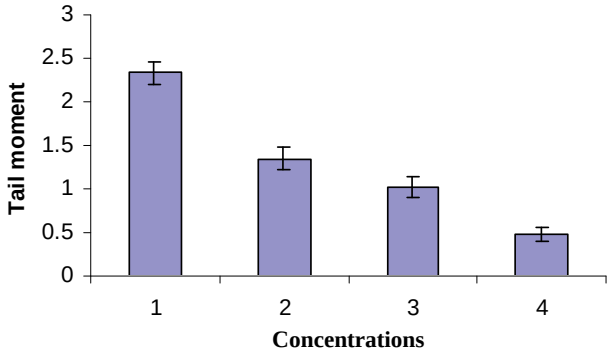
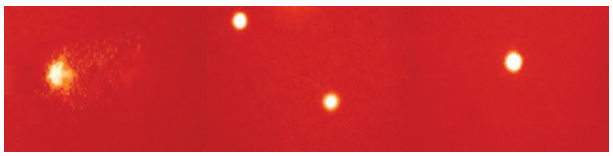


Figure 5. Effect of Castasterone isolated from *Centella asiatica* on the genotoxicity induced by Hydrogen peroxide (5×10^{-5} M) in human lymphocytes using comet assay. 1 = treatment with H_2O_2. 2-4 = treatment with different concentrations of Castasterone.



(a) (b) (c)

Digital images illustrating the inhibition of DNA damage by Castasterone in the Comet assay (a) +ve control *i.e.* H_2O_2 (b) –ve control *i.e.* Castasterone only (c) Castasterone + H_2O_2.

water organisms, soil microbiological processes and plants [25]. Mutagenic studies carried out at the Scientific Research Center of Toxicological and Hygienic Regulation of Biopreparations of Russia showed that Ames test, with or without metabolic activitation, was negative with the tester strains of *Salmonella typhimurium* TA1534, TA1537, TA1950, TA98 and TA100 [25]. Antigenotoxic properties of EBL isolated from *A.marmelos* had also been studied by [26].

Reactive oxygen species can damage the normal cellular functions and can cause atherosclerosis in vessels or malignant growth in other tissues and ageing processes [27]. The lymphocytes when treated with H_2O_2 showed the significant DNA damage. However this damage was ameliorated significantly by the simultaneous application of different concentrations of this BR. The H_2O_2 stress protective properties of BRs in human lymphocytes are the first such study carried out with plant steroids. In the present study, the protective effect observed against the ROS may in part be responsible to the anticancer activity of brassinosteroids reported by some workers [11,28]. In the studies carried out by [29] three types of 5α-androstane and ergostane analogues of brassinolide, containing a fluorine atom in either the 3α or the 5α positions or in 3α or the 5α positions, were prepared using standard operations. The 5α fluorine was found to effect chemical reactivity as well as physical properties of the products. Cytotoxicity of the products was studied using human normal and cancer cell lines with 28-homocastasterone as positive control and their brassinolide type activity was established using the bean second-internode test with 24-epibrassinolide as standard. The equivalence of F and OH groups was observed in some of the active compounds. Ergostane derivatives were most active in the anticancer activity while androstane derivatives were active in brassinolide type activity. Brassinolide was found to induce a time and concentration dependent cytotoxicity in androgen–independent human prostate cancer in PC-3 cells. The mode of cell death appeared to be predominately apoptosis. Western blot studies indicated that treatment with brassinolide triggered a time dependent decrease in the expression of

antiapoptotic protein Bcl-2. 24-epibrassinolide and 28-homoCS were found to inhibit the growth, at micromolar concentrations, of several human cancer cell lines without affecting the growth of normal cells [10]. Studies carried out by Malikova *et al*. (2008) indicate that BRs may prove to be promising leads for the development of new generation of anticancer drugs. Varoius animal steroids have been found to exhibit antioxidant properties [30]. BRs have also been reported to regulate antioxidative defence system of plants under stress conditions [31]. The reduction in DNA damage indicates amelioration of oxidative stress generation by H_2O_2 in lymphocytes. The tissues are protected from oxidative damage by variety of mechanism including antioxidants and antioxidative enzymes, repair enzymes and growth regulators. Further studies are needed to understand the mechanism of protective effect of these steroids in animal system which opens a field of study on possible medical applications of these plant steroids.

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REFERENCES

[1] Halliwell, B. and Gutteridge, J.M. (2007) Free Radicals in Biology and Medicine. 4th Edition, Oxford University Press, USA.

[2] Vahala, J., Keinanen, M., Schutzendubel, A., Polle, A. and Kangasjarvi, J. (2003) Differential effects of elevated ozone on two hybrid aspen genotypes predisposed to chronic ozone fumigation. Role of ethylene and salicylic acid. *Plant Physiology*, **132(1)**, 196-205.

[3] Kovtun, Y., Chiu, W.L., Tena, G. and Sheen, J. (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proceedings of National Academy of Sciences, USA*, **97(6)**, 2940-2945.

[4] Cao, S., Xu, Q., Cao, Y., Qian, K., An, K., Zhu, Y., Binzeng, H., Zhao, H. and Kuai, B. (2005) Loss-of-function mutation in DET2 gene lead to an enhanced resistance to oxidative stress in Arabidopsis. *Physiologia Plantarum*, **123(1)**, 57-66.

[5] Sasse, J.M. (2003) Physiological actions of brassinosteroids: An update. *Journal of Plant Growth Regulation*, **22 (4)**, 276-288.

[6] Wachsman, M.B., Ramirez, J.A., Galagovsky, L.R. and Coto, C.E. (2002) Antiviral activity of brassinosteroids derivatives against measles virus in cell cultures. *Antiviral Chemistry and Chemotherapy*, **13(1)**, 61-66.

[7] Wachsman, M.B., Castilla, V., Talarico, L.B., Ramirez, J.A., Galagovsky, L.R. and Coto, C.E. (2004) Antiherpatic mode of action of (22S, 23S)-3beta-Bromo-5alpha, 22, 23-trihydroxystigmastan-6-one in vitro. *International Journal of Antimicrobial Agents*, **23(5)**, 524-526.

[8] Romanutti, C., Castilla, V., Coto, C.E. and Wachsman, M.B. (2007) Antiviral effect of a synthetic brassinosteroid on the replication of vesicular stomatitis virus in Vero cells. *International Journal of Antimicrobial Agents*, **29 (3)**, 311-316.

[9] Franek, F., Eckschlager, T. and Kohout, L. (2003) 24-epibrassinolide at subnanomolar concentrations modulates growth and production characteristics of a mouse hybridoma. *Collection of Czechoslovak Chemical Communications*, **68(11)**, 2190-2200.

[10] Malıkova, J., Swaczynova, J., Kolar, Z. and Strnad, M. (2008) Anticancer and antiproliferative activity of natural brassinosteroids. *Phytochemistry*, **69(2)**, 418-426.

[11] Swaczynova, J., Malikova, J., Hoffmannova, L., Kohout, L. and Strnad, M. (2006) Anticancer properties of brassinosteroids. *Planta Medica*, 72.

[12] Wang, X.S., Zheng, Y., Zuo, J. and Fang, J. (2005) Structural features of an immunoactive acidic arabinogalactan from Centalla asiatica. *Carbohydrate Polymers*, **59(3)**, 281-288.

[13] Zainol, M.K., Abd-Hamid, A., Yusof, S. and Muse, R. (2003) Antioxidant activity and total phenolic compounds of leaf, root and petiole of four accessions of Centella asiatica (L) Urban. *Food Chemistry*, **81(4)**, 575-581.

[14] Gnanapragasam, A., Ebenezar, K.K., Satish, V., govindaraju, P. and Devaki, T. (2004) Protective effects of Centella asiatica on antioxidant tissue defence system against adriamycin induced cardiomyopathy in rats. *Life Sciences*, **76(5)**, 585-597.

[15] Bunpo, P., Kataoka, K. and Arimochi, H. (2004) Inhibitory effect of Centella asiatica on azoxymethane-induced aberrant crypt focus formation and carcinogenesis in the intestines of F344 rats. *Food and Chemical Toxicology*, **42(12)**, 1987-1997.

[16] Cheng, C.L., Guo, J.S., Luk, J. and Koo, M.W.L. (2004) The healing effets of *Centella asiatica* extrats and asiaticoside on acetic acid induced gastric ulcers in rats. *Life Sciences*, **74(18)**, 2237-2249.

[17] Takatsuto, S., Yazawa, N., Ikekawa, N., Takematsu, T., Takeuchi, Y. and Koguchi, M. (1983) Structural-activity relationship of Brassinosteroids. *Phytochemistry*, **22(11)**, 2437-2441.

[18] Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, **175(1)**, 184-191.

[19] Anderson, D., Dobrzynska, M.M., Basaran, N., Basaran, A. and Yu, T.W. (1998) Flavonoids modulate Comet assay response to food mutagens in human lymphocytes and sperm. *Mutation Research*, **402(1-2)**, 269-277.

[20] Boyum, A. (1968) Separation of leucocytes from blood and bone marrow. *Scandinavian Journal of Clinical and Laboratory Investigation*, **21(Suppl 97)**, 78-89

[21] Mayers, L.S. and Grossen, N.E. (1974) Analysis of independent group designs. In. Meyers, L.S. and Grossen, N.E. Eds., *Behavioural Research, Theory, Procedure and*

Design, W.H. Freeman, San Francisco.

[22] Fujioka, S., Noguchi, T., Yokota, T., Takatsuto, S. and Yoshida, S. (1998) Brassinosteroids in Arabidopsis thaliana. *Phytochemistry*, **48(4)**, 595-599

[23] Schmidt, J., Porzel, A. and Adam, G. (1998) Brassinosteroids and a pregnane glucoside from Daucus carota. *Phytochemical Analysis*, **9(1)**, 14-20.

[24] Yokota, T., Higuchi, K., Takahashi, N., Kamuro, Y., Watanabe, T. and Takatsuto, S. (1998) Identification of brassinosteroids with epimerized substituents and/or the 23-Oxo group in pollen and anthers of Japanese Cedar. *Bioscience Biotechnology and Bichemistry*, **62(3)**, 526-531

[25] Onatskiy, N.M., Marchenko, A.I. and Mikhina, L.V. (1997) Technical Report: Evaluation of mutagenic activeity of epibrassinolide (active ingredient of Epin) in Ames test, chromosome aberrations and in micronuclear tests. Scientific Research Centre of Toxicologie and Hygienic Regulation of Biopreparations of Russia, Serpukhov.

[26] Sondhi, N.P., Bhardwaj, R., Kaur, S., Kumar, N. and Singh, B. (2008) Isolation of 24-epibrassinolide from leaves of Aegle marmelos and evaluation of its anti-genotoxicity employing Allium cepa chromosomal aberration assay. *Plant Growth Regulation*, **54(3)**, 217-224.

[27] Merzenich, U.G., Zeitler, H., Vetter, H. and Kraft, K. (2008) Synergy research: Vitamins and secondary plant components in the maintenance of the redox homeostasis and cell signaling. *Phytomedicine*, **16(1)**, 2-16.

[28] Wu, Y.D. and Lou, Y.J. (2007) Brassinolide, a plant sterol from pollen of Brassica napus L., induces apoptosis in human prostrate cancer PC-3 cells. *Die Pharmazie*, **62(5)**, 392-395.

[29] Slavikova, B., Kohout, L., Budesinsky, M., Swaczynova, J. and Kasal, A. (2008) Brassinosteroids: Synthesis and activity of some fluoro analogues. *Journal of Medicinal Chemistry*, **51(13)**, 3979-3984

[30] Mooradian, A.D. (1993) Antioxidant properties of steroids. *Journal of steroid Biochemistry and Molecular Biology*, **45(6)**, 509-511.

[31] Krishna, P. (2003) Brassinosteroid-mediated stress responses. *Journal of Plant Growth Regulation*, **22(4)**, 289-297.

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Estimates of energy expenditure using the RT3 accelerometer in patients with systemic lupus erythematosus

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ABSTRACT

This study aimed to characterize energy expenditure patterns using the tri-axial accelerometer and to identify the association of energy expenditure with clinical parameters in patients with systemic lupus erythematosus (SLE). Estimates of energy expenditures represented by total activity calorie (TA), physical activity calorie (PA), total activity calorie per body weight (TABW), and physical activity calorie per body weight (PABW) of 49 female SLE patients were assessed using the RT3 tri-axial accelerometer (StayHealthy, Monrovia, CA) in a seven-day period. SLE patients in the highest body mass index (BMI) tertile showed significantly lower values of TABW compared to those in the lowest tertile, while SLE patients in the lowest TABW tertile showed significantly higher body weight, waist circumference, BMI, SLE disease activity index (SLEDAI), dosage of prednisone, and blood pressure. There was a high prevalence of metabolic syndrome and SLE patients with metabolic syndrome showed significantly lower TABW. In addition, both TABW and PABW significantly but negatively correlated with SLEDAI. In conclusion, the RT3 accelerometer is suitable for evaluating total and physical activity-related energy expenditure in patients with SLE. TABW measured by the tri-axial accelerometer is inversely related with body weight status and disease activity in SLE patients. This suggests that estimates of energy expenditure by the tri-axial accelerometer may be applied in the management of SLE.

Keywords: Accelerometer; Body mass index; Energy Expenditure; Systemic Lupus Erythematosus

1. INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized not only by the activation of T and polyclonal B cells but also by a wide variety of immunologic abnormalities [1]. Previous studies demonstrate that SLE patients present with limitations in exercise capacity and reduced quality of life due to various clinical complaints [2]. The exercise intolerance of SLE patients is associated with a reduced aerobic capacity of peripheral muscles [3], such that SLE patients are less aerobically fit, with reduced exercise capacity, reduced muscle strength, more fatigue, and greater disability compared to sedentary controls [4].

The incidence of SLE is higher in females than in males. There is strong evidence on the relationship between physical activity and the primary prevention of chronic health problems in women [5]. Estimates of energy expenditure provided a better indicator of physical activity. However, methods to assess energy expenditure have strengths and limitations [6].

The RT3 accelerometer, an integrated tri-axial accelerometer giving output measures in medio-lateral, anterior-posterior, and vertical dimensions, is a valid tool for assessing physical activity [7-9]. Although its reliability, validation, and application in measuring physical activeity in different population have been reported [10-15], the relationship between disease activity and energy expenditure measured by the RT3 accelerometer in SLE patients has not been previously established.

This study aimed to characterize energy expenditure patterns using the RT3 accelerometer and identify any relationship between energy expenditure and clinical parameters in SLE patients.

2. MATERIALS AND METHODS

Forty-nine (49) Chinese female SLE patients aged 20-50 years who fulfilled the American College of Rheumatology criteria [16] and had no contraindications to physical activity were randomly selected from the outpatient clinics of Taichung Veterans General Hospital (Taichung, Taiwan). The hospital's ethical committee approved the study and all SLE patients provided written informed consent.

Energy expenditure was assessed using the RT3 Triaxial Activity Measurement and Recording System (Stay-Healthy, Monrovia, CA, USA). The RT3 was a relatively small device (the size of a pager) and used an integrated tri-axial accelerometer. These were worn by the SLE patients in nylon pouches secured to a belt at the waistline above each hip during the seven-day period. The patients' profiles, including age, height, and weight, were entered into the RT3 accelerometer and the acceleration was measured periodically. Information obtained was then converted into energy expenditure estimates that included total activity calorie (TA), physical activity calorie (PA), total activity calorie per body weight (TABW), and physical activity calorie per body weight (PABW).

Anti-dsDNA was measured by enzyme-linked immunosorbent assay using a Quanta Lite^{TM} dsDNA Kit (INOVA Diagnostics Inc., San Diego, CA, USA). Quantitative determinations of serum C3 and C4 were conducted using N Antisera to Human Complement Factor reagents with the Behring Nephelometers (Dade Behring, Inc., Newark, DE, USA). Disease activity was determined using the SLE Disease Activity Index (SLEDAI) [17], while enzymatic methods were used to determine circulating concentrations of total cholesterol (Beckman TC Reagent) and triglyceride (TG) (Beckman TG Reagent). Magnesium-dextran sulfate precipitation reagent was used to separate high-density lipoprotein-cholesterol (HDL-C), which was then assessed enzymatically. Low-density lipoprotein-cholesterol (LDL-C) was determined by the Friedewald equation [18].

Metabolic syndrome was based on the National Cholesterol Education Program (NCEP/ATP III) definition [19]. SLE patients were defined as having metabolic syndrome in the presence of three or more of the following criteria: waist circumference > 88 cm; systolic blood pressure > 130 mm Hg or diastolic blood pressure > 85 mmHg; HDL-C < 50 mg/dL; TG > 150 mg/dL; and fasting blood glucose > 110 mg/dL.

Statistical analyses were performed using the Statistical Package of Social Sciences (SPSS) 10.0 for Windows (SPSS Inc., Chicago, IL, USA). Tested variables for comparison of means were expressed as mean ± standard deviation and 95% confidence interval. The distribution of tested variables was examined graphically for normality. Kruskal-Wallis test and Mann-Whitney U test were used to examine the mean differences of energy expenditure between the SLE patients stratified by BMI and metabolic syndrome. Analysis of variance (ANOVA) was also used to examine the mean differences of test parameters among SLE patients stratified by tertiles of TABW as measured by the RT3 accelerometer. In addition, Pearson's or Spearman correlation analysis was used accordingly to examine the relationships between estimates of energy expenditure and test variables. A p value < 0.05 was considered significant for all statistical analyses in this study.

3. RESULTS

All of the patients were non-smokers, performed regular daily activities, and did not attend any supervised exercise training program during the experiment period. Average estimates of energy expenditures measured by the RT3 accelerometer were 1730 ± 218 Kcal/day TA, 284 ± 108 Kcal/day PA, 26 ± 3 Kcal/kg/day TABW, and 4 ± 1 Kcal/kg/day PABW. Thirty-six patients (74%) had a normal BMI (< 25 kg/m^2), nine (18%) were overweight (25-29.9 kg/m^2), and four (8%) were obese (> 30 kg/m^2). Compared to patients with normal BMI, overweight or obese patients had higher SLEDAI (mean: 3.38 ± 2.50, n = 13 vs. 1.28 ± 1.80, n = 36, p = 0.013) and lower TABW (mean: 23.42 ± 1.92 Kcal/kg/day, n = 13 vs. 27.25 ± 2.60 Kcal/kg/day, n = 36, p < 0.001). The median SLEDAI value in all patients was 2 and only 31% of patients had SLEDAI scores higher than 3, indicating that most of the patients had stable or moderately active disease status [20]. However, SLEDAI significantly correlated with body weight (r = 0.434, p = 0.002) and BMI (r = 0.379, p = 0.007). Both TABW (r = –0.303, p = 0.034) and PABW (r = –0.301, p = 0.035) negatively correlated with SLEDAI.

Estimates of energy expenditure measured by the RT3 accelerometer in SLE patients stratified by BMI were shown in **Table 1**. SLE patients in the highest BMI tertile showed significantly higher TA and PA, and signifycantly lower TABW compared to those in the lowest tertile. In addition, there was a significant negative correlation between BMI and TABW (r = –0.668, p < 0.001).

Estimates of energy expenditure in SLE patients with and without metabolic syndrome were shown in **Table 2**. According to the NCEP/ATP III definition, 21 patients (43%) met the criteria for metabolic syndrome. They had significantly higher TA and PA, and significantly lower TABW.

Table 1. Estimates of energy expenditure by RT3 accelerometer in SLE patients stratified by body mass index (n = 49)[a].

	Body mass index (kg/m^2) tertiles			Overall P value[b]
	< 20.5 kg/m^2	20.5 - 23.6 kg/m^2	> 23.6 kg/m^2	
TA (Kcal/day)	1611 ± 118 (1549 – 1674)	1696 ± 263 (1561 – 1831)	1886 ± 147 (1808 – 1964)	< 0.001*
TABW (Kcal/kg/day)	28.11 ± 2.10 (26.99 – 29.22)	26.50 ± 2.97 (24.97 – 28.02)	24.09 ± 2.35 (22.84 – 25.34)	< 0.001*
PA (Kcal/day)	244 ± 79 (202 – 287)	263 ± 98 (213 – 313)	347 ± 119 (284 – 411)	0.027*
PABW (Kcal/kg/day)	4.22 ± 1.19 (3.58 – 4.85)	4.10 ± 1.30 (3.43 – 4.77)	4.56 ± 1.54 (3.74 – 5.38)	0.694

Abbreviations: TA, Total activity calorie; TABW, Total activity calorie per body weight; PA, Physical activity calorie; PABW, Physical activity calorie per body weight

[a]Values are expressed as mean ± standard deviation and 95% confidence interval.

[b]Statistical significance (*$p < 0.05$) as determined by the Kruskal-Wallis test.

Table 2. Estimates of energy expenditure by RT3 accelerometer in SLE patients with and without metabolic syndrome[a].

	SLE patients without metabolic syndrome (n = 28)	SLE patients with metabolic syndrome (n = 21)	P value[b]
TA (Kcal/day)	1668 ± 149 (1602 – 1734)	1894 ± 267 (1772 – 2016)	0.001*
TABW (Kcal/kg/day)	31.56 ± 2.70 (30.36 – 32.76)	29.78 ± 4.25 (27.85 – 31.72)	0.009*
PA (Kcal/day)	264 ± 101 (220 – 309)	390 ± 226 (287 – 493)	0.039*
PABW (Kcal/kg/day)	4.97 ± 1.79 (4.18 – 5.77)	6.06 ± 3.53 (4.45 – 7.67)	0.409

Abbreviations: TA, Total activity calorie; TABW, Total activity calorie per body weight; PA, Physical activity calorie; PABW, Physical activity calorie per body weight

[a]Values are expressed as mean ± standard deviation and 95% confidence interval.

[b]Statistical significance (*$p<0.05$) was determined by Mann-Whitney U test.

The mean differences of anthropometric measurements, disease activity-related variables, and lipid profile between patients stratified into tertiles based on TABW were shown in **Table 3**. SLE patients in the lowest TABW tertile showed higher body weight, waist circumference, BMI, SLEDAI, dosage of prednisone, and blood pressure. Patients in the higher TABW tertile tended to have reduced concentrations of total cholesterol, TG and LDL-C but the differences were not statistically significant.

4. DISCUSSION

This study evaluated estimates of energy expenditure by using the RT3 accelerometer in 49 female SLE patients. The major finding was that estimates of energy expenditure represented as daily TABW and PABW were negatively associated with disease activity.

There is a number of assessments available for evaluating physical activity, such as self-administered questionnaires of physical activity, pedometers, accelerometers, and supervised cardio-vascular training programs [11,21,22]. However, each method has its own strengths and limitations. The RT3 accelerometer is used to assess physical activity in children [10,11], adolescents [11], overweight adults [12], pregnant women [13], individuals with multiple sclerosis [14], and older adults with coronary heart disease [15]. Klassen et al. report that the RT3 accelerometer can detect a significant difference between moderately active and active individuals with multiple sclerosis [14]. Chu et al. have demonstrated that RT3 movement counts increases in a linear manner with scaled oxygen uptake from stationary to vigorous intensity movement in children [10]. In the present study, the RT3 accelerometer is used to assess estimates of energy expenditure in SLE patients and is proven to be user-friendly and an acceptable measure of free-living physical activity in such population. The RT3 accelerometer appears to distinguish estimates of energy expenditure in SLE patients with varying clinical characteristics.

Recent studies demonstrate the beneficial effects of exercise in SLE patients in terms of improving fatigue, physical function, aerobic fitness, and quality of life [22-25]. Aerobic exercises performed on a treadmill do not aggravate disease activity at any time during an exercise period and may provide some benefits on fatigue

Table 3. Characteristics of SLE patients in the respective tertiles of total activity calorie per body weight (n = 49)[a].

	Total activity calorie per body weight (Kcal/kg/day) tertiles			Overall P value[b]
	< 24.91 (Kcal/kg/day)	24.91-27.17 (Kcal/kg/day)	> 27.17 (Kcal/kg/day)	
Weight (kg)	66.87 ± 9.95 (61.56 – 72.17)	55.74 ± 6.10 (52.49 – 58.99)	50.15 ± 6.12 (47.00 – 53.29)	<0.001*
BMI (kg/m^2)	26.96 ± 4.69 (24.45 – 29.46)	22.03 ± 1.93 (20.99 – 23.06)	20.62 ± 2.30 (19.43 – 21.80)	<0.001*
Waist circumference (cm)	88.39 ± 12.65 (81.09 – 95.69)	78.70 ± 8.77 (73.85 – 83.55)	74.89 ± 5.49 (71.72 – 78.06)	0.002*
Anti-dsDNA (IU/ml)	129.14 ± 181.57 (32.38 – 225.89)	115.49 ± 133.08 (44.57 – 186.40)	151.50 ± 195.41 (51.03 – 251.97)	0.833
C 3 (mg/dl)	84.58 ± 22.73 (72.46 – 96.69)	93.93 ± 20.71 (82.89 – 104.96)	89.34 ± 16.77 (80.72 – 97.97)	0.429
C 4 (mg/dl)	16.09 ± 9.19 (11.19 – 20.99)	16.20 ± 9.61 (11.08 – 21.32)	18.11 ± 9.08 (13.44 – 22.78)	0.781
SLEDAI	3.38 ± 2.28 (2.16 – 4.59)	1.00 ± 1.79 (0.05 – 1.95)	1.18 ± 1.74 (0.28 – 2.07)	0.002*
Prednisone (mg/day)	12.03 ± 5.18 (9.27 – 14.79)	6.88 ± 4.13 (4.67 – 9.08)	7.79 ± 6.67 (4.37 – 11.22)	0.023*
TC (mmol/l)	5.32 ± 1.18 (4.70 – 5.95)	5.21 ± 1.01 (4.67 – 5.75)	4.65 ± 0.86 (4.21 – 5.09)	0.139
TG (mmol/l)	1.48 ± 0.77 (1.07 – 1.89)	0.99 ± 0.34 (0.81 – 1.17)	1.20 ± 0.50 (0.94 – 1.46)	0.061
LDL-C (mmol/l)	2.94 ± 1.03 (2.39 – 3.49)	2.82 ± 0.72 (2.44 – 3.20)	2.49 ± 0.60 (2.19 – 2.80)	0.264
HDL-C (mmol/l)	1.71 ± 0.49 (1.45 – 1.97)	1.93 ± 0.62 (1.61 – 2.26)	1.61 ± 0.47 (1.37 – 1.85)	0.207
TC/HDL-C	3.36 ± 0.88 (2.86 – 3.87)	2.83 ± 0.50 (2.55 – 3.10)	3.15 ± 0.76 (2.71 – 3.59)	0.144
SBP (mmHg)	153 ± 29.22 (136.13 – 169.87)	126.13 ± 28.37 (110.42 – 141.84)	121.29 ± 14.92 (112.67 – 129.90)	0.004*
DBP (mmHg)	91.14 ± 19.35 (79.97 – 102.32)	78.67 ± 16.70 (69.42 – 87.92)	74.43 ± 12.99 (66.93 – 81.93)	0.029*

Abbreviations: BMI, body mass index; C3, complement factor 3; C4, complement factor 4; SLEDAI, systemic lupus erythematosus disease activity index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure

[a]Values are expressed as mean ± standard deviation and 95% confidence interval.

[b]Statistical significance (*p < 0.05) as determined by ANOVA test.

and physical function in SLE patients with low disease activity [25]. In a 12-week supervised cardio-vascular training program, SLE patients have shown improved exercise tolerance, aerobic capacity, oxygen pulse, fatigue, quality of life, depression, and functional capacity [22]. In a pilot study on the effect of exercise in SLE patients, both aerobic and range of motion/muscle strengthening types of exercises are safe and do not worsen SLE disease activity [23]. In the present study, SLE patients with lowest TABW tertile show higher SLEDAI and dosage of prednisone. It also identifies both TABW and PABW to be inversely correlated with SLEDAI, suggesting that improved daily energy expenditure may be beneficial to disease management in SLE.

Obesity is independently associated with impaired functional capacity and health-related quality of life in patients with SLE [26] and disease activity is predictive of deleterious increases in BMI [27]. Although only 26% of patients in this study are overweight or obese with BMI > 25 kg/m^2, they have higher SLEDAI and lower TABW. SLE patients in the lowest TABW tertile show the highest body weight, waist circumference, BMI, SLEDAI, dosage of prednisone, and blood pressure, as well as a positive correlation between BMI and SLEDAI (r = 0.379, p = 0.007). Taken together, the findings here suggest that less energy expenditure corresponding to increased BMI may aggravate, at least partly, the disease activity in female SLE patients. However, due to lack of self-reported information about the time record and types of activity when the RT3 accelerometer was on and off, physical activity intensity and metabolic equivalent energy expenditure cannot be completely evaluated.

Recent studies demonstrate a higher prevalence of insulin resistance and metabolic syndrome in SLE patients [28,29]. The high prevalence of metabolic syndrome is confirmed in the present study wherein 21 of 49 (43%) SLE patients have metabolic syndrome using the NCEP/ATP III definition. Physical activity energy expenditure can predict progression towards metabolic syndrome in middle-aged healthy Caucasians [30]. SLE patients with metabolic syndrome in the present study show signifycantly lower TABW. In addition, patients with higher

TABW tend to have reduced concentrations of total cholesterol, TG, and LDL-cholesterol. However, the lipid profiles of most patients in this study are within normal range and not considered dyslipidemic [19], which can limit possible effect of physical activity-related energy expenditure on circulating lipid levels.

In conclusion, as increasing physical activity can be a component of lifestyle interventions designed to improve quality of life and functional capacity in SLE patients, this study demonstrates the relationship among estimates of energy expenditure measured by RT3 accelerometer, disease activity, and components of metabolic syndrome. Incorporating physical activity-related energy expenditure into the clinical management of SLE may be beneficial.

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REFERENCES

[1] D'Cruz, D.P., Khamashta, M.A. and Hughes, G.R. (2007) Systemic lupus erythematosus. *Lancet*, **369(9561)**, 587-596.

[2] Vu, T.V. and Escalante, A. (1999) A comparison of the quality of life of patients with systemic lupus erythematosus with and without end-stage renal disease. *Journal of Rheumatology*, **26(12)**, 2595-2601.

[3] Forte, S., Carlone, S., Vaccaro, F., Onorati, P., Manfredi, F., Serra, P. and Palange, P. (1999) Pulmonary gas exchange and exercise capacity in patients with systemic lupus erythematosus. *Journal of Rheumatology*, **26(12)**, 2591-2594.

[4] Tench, C., Bentley, D., Vleck, V., McCurdie, I., White, P. and D'Cruz, D. (2002) Aerobic fitness, fatigue, and physical disability in systemic lupus erythematosus. *Journal of Rheumatology*, **29(3)**, 474-481.

[5] Brown, W.J., Burton N.W. and Rowan P.J. (2007) Updating the evidence on physical activity and health in women. *American Journal of Preventive Medicine*, **33(5)**, 404-411.

[6] Lagerros Y.T. and Lagiou P. (2007) Assessment of physical activity and energy expenditure in epidemiological research of chronic diseases. *European Journal of Epidemiology*, **22(6)**, 353-362.

[7] DeVoe, D., Gotshall, R. and McArthur, T. (2003) Comparison of the RT3 research tracker and tritrac R3D accelerometers. *Percept Mot Skills*, **97(2)**, 510-518.

[8] Powell, S.M. and Rowlands, A.V. (2004) Intermonitor variability of the RT3 accelerometer during typical physical activities. *Medicine and Science in Sports and Exercise*, **36(2)**, 324-330.

[9] Rowlands, A.V., Thomas, P.W., Eston, R.G. and Topping, R. (2004) Validation of the RT3 tri-axial accelerometer for the assessment of physical activity. *Medicine and Science in Sports and Exercise*, **36(3)**, 518-524.

[10] Chu, E.Y., McManus, A.M. and Yu, C.C. (2007) Calibration of the RT3 accelerometer for ambulation and non-ambulation in children. *Medicine and Science in Sports and Exercise*, **39(11)**, 2085- 2091.

[11] De Vries, S.I., Bakker, I., Hopman-Rock, M., Hirasing, R.A. and Van Mechelen, W. (2006) Clinimetric review of motion sensors in children and adolescents. *Journal of Clinical Epidemiology*, **59(7)**, 670-680.

[12] Jacobi, D., Perrin, A.E., Grosman, N., Dore, M.F., Normand, S., Oppert, J.M. and Simon, C. (2007) Physical activity-related energy expenditure with the RT3 and TriTrac accelerometers in overweight adults. *Obesity*, **15 (4)**, 950-956.

[13] DiNallo, J.M., Le, Masurier, G.C., Williams, N.I. and Downs, D.S. (2008) Walking for health in pregnancy: assessment by indirect calorimetry and accelerometry. *Research Quarterly for Exercise and Sport*, **79(1)**, 28-35.

[14] Klassen, L., Schachter, C. and Scudds, R. (2008) An exploratory study of two measures of free-living physical activity for people with multiple sclerosis. *Clinical Rehabilitation*, **22(3)**, 260-271.

[15] Orrell, A., Doherty, P., Miles, J. and Lewin, R. (2007) Development and validation of a very brief questionnaire measure of physical activity in adults with coronary heart disease. *European Journal of Cardiovascular Prevention and Rehabilitation*, **14(5)**, 615-623.

[16] Tan, E.M., Cohen, A.S., Fries, J.F., Masi, A.T., McShane, D.J., Rothfield, N.F., Schaller, J.G., Talal, N. and Winchester, R.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis and Rheumatism*, **25(11)**, 1271-1277.

[17] Bombardier, C., Gladman, D.D., Urowitz, M.B., Caron, D., Chang, C.H. and the Committee on Prognosis Studies in SLE (1992) Derivation of the SLEDAI: A disease activity index for lupus patients. *Arthritis and Rheumatism*, **35(6)**, 630-640.

[18] Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clinical Chemistry*, **18(6)**, 499-502.

[19] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *Journal of the American Medical Association*, **285**, 2486-2497.

[20] Chang, D.M., Lan, J.L., Lin, H.Y. and Luo, S.F. (2002) Dehydroepiandrosterone treatment of women with mild-to-moderate systemic lupus erythematosus. *Arthritis and Rheumatism*, **46(11)**, 2924-2927.

[21] Faulkner, G., Cohn, T. and Remington, G. (2006) Validation of a physical activity assessment tool for individuals with schizophrenia. *Schizophrenia Research*, **82(3)**, 225-231.

[22] De Carvalho, M.R.P., Sato, E.I., Tebexreni, A.S., Heidecher, R.T.C., Schenkman, S. and Barros Neto, T.L. (2005) Effects of supervised cardio-vascular training

program on exercise tolerance, aerobic capacity, and quality of life in patients with systemic lupus erythematosus. *Arthritis and Rheumatism*, **53(6)**, 838-844.

[23] Ramsey-Goldman, R., Schilling, E.M., Dunlop, D., Langman, C., Greenland, P., Thomas, R.J. and Chang, R.W. (2000) A pilot study on the effects of exercise in patients with systemic lupus erythematosus. *Arthritis Care and Research*, **13(5)**, 262-269.

[24] Tench, C.M., McCarthy, J., McCurdie, I., White, P.D. and D'Cruz, D.P. (2003) Fatigue in systemic lupus erythematosus: A randomized controlled trial of exercise. *Rheumatology*, **42(9)**, 1050-1054.

[25] Clarke-Jenssen, A.C., Fredriksen, P.M., Lilleby, V. and Mengshoel, A.M. (2005) Effects of supervised aerobic exercise in patients with systemic lupus erythematosus: A pilot study. *Arthritis and Rheumatism*, **53(2)**, 308-312.

[26] Oeser, A., Chung, C.P., Asanuma, Y., Avalos, I. and Stein, C.M. (2005) Obesity is an independent contributor to functional capacity and inflammation in systemic lupus erythematosus. *Arthritis and Rheumatism*, **52(11)**, 3651-3659.

[27] Kipen, Y., Briganti, E.M., Strauss, B.J.G., Littlejohn, G.O. and Morand, E.F. (1999) Three-year follow-up of body composition changes in pre-menopausal women with systemic lupus erythematosus. *Rheumatology*, **38(1)**, 59-65.

[28] Chung, C.P., Avalos, I., Oeser, A., Gebretsadik, T., Shintani, A., Raggi, P. and Stein, C.M. (2007) High prevalence of the metabolic syndrome in patients with systemic lupus erythematosus: Association with disease characteristics and cardio-vascular risk factors. *Annals of Rheumatic Disease*, **66(2)**, 208-214.

[29] Sabio, J.M., Zamora-Pasadas, M., Jimenez-Jaimez, J., Albadalejo, F., Vargas-Hitos, J., Rodriguez del Aguila, M.D.M., Hidalgo-Tenorio, C., Gonzalez-Gay, M.A. and Alonso, J.J. (2008) Metabolic syndrome in patients with systemic lupus erythematosus from Southern Spain. *Lupus*, **17(9)**, 849-859.

[30] Ekelund, U., Brage, S., Franks, P.W., Hennings, S., Emms, S. and Wareham, N.J. (2005) Physical activity energy expenditure predicts progression toward the metabolic syndrome independently of aerobic fitness in middle-aged healthy Caucasians: The Medical Research Council Ely Study. *Diabetes Care*, **28(5)**, 1195-1200.

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Chondrocyte viability depends on the preservative solution

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ABSTRACT

Fresh osteochondral grafts find broad application in the treatment of extensive and focal damages of joint surfaces. The maintenance of chondrocyte viability of the collected grafts is of key importance. Aim: The evaluation of chondrocyte survivability in a stable temperature of 4^0C in different preservative solutions is the aim of this work. Method: Chondrocyte survivability has been evaluated in saline solution (group I), Ringer solution (group II), saline with an addition of hyaluronic acid (group III) and saline enriched with glucosamine sulphate (group IV). The amount of live chondrocytes was examined on the day of collection and subsequently after 1, 2, 3,6,12, and 21 days using the Promega MTT test. Results: The highest number of live chondrocytes as calculated for 1g of hyaline cartilage after 21 days was ascertained in group IV (saline with glucosamine sulphate). The lowest number of live chondral cells was observed in group II (saline with hyaluronic acid). Chondrocyte survivability in saline (group I) was higher than in the Ringer solution (group II). Conclusions: The enrichment of saline solution with glucosamine sulphate protracts the viability of chondrocytes in fresh osteochondral grafts prepared for chondral transplantation.

Keywords: Chondrocyte; Preservative Solution; Osteochondral Grafts; Fresh Grafts

1. INTRODUCTION

The use of fresh allogeneic osteochondral grafts to repair extensive damage to joint surfaces spans more than 100 years [1]. Grafts of many different types and sizes have been used, from various cylindrical osteochondral grafts with radii ranging from several millimeters to larger than 1 cm, through shell grafts, to the transplantation of whole joint surfaces of tibial or femoral condyles [2]. The size of fresh allogeneic osteochondral grafts notwithstanding, the determination and maintenance of safe conditions for the donor joint surfaces remains an ongoing issue. It is crucial to maintain strict sanitary standards for grafts until their implantation. Although rules regarding such standards are determined by laws, and thus differ by country, it is important for hospitals to organize a specialized transplant service with procedural guidelines, which will mitigate difficulties encountered while performing this repair method. The tasks of such a service should include recruiting donors and preparing potential recipients for transplantation. Efficient use of fresh allogeneic grafts of joint surfaces is essential because the chondrocytes of transplanted cartilage atrophy rapidly. Tissue survival can be extended by cooling collected fragments. However, freezing typically destroys live chondral cells and disintegrates the intercellular matrix structure [3]. Thus, maintaining the viability of collected chondrocytes is a key issue to be addressed at centers that use this method of joint cartilage repair. Significant chondrocyte survival can be maintained for only 1-2 weeks by preserving osteochondral grafts in normal saline with antibiotics, buffered normal saline, or Ringer solution cooled to 4°C, although this period can be extended [4-6]. Thus there is a continuing need for methods that prolong the safe preservation and transport of graft materials with live chondrocytes.

Pharmaceuticals and dietary supplements that purportedly improve chondrocyte vitality and the ability of damaged chondral surfaces to self-repair have gained

popularity in recent years. Various derivatives of hyaluronic acid and substances containing glucosamine are most frequently used.

2. AIM

The widespread use of hyaluronic acid and glucosamine compounds in the pharmacotherapy of focal and generalized joint cartilage damages raises the prospect of possibly extending chondrocyte vitality by adding these substances to the transport solutions for osteochondral grafts. Therefore, in this study, we determined the survival of human chondral cells stored *in vitro* at the same temperature, but in different conserving solutions.

3. MATERIALS AND METHODS

The distal femoral epiphysis was collected along with the cartilage coating from six male donors, aged 21-48 years, in the operating room after the conclusion of multi-organ collections. The obtained osteochondral specimens were divided into four equal parts. Each was randomly assigned for immersion in one of the following four media: normal saline solution (group I), Ringer solution (group II), normal saline solution with 20 mg sodium salt of hyaluronic acid (group III), or normal saline solution enriched with 1.5 g glucosamine sulphate (group IV). Each medium was enriched with 1.0 g third-generation cephalosporin. Each of the obtained quarters of the distal femoral epiphysis was subsequently divided into seven equal parts, which were placed in separate sterile containers filled with the evaluated solution. All samples were stored at 4°C. Live chondrocyte numbers in the initial samples of each group were quantitatively analyzed within 24 h after collection using colorimetric reduction in the Promega MTT (3-(4, 5-dimethylthiazol-2-yl)2, 5-diphenyl tetrazolium bromide) test. Evaluations were subsequently conducted on Days 1, 2, 3, 6, 12, and 21. The obtained absorption capacity was proportional to the cellular oxidoreductive activity and subsequently scaled to 1 g hyaline cartilage. Measurement results were evaluated using analysis of variance (ANOVA) and Friedman's and Dunnet's statistical tests.

The research was approved by the Bioethical Commission of the Medical University of Lublin (PL) no. KE-0254/143/2006, according to Good Clinical Practice conditions.

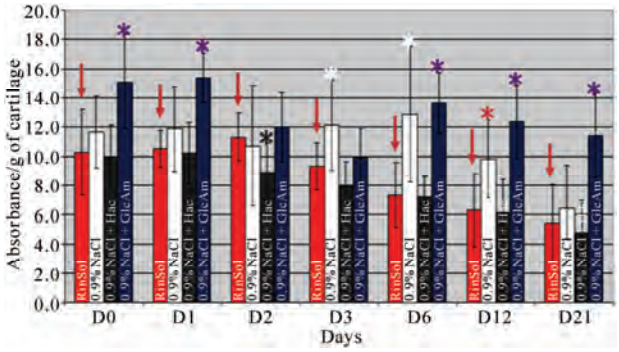
4. RESULTS

None of the evaluated groups showed a statistically significantly change in absorption from initial values during the first 3 days of observation. Group IV (saline solution enriched with glucosamine) had the highest absorption values, both initially and during the subsequent evaluation days, as calculated per gram of cartilage. A statistically significant decline in live chondrocyte numbers occurred between Days 3 and 6 of storage in Ringer fluid (group II) and saline with sodium salt of hyaluronic acid (group III). A similar decline occurred in normal saline (group I) between Days 12 and 21 of storage. No statistically significant decrease in the number of live chondrocytes was evident in group IV (saline enriched with glucosamine sulphate) over the entire observation period.

At Day 21, group IV samples had the highest number of live chondrocytes, and the difference in counts between this group and each other group was statistically significant.

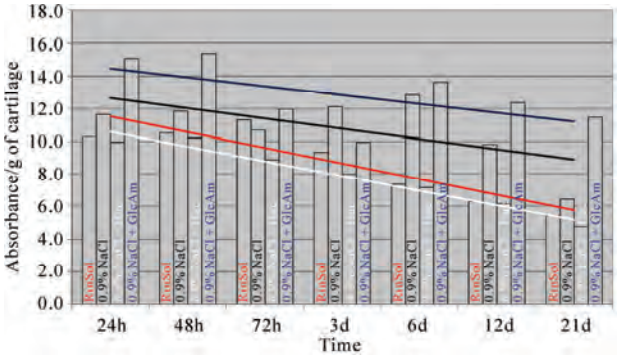
The lowest numbers of live chondrocytes throughout the whole research period were found in group III, saline solution with sodium salt of hyaluronic acid. The most

Table 1. Absorbance values of Ringer's solution vs. other media.



Chondrocyte viability was highest in the 0.9% NaCl + glucosamine group on all observation days.

Table 2. Trend analysis of absorbance values.



The lowest absorbance values and the biggest decrease in values were seen in group III (0.9% NaCl + HAc). The highest absorbance values and the smallest drop in values were seen in group IV (0.9% NaCl + glucosamine).

rapid drops in numbers of live chondral cells were also observed in this group. The number of live chondral cells in group II (Ringer solution) was lower than in group I (normal saline solution).

5. DISCUSSION

The storage period of fresh osteochondral grafts greatly affects their clinical value, where longer storage reduces the number of live chondral cells [7].Allen *et al.* reported a significant decline in live chondral cell numbers in osteochondral grafts within 3 weeks of collection [4]. Previous research has focused on protecting chondrocyte vitality in allogeneic osteochondral grafts primarily by changing the temperature. Voss *et al*. demonstrated that storing cartilage at room temperature accelerated cell death [8], and a critical increase occurred between 50°C and 55°C. In contrast, lowering the temperature is generally favorable to chondrocyte survival. Pearsall reported that the number of live chondrocytes dropped to 67% of the initial value after a storage period of 44 days at 4°C [9]. Judas *et al*. showed that adding protective substances to the agents in which osteochondral grafts were stored before freezing extended chondrocyte survival [10]. Adding tetracycline-type antibiotics may improve the vitality of chondral graft cells [11]. Teng *et al*. examined the impact of IGF-1(insulin-like growth factor 1) and the apoptosis inhibitor zVAD-fmk in Ringer fluid and Dulbecco's modified Eagle's medium (DMEM) on the survival of bovine chondrocytes [7]. Chondrocytes atrophied most rapidly in Ringer fluid; the process was slower in DMEM. Adding either IGF-1 or zVAD-fmk to the DMEM significantly extended the survival of chondral cells. Pennock analyzed the survivability of human chondrocytes in osteochondral grafts suspended in normal saline, glucose, amino acid solution, and 10% fetal bovine serum solution [12]. Chondrocyte survival was significantly lower in solutions without bovine serum. However, storing tissue with bovine serum requires further research on the possibility of the transmission of infections as well as immune reactions to proteins from foreign species.

The results of our research demonstrate that chondrocyte cell death was quickest in saline solution enriched with sodium salt of hyaluronic acid. We are not in a position to determine why adding hyaluronic acid derivatives did not improve chondrocyte graft vitality. Samples of human joint cartilage stored for 3 weeks in saline solution enriched with glucosamine sulphate retained their vitality to the greatest degree compared to samples preserved in normal saline alone, Ringer solution, and normal saline solution plus sodium salt of hyaluronic acid. Thus, chondrocyte vitality can be increased by enriching the fluid environment of graft preservation with glucosamine sulphate. The lowest decline in live chondrocyte numbers over a 3-week period decreased the biological value of the graft only slightly. Our results show that the inevitable process of *in vitro* chondral cell atrophy during the storage period for surgical transplantation of joint surfaces may be slowed to maintain high biological and mechanical value of the graft. Otsuki *et al*. showed that a decrease in glycosaminoglycan concentration did not lead directly to the intensification of chondral joint cell atrophy [13]. Based on our analyses, increasing the amount may slow these processes.

REFERENCES

[1] Lexer, E. (1908) Substitution of whole or half joints from freshly amputated extremities by free plastic operations. *Surgery, Gynecology and Obstetrics*, **6**, 601-607.

[2] Gross, A.E., Silverstein, E.A., Falk, J., Falk, R. and Langer, F. (1975) The allotransplantation of partial joints in the treatment of osteoarthritis of the knee. *Clinical Orthopaedics and Related Research*, **108**, 7-14.

[3] Csönge, L., Bravo, D., Newman-Gage, H., Rigley, T., Conrad, E.U., Bakay, A., Strong, D.M. and Pellet, S. (2002) Banking of osteochondral allografts, Part II. Preservation of Chondrocyte Viability during Long-Term Storage. *Cell and Tissue Banking*, **3(3)**, 161-168.

[4] Allen, T.R., Robertson, C.M., Pennock, A.T., Bugbee, W.D., Harwood, F.L., Wong, W., Chen, A.C., Sah, R.L. and Amiel, D. (2005) Analysis of stored osteochondral allografts at the time of surgical implantation. *America Journal of Sports Medicine*, **33(10)**, 1479-1484.

[5] LaPrade, R.F., Botker, J., Herzog, M. and Agel, J. (2009) Refrigerated osteoarticular allografts to treat articular cartilage defects of the femoral condyles. *Journal of Bone and Joint Surgery American*, **91(4)**, 805-811.

[6] Williams III, R.J., Dreese, J.C., Chen and C.-T. (2004) Chondrocyte survival and material properties of hypothermically stored cartilage. An evaluation of tissue used for osteochondral allograft transplantation. *American Journal of Sports Medicine*, **32(1)**, 132-139.

[7] Teng, M.S., Yuen, A.S. and Kim, H.T. (2008) Enhancing osteochondral allograft viability: effects of storage media composition. *Clinical Orthopaedics and Related Research*, **466(8)**, 1804-1809.

[8] Voss, J.R., Lu, Y., Edwards, R.B., Bogdanske, J.J., Markel, M.M. (2006) Effects of thermal energy on chondrocyte viability. *American Journal of Veterinary Research*, **67(10)**, 1708-1712.

[9] Pearsall, A.W., Tucker, J.A., Hester, R.B. and Heitman, R.J. (2004) Chondrocyte viability in refrigerated osteochondral allografts used for transplantation within the knee. *American Journal of Sports Medicine*, **32(1)**, 125-131.

[10] Judas, F., Rosa, S., Teixeira, L., Lopes, C. and Ferreira Mendes, A. (2007) Chondrocyte viability in fresh frozen large human osteochondral allografts: effect of cryoprotective agents. *Transplantations Proceedings*, **39(8)**, 2531-2534.

[11] Snasshall, J.T., Teng, M.S., Yuen, A.S. and Kim, H.T. (2006) Minocycline increases articular chondrocyte viability during storage of osteochondral allografts. 52*nd Annual Meeting of the Orthopedic Research Society*, Chicago, paper 0267.

[12] Pennock, A.T., Wagner, F., Robertson, C.M., Harwood, F.L., Bugbee, W.D., Amiel, D. (2006) Prolonged storage of osteochondral allografts: Does the addition of fetal bovine erum improve chondrocyte viability? *The Journal of Knee Surgery*, **19**, 265-272.

[13] Otsuki, S., Brinson, D.C., Creighton, L., Kinoshita, M., Sah, R.L., D'Lima, D., Lotz, M. (2008) The effect of glycosaminoglycan loss on chondrocyte viability: A study on porcine cartilage explants. *Arthritis and Rheumatism*, **58(4)**, 1076-1085.

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Sublingual epidermoid cyst—a case report

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ABSTRACT

In this article we present the case of an epidermoid cyst of sublingual space along with submental extension in a 35 year old lady who presented with a mass in the oral cavity. On examination a mass was seen on the floor of the mouth displacing the tongue superiorly. Diffuse fullness was noted in the submental region. The lesion was excised under general anaesthesia with nasotracheal intubation. The entire mass was removed intra orally. The histological features were suggestive of epidermoid cyst. The patient did well postoperatively and no recurrence was seen during 8 months follow up.

Keywords: Sublingual; Epidermoid Cyst; Intraoral Approach

1. INTRODUCTION

The epidermoid cysts are those benign cysts which are lined by epithelium only, where as dermoid cysts are those which contain skin and adnexa and teratoid cysts are those which cartilage, bone and even muscle are present [1]. Dermoid and epidermoid cysts are developmental pathologies that occur in the head and neck with an incidence of 6.9-7% [1,2]. About 11.5% of dermoid cysts of the head and neck are in the floor of the mouth, the second most common location after the cervical region. They represent less than 0.01% of all oral cavity cysts [3]. Surgical excision is the treatment of choice.

2. CASE REPORT

A 35 year old lady was presented to the department of ENT, K.S.Hegde Hospital, Mangalore, with the history of mass in the oral cavity since one and half months. The swelling was painless. She had no difficulty in moving the tongue and there was no history of dysphagea or dyspnoea.

On examination of the oral cavity, an ovoid solitary mass measuring about 4 cm × 3 cm was present in the midline on the floor of the mouth. Edges could not be distinctly made out. Mucosa over the swelling was normal but appears stretched. The swelling was soft to cystic in consistency. The lesion extends into the submental region causing diffuse fullness in the submental region. Transmission of movements between intra-oral and submental swelling was felt. Transillumination was negative. A provisional diagnosis of sublingual dermoid cyst or plunging ranula was made.

Routine haematological tests were within normal limits.

Excision of the mass was done under general anaesthesia with nasotracheal intubation. A horizontal incision was placed intra orally on the floor of the mouth. The mass appeared to be a thick walled tense cyst and it was excised in toto. The submental extension through the mylohyoid muscle was excised from the same incision. Post-operative period was uneventful and she was discharged after 5 days. She was seen in the outpatient department after 2 weeks and then after 4 months and 8 months and no recurrence is noted.

3. DISCUSSION

Meyer classified cysts of the floor of the mouth into three groups namely Epidermoid, Dermoid and Teratoma [4]. Histologically midline cysts of the floor of the mouth are divided into epidermoid cysts which consist of an epithelial lined wall that may be partly keratinized; dermoid cysts, which show evidence of skin appendages such as hair follicles, hair, sweat and sebaceous glands; and teratomas which contain, in addition to skin appendages, mesodermal elements like bone, muscle, respiratory and gastrointestinal tissues and a fibrous capsule. The later type may have malignant potential [1,4].

Congenital cysts are dysembryogenetic lesions that arise from ectodermal elements entrapped during midline fusion of first and second branchial arches between the third and fourth weeks of intrauterine life. Congenital cysts may rise from the tuberculm impar during the for-

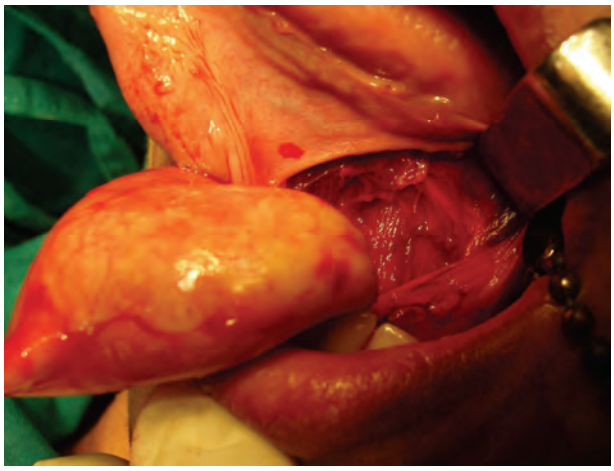

Figure 1. Excision of the cyst by Intraoral Approach.

mation of the floor of the mouth [5,6].

Epidermoid cysts have thin squamous lining because of lack of dermal appendages, which rarely contains calcifications. These cysts contain debris from the desquamation of the epithelial lining. The debris contain mainly keratin, a proteinaceous material and some cholesterol. These are often described as pearly tumours because of the shiny smooth waxy character of the "dry keratin" on gross inspection [1].

Dermoid cysts are well circumscribed lesions most commonly seen at the lateral canthus of the eye. They are typically lined by squamous epithelium and contain skin appendages but unlike epidermoid cyst they have a thicker lining and may contain dystrophic calcification [7].

Ultrasonographically the solid and cystic structures within a heterogenous mass and calcifications are seen

Treatment is by surgical excision of intra oral cysts in the floor of the mouth to relieve symptoms caused by the cyst and possible infection. Usually intra oral incision is placed for small cysts but large ones require an external approach. Post operative complications are rare and are reduced by closely following the capsule and its complete removal [8].

4. CONCLUSIONS

We report a case of epidermoid cyst in the sublingual region with extension into submental region through the mylohyoid muscle, presenting as an intraoral and submental swelling. The cyst was completely excised through intraoral incision without any complications and without any evidence of recurrence in 8 months follows up.

REFERENCES

[1] Koeller, K.K., *et al*. (1999) Congenital cystic masses of the neck: Radiological-Pathologic correlation. *Radiographics*, **19(1)**, 121-146.

[2] Dimov, Z.H., *et al.* (2000) Dermoid, epidermoid and teratoma cysts of the tongue and oral cavity. *Sofiia*, **56(2)**, 30-32.

[3] Saverio, D.P.F., *et al*. (2002) Sublingual Epidermoid Cyst. *Journal of Craniofacial Surgery*, **13(2)**, 308-310.

[4] Calderon, S. (1993) Concomitant Sublingual and Submental Epidermoid cyst of the floor of the mouth. *Journal of Oral and Maxillofacial Surgery*, **51(7)**, 790-792.

[5] Longo, F., *et al.* (2003) Midline Dermoid cysts of the floor of the mouth: Report of 16 cases and review of surgical techniques. *Plastic and Reconstructive Surgery*, **112 (6)**, 1560-1565.

[6] De Ponte, F.S., *et al*. (2002) Sublingual epidermoid cyst. *Journal of Craniofacial Surgery*, **13(2)**, 308-310.

[7] Smirinitopolus, J., Chiechi, M., *et al*. (2005) Tertomas, Dermoids and Epidermoids of the head and neck. *Radiographics*, **25(4)**, 931-948.

[8] Leveque, H., *et al*. (1979) Dermoid Cyst of the floor of the mouth and lateral neck. *Laryngoscope*, **89(2 Pt 1)**, 296-305.

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Aging and the decline in health

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ABSTRACT

The biological reasons for aging are now understood. Aging is the result of multiple stochastic events in molecules, cells, tissues and organs. These together produce the aged phenotype, senescence and ultimately death. Many of these changes can be directly linked to specific age-associated disease. However, there are also age-related changes that are not pathological. It can be said that aging has multiple causes, or is instead due to a general loss of molecular fidelity, that is, an increase in disorder. The complexity of organism means that they develop as ordered structures by obtaining energy from the environment. These ordered structures must be maintained by a wide variety of mechanisms which also depend on energy resources. Eventually these mechanisms fail, and senescence sets in. It is known that the efficiency of maintenance is correlated directly with the lifespan of different mammalian species. Also, these lifespans are inversely correlated with fecundity or reproductive potential. There is a trade off between investment of resources in maintenance of the body, or soma, and investment in reproduction.

Keywords: Aging; Senescence; Disease; Pathologies; Evolution

1. INTRODUCTION

It is now evident that aging is no longer an unsolved biological problem [1-6]. However, the relationship between natural aging in humans to age-associated diseases is controversial. Most books and reviews about aging completely ignore the vast literature on human age related pathologies. Also, most research on each of these pathologists is done by specialists who have no particular knowledge or interest in the process of ageing *per se.* An exception that relates late onset disease to aging is the excellent monograph *The Oxford Textbook of Geriatric Medicine* [7]. Hayflick [8] has argued that aging is an intrinsic process occurring in almost all animals, and that it is not directly related to particular age-associated pathologies. Instead, he states that aging makes an animal susceptible to these pathological events. In contrast, it is argued here and elsewhere [2,5,9] that the process or processes of aging are responsible for most of these pathological changes. This leads to the loss or decline of health that is eventually lethal. Many diseases are the result of multiple molecular or cellular events. These events may occur over a long period of time, and it is their multiplicities that eventually produce the symptoms of disease. In other words many diseases can be due to time dependent multiple "hits" which are stochastic random events. There is an intermediate position which states that "senescence gives rise to disease, but disease does not give rise to senescence" [10], and also that the distinction between senescence and disease is blurred.

At the outset it is important to define some key words. Health is the state of being free from illness or injury. Aging is the process of growing old. Senescence is the condition or process of deterioration with age. Aging (ageing) and senescence are often used interchangeably. Disease is a disorder of structure or function which is not simply the result of specific injury. Pathology is the science of the causes and effects of diseases. (It is also the branch of medicine that deals with the laboratory examination of samples of body tissue for diagnostic or forensic purposes, but this is not relevant to the discussion here).

2. THE BIOLOGICAL REASONS FOR AGING

To understand aging one must first explain its biological origin and function. Organisms develop from the fertilized egg to become adults that are capable of reproduction. In the natural environment in which evolution occurred, animals are confronted with various hazards, for example, predators, insufficient food and water, or parasites and pathogens. Mortality is therefore high, so that

most offspring are born to young adults rather than old ones. In this situation there is little, if any, natural selection for a long lifespan. Instead, Darwinian fitness is increased if resources are channeled into reproduction rather than preserving the body indefinitely [11-13]. It has been shown that in 47 mammalian species there is an inverse relationship between fertility and fecundity and longevity in a non-hazardous environment such as a zoo or under domestication [2,14]. (In the case of humans, longevity is highest in developed countries with good health care). It has also become evident that longevity is directly related to the maintenance of function of the various tissues and organs of the body.

There are many maintenance mechanisms, which will be listed but not reviewed here: 1) The repair of damage in DNA; 2) The degradation of abnormal protein molecules; 3) The defences against reactive oxygen species (ROS); 4) The immune system which provides defences against pathogens and parasites; 5) The detoxification of harmful chemicals in the diet; 6) Proofreading in the synthesis of macromolecules, which removes errors; 7) Wound healing, including the clotting of blood and the repair of broken bones; 8) Epigenetic controls for normal cell functions, and which also prevent the development of cancer; 9) Apoptosis, which removes potentially harmful cells; 10) Physiological homeostasis co-ordinating the functions of different cells, tissues and organs; 11) The grooming of hair and skin to remove harmful pests and parasites; 12) The storage of fat as an energy reserve.

In this list there is no reference to a central component of cell function, namely, RNA (apart from proofreading in its synthesis). This is largely due to lack of information. RNA transmits information in DNA to proteins, The translation of RNA into proteins depends on transfer RNAs and ribosomes (which consist of proteins and RNA). There is also the accurate splicing of RNA transcripts. The regulatory role of small RNAs has recently been demonstrated in normal cells. It would be surprising if 1) there were not important controls of all these functions, and 2) there were no age-related changes in RNA metabolism and function. However, they remain to be discovered.

Most of the listed maintenance mechanisms are scientific disciplines in their own right, and together they depend on a considerable proportion of the resources available to an animal. It should also be noted that a large number of genes are necessary to code for all the proteins and enzymes that are needed for each mechanism. These genes in one way or another have an effect on the process of aging.

There have been many comparative studies that clearly demonstrate that long-lived species have more efficient maintenance mechanisms than short lived ones. These have been comprehensively reviewed elsewhere [2], and since that time more evidence has been published [5,15-17]. Only a few examples can be mentioned here. The same chemical cross links occur more rapidly in bovine skin than human skin [18]. In rats, carcinomas are far more frequently than they do in humans, with an approximately 30-fold difference in the rate of onset [19]. Also, somatic mutations in lymphocytes increase about 10 fold during the lifespan of mice and humans. However, this increase occurs over about three years in mice and 80 years in humans [20]. It has been shown that the defences against ROS correlate with mammalian lifespan [16,21,22]. It has also been shown that these defences are much more efficient in the pigeon, which is long lived, than the rat, a short lived animal of similar size and metabolic rate [23]. A similar difference was demonstrated between small long-lived birds (canary and parakeet) and the short lived mouse [24]. From all these studies it can be concluded that it is the eventual decline in maintenance that brings about aging.

3. CAUSES OF AGING

How does the decline or loss of maintenance give rise to aging? To answer this question we need to consider many of the events that actually occur during aging (2,15). It is known that chromosomal changes and also mutations increase during aging. There may be additional damage to DNA which is not recognized by repair enzymes and simply accumulates with time [25]. Many studies document deletions in mitochondrial DNA. Abnormalities in nuclear DNA can result in age-associated carcinomas. Altered proteins appear in many locations. Collagen and elastin become cross-linked, which is a cause of hardening of the arteries. The loss of elasticity of artery walls can increase blood pressure, and this can result in kidney damage and also strokes. Many types of protein react with glucose and other carbohydrates to produce advanced glycation products (AGEs). These are high molecular weight aggregates that can occur in many locations. There is also the accumulation of lipofuscin, also known as the “age pigment”, which is a complex mixture of many degradation products. This is also seen in many tissues during aging. Recently there has been much interest in epigenetic events during aging, and particularly “epigenetic drift” [26-28]. These events may be due to changes in DNA methylation and histone modification, which in turn can change gene expression. There may be irregularities in hormone function or metabolism, for example, late onset diabetes is caused by a failure of the normal controls of insulin levels, or to changes in insulin receptors. In the brain, neurons may

be lost or become abnormal, producing the amyloid plaques and neurofibrillary tangles which give rise to Alzheimer's disease and other dementias. In the vascular system, atheromatous plaques can form on the inner wall of the major arteries and these impair normal blood flow. This is a major cause of heart disease. The valves of the heart can become calcified, which is another component of the disease. Although muscle tissue can to some extent repair itself, over a long period of time cells are lost and not replaced. Aging is frequently associated with loss of hearing and sight. The long lived protein crystallin becomes denatured and loses transparency, and this result in the appearance of lens cataracts. Retinopathy is largely due to the failure to remove protein aggregates that are the end products of the continual turnover of photoreceptors in the cells of the retina. The age-associated diseases of osteoporosis and osteoarthritis, are due to a decline in normal bone metabolism and the accumulation of damage in bone joints. Another example of multiple events giving rise to disease is the gradual loss of kidney glomeruli and eventually renal failure.

4. MULTIPLE EVENTS

The previous section is only a summary of some of the changes that can occur during aging. They are sufficient to demonstrate that multiple events at the molecular and cellular level can bring about very significant changes in tissues and organs. These in turn can bring about ill health and disease during ageing. However, not all age associated multiple events are pathological. A good example is the whitening of hair. This is due to the loss of melanocytes in hair follicles, and the loss of hair follicles themselves results in baldness. One of the most obvious effects of aging is on the skin, and this provides a rough measure of a person's age. Skin changes are due to loss of elasticity, wrinkling, and often the accumulation of pigmented "age spots". Leaving aside skin cancers, these cumulative effects are simply part of the aging phenotype, and do not impair health. A third example is the loss of muscle strength with age, which by itself is not a harmful change, but is can lead to other problems such as falls and broken bones, particularly if the individual also has osteoporosis.

It is interesting that these outward manifestations of aging are not pathological. It is the internal changes that eventually produce age-associated disease and a senescent phenotype. The longevity of identical twins is more similar that the longevities of sibs. Also, inbred mice which are genetically identical and live in the same environment have quite variable lifespans, and the survival curve of a population of these mice is an S-shaped curve. There is a plateau with no deaths, then slowly increasing mortality, followed by a steeper increase in mortality. Finally, the slope of the curve flattens out and a few "outliers" achieve the longest lifespan [29] If this curve is plotted on a logarithmic scale, there is again a plateau followed by an exponential decline in surviving animals. This is very similar to observations in radiology, where, for example, a population of viruses is irradiated. Again there is a plateau in survival, followed by an exponential decline in survival. This is an example of a multiple hit survival curve. The same can be said of many of the vulnerable components of the body, and taken together this constitutes aging of the whole body. Obviously, there is not complete synchrony in the change and loss of function of one or another tissue or organ system.

This can gives rise to a common misconception. For example, it has frequently been said that dementia has nothing to do with ageing because many old people retain their mental facilities throughout their lifespan. The same can be said of carcinomas, cardiovascular disease, and so on. A further related and interesting point is that death certificates must specify a particular cause or causes of death, it is not permissible for a physician to use the term "natural aging".

During long periods of evolution some animal species reduce their lifespan, whereas others increase it. Since aging is multi-causal, the evolved changes in the efficiency of maintenance must depend on a degree of synchrony in the rate that tissues and organs gradually become senescent. There would be no selective advantage if one organ system increased (or reduced) its survival time, whilst others did not. This was first clearly stated by John Maynard Smith nearly fifty years ago [30].

5. THE LOSS OF MOLECULAR FIDELITY

In his book *What is Life*? Schrodinger [31] discussed the complexity of living organisms. The second law of thermodynamics states that ordered states of atoms and molecules will eventually become disordered. Schrodinger wrote about organisms feeding on negative entropy. This means that they depend on energy to maintain their complex structures, which is a contravention of the second law. It is obvious that this is not a permanent situation because aging eventually results in death, and it is after this that disorder prevails. Instead of stating that there are multiple causes of aging, one can instead regard the whole process of aging to have just one cause, and this is the loss of molecular fidelity, and gain in entropy. This is the viewpoint of Hayflick [4,8].

There is, however, no radical distinction between this one cause of aging and the multiple causes that have been discussed here. The analogy of a motor car helps to explain this. The car is a complex machine that requires

continual service and maintenance. With time it is subject to wear and tear in its component parts which are to some extent independent of each other, but are also essential for its normal function.

We can equate the loss of molecular fidelity or increasing molecular disorder with wear and tear. The component parts of a car cannot be expected to last indefinitely, for example, the gearbox, the electrical system, the cooling system, the engine, and so on. With time the defects increase and are more and more expensive to repair. At a given point in time the car is not worth repairing and reaches the end of its working life. We can conclude that the multiple parts of a car deteriorate at a given rate, and its life ends as a result on innumerable cumulative defects, which are the equivalent of "molecular infidelity" in an organism. It is also significant to note that a vintage car can be maintained indefinitely, but at huge expense. Similarly an animal body could, in principle, be maintained indefinitely, but a cost in resources that would be selectively disadvantageous. Thus all animals, except a few of the simplest, have finite lifespan.

6. CONCLUSIONS

The biological reasons for aging are no longer a mystery. The adult organism is a structure capable of reproduction for a given period, but in a natural environment, most offspring are born to young adults because environmental hazards limit the lifespan of parents. The energy resources available to every animal are used for general metabolism, for reproduction and also to maintain the body, or soma. Normal metabolism is obviously essential for life, but there is a trade off between the investment of resources in reproduction and in body maintenance, which varies between species. Thus, short lived small species, such as rodents, can have many offspring, whilst large long lived species have few offspring.

In the case of the human species, a great deal is known about the changes that occur with aging. Many of these changes affect vital functions which result in a decline in health and eventually to events that cannot be treated at all. Nevertheless, the last few months of life very often depend on medical resources that are increasingly expensive, especially in developed countries. In the end, all care and treatments fail, and the elderly individual dies. It is true to say that aging is almost always associated with a decline in health leading to death.

REFERENCES

[1] Hayflick, L. (1996) How and Why We Age. Ballantine Books, New York.

[2] Holliday, R. (1995) Understanding Ageing. Cambridge University Press, Cambridge.

[3] Austad, S.N. (1997) Why We Age. John Wiley, New York.

[4] Hayflick, L. (2007) Biological aging is no longer an unsolved problem. *Annals of the New York Academy of Sciences*, **1100(1)**, 1-13.

[5] Holliday, R. (2006) Aging is no longer an unsolved problem in biology. *Annals of the New York Academy of Sciences*, **1067(1)**, 1-9.

[6] Kirkwood, T.B. (2005) Understanding the odd science of aging. *Cell*, **120(4)**, 437-447.

[7] Evans, G.J., Franklin W.T., Beattie, B., Michel, J.P. and Wilcock, G.K. (2000) The Oxford Textbook of Geriatric Medicine. Oxford University Press, Oxford.

[8] Hayflick, L., (2004) The not-so-close relationship between biological aging and age-associated pathologies in humans. *Journal of Gerontology*: *Biological Sciences*, **59 (6)**, 547-550.

[9] Holliday, R. (2004) The close relationship between biological aging and age-associated pathologies in humans. *Journal of Gerontology*: *Biological Sciences*, **59(6)**, 543-546.

[10] Carnes, B.A., Staats, D.O. and Sonntag, W.G. (2009) Does senescence give rise to disease? *Mechanisms of Ageing and Development*, **129(12)**, 693-699.

[11] Kirkwood, T.B.L. (1977) The evolution of ageing. *Nature*, **270(5635)**, 301-304.

[12] Kirkwood, T.B. and Holliday, R. (1979) The evolution of ageing and longevity. *Proceedings of the Royal Society London B*: *Biological Sciences*, London, **205(1161)**, 532-546.

[13] Kirkwood, T.B.L. and Holliday, R. (1986) Ageing as a consequence of natural selection. In. Collins, K.J. and Bittles, A.H., Eds., *Biology of Human Ageing*, Cambridge University Press, Cambridge.

[14] Holliday, R. (1994) Longevity and fecundity in eutherian mammals. In. Rose, M.R. and Finch, C.E. Eds., *Genetics and the Evolution of Aging*, Kluwer Academic, Dordrecht.

[15] Holliday, R., (2004) The multiple and irreversible causes of aging. *Journal of Gerontology*: *Biological Sciences*, **59(6)**, 568-572.

[16] Barja, G., (2004) Aging in vertebrates, and the effect of calorie restriction: a mitochondrial free radical production-DNA damage mechanism. *Biological Reviews of the Cambridge Philosophical Society*, **79(2)**, 235-251.

[17] Kapahi, B., Boulton, M.E. and Kirkwood, T.B.L. (1999) Positive correlation between mammalian lifespan and cellular resistance to stress. *Free Radical Biology and Medicine*, **26(5-6)**, 495-500.

[18] Yamauchi, M., Woodley, D.T. and Mechanic, G.L. (1988) Aging and cross-linking of skin collagen. *Biochemical and Biophysical Research Communications*, **152(2)**, 898-903.

[19] Ames, B.N., Saul, R.L., Schwiers, E., Adelman, R. and Cathcart, R. (1985) Oxidative damage as related to cancer and aging: the assay of thymine glycol, thymidine glycol and hydroxymethyl uracil in human and rat urine. In. Sohal, R.S., Birnbaum, L.S. and Cutler, R.G., Eds., *Molecular Biology of Aging*, Raven Press, New York.

[20] Morley, A.A., (1998) Somatic mutations and aging. *Annals of the New York Academy of Sciences*, **854**, 20-22.

[21] Pamplona, R., Portero-Ortin, M., Ruiz, C., Gredilla, R., Herrero, A. and Barja, G. (2000) Double bond content of phospholipids and lipid peroxidation negatively correlate with maximum longevity in the heart of mammals. *Mechanisms of Ageing and Development*, **112(3)**, 169-183.

[22] Barja, G. and Herrero, A. (2000) Oxidative damage to mitochondria is inversely correlated to maximum lifespan in the heart and brain of mammals. *Journal of the Federation of American Societies for Experimental Biology*, **14(2)**, 312-318.

[23] Pamplona, R., Portero-Otin, M., Requena, J.R., Thorpe, S.R., Herrero, A. and Barja, G. (1999) A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation derived proteiun modification in heart mitochondria in the longevous pigeon than the short lived rat. *Mechanisms of Ageing and Development*, **106 (3)**, 283-296.

[24] Pamplona, R., Portero-Otin, M., Riba, D., Gredilla, R., Herrero, A. and Barja, G. (1999) Heart fatty acid unsaturation and lipid peroxidation, and aging rate, are lower in the parakeet and canary than in the mouse. *Aging Clinical and Experimental Research*, **11**, 44-49.

[25] Lindahl, T. (1993) Instability and decay of the primary structure of DNA. *Nature*, **362(6422)**, 709-715

[26] Fraga, M.F., Ballistar, E., Paz, M.F., *et al*. (2005) Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Nat. Acad. Sci. USA*, **102(30)**, 10604-10609.

[27] Martin, G.M. (2005) Epigenetic drift in aging identical twins. *Proceedings of the National Academy Science USA*, **102**, 10413-10414.

[28] Tollefsbol, T. (2009) Epigenetics of aging. Springer Science, Humana Press, New Jersey.

[29] Zurcher, C., Van Zwieten, M.J., Solleveld, H.A. and Hollander, C.F. (1982) Ageing research. In. Foster H. L., Small, J.D. and Fox, J.G. Eds., *The Mouse in Biomedical Research*, Academic Press, New York, **4**, 11-35.

[30] Smith, M.J. (1962) The causes of ageing. *Proceedings of the Royal Society London B*: *Biological Sciences*, London, **157(966)**, 115-127.

[31] Schrodinger, E. (1944) What is life? Cambridge University Press. Cambridge.

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A comparison of duloxetine hydrochloride with fluoxetine hydrochloride in major depressive disorders: a pilot study

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ABSTRACT

To compare remission rate, relapse rate and tolerability of duloxetine, a dual reuptake inhibitor of 5-hydroxy serotonin (5-HT) and norepinephrine (NE), versus fluoxetine, a reuptake inhibitor of 5-HT during follow up period of 16 weeks in major depressive disorder (MDD) a open label comparative trial was conducted. Trial was comprising of 60 patients, diagnosed with MDD, were allocated to fluoxetine group (n*-30, 20-60mg od) or duloxetine group (n*-30, 40-60mg od) for 16 weeks. The end points were remission and relapse assessed by Hamilton Rating scale for Depression-24 items (HAMD-24). In results the mean fall in HAMD-24 scores between groups was comparable till 4 weeks. Thereafter, at 8 weeks the mean fall in HAMD-24 score was significantly greater in duloxetine group (p value < 0.05). At 16 weeks the mean fall was highly significant (p value < 0.01) in duloxetine group. Thirty percent patients in duloxetine group achieved remission in comparison to none in fluoxetine group. None of the patient, in any group, reported relapse. Adverse effects were mild to moderate in severity.In conclusion duloxetine has a better pharmacological profile over fluoxetine in terms of efficacy and safety.

Keywords: Major Depressive Disorder; Duloxetine; Remission; Relapse

1. INTRODUCTION

Major depressive disorder (MDD) represents one of the most serious conditions encountered in clinical practice affecting 340 million people globally [1]. It is currently estimated to be the fourth leading cause of disability adjusted life years (DALY) in all age groups. Selective serotonin reuptake inhibitors (SSRIs) are the treatment of choice for moderate to severe depression [2]. However, studies have shown that up to half of all depressed patients fail to respond to SSRIs treatment. Thus there exists need for alternatives with multiple mechanisms along with avoidance of unwanted effects of tricyclic antidepressants (TCA) [3].

Duloxetine, a newer antidepressant which belongs to selective serotonin nor-epinephrine reuptake inhibitor (SSNRI) group, has been approved for MDD [4]. In double blind, placebo controlled multicentre, randomized, 9 weeks trials Gartlehner *et al.* [5] and Detke *et al.* [6] concluded that, for treatment of MDD Duloxetine is safe and effective. In 8 weeks, fluoxetine comparator trial, remission rates were high in duloxetine group, but not statistically significantly than fluoxetine group [7]. For long term treatment of MDD duloxetine has been shown to be effective in 52 weeks non-comparator open label multicentre trial [8].

As previous studies indicate that duloxetine may have some advantages in MDD, however there are limited numbers of studies which assess SSRIs as active comparators [9]. These studies are lacking with data's either duo to of short duration of study or duo to of lack of standard comparator. Therefore the current study was planned to compare remission rate, relapse rate and safety of oral duloxetine (SSNRI) with fluoxetine (SSRI) in 16 weeks comparative study.

2. MATERIALS AND METHODS

An open label study was conducted in the Department of

Pharmacology in collaboration with the Department of Psychiatry, Lady Hardinge Medical College and associated Smt. Sucheta Kriplani (SSK) Hospital, New Delhi. Sixty six Patients, aged between 18 and 65 years with either sex, presenting with MDD, as per ICD-10 DCR criteria [10], on an outpatient basis, were included after obtaining an informed consent. The study protocol was approved by Institution Ethics Committee. Baseline disease severity was defined by patients' scores on the Hamilton Rating Scale for Depression-24 items (HAMD-24) [11]. Exclusion criteria were history of psychosis or bipolar disorder, substance abuse disorder, intake of psychoactive medication during past two weeks. Patients with serious medical illness, serious suicidal risk requiring hospitalization and pregnant or lactating women were also excluded from study. A complete workup was done for all patients with respect to history, baseline investigations (weight, blood pressure, hemoglobin, complete blood count, blood sugar, serum electrolyte, kidney and liver function test) and clinical examinations.

All patients were randomly assigned to Fluoxetine 20-60 mg per oral (FLX) and Duloxetine 40-60 mg per oral (DLX) group. Both the study drugs were administered daily for 16 weeks and dose was titrated as per the need of patient.

3. CLINICAL ASSESSMENT OF PATIENTS

Clinical response of the patients was assessed at baseline (0 week), 1 week, 2 week, 4 week, 8 week and 16 week by using HAMD-24 scale. Improvement in HAMD-24 score from baseline was used as the primary efficacy measure to assess remission and relapse. Remission was defined as a total score on HAMD-24 of ≤ 7 and relapse was defined as an episode of MDD that occurs within 6 months after either response or remission [12].

4. SAFETY PROFILE

The patients were monitored for any adverse drug effects during the course of study. The patients as well as the informants were asked in detail about any adverse drug effects the course of treatment on each visit.

5. STATISTICAL ANALYSIS

Baseline and end-point HAMD-24 scores were compared within groups using the paired student's t-test. Unpaired student's t-test was used for comparison of parameters between two study groups. Safety profile and response rate were compared between two treatment groups by Fisher's exact test. P value of < 0.05 was taken to be significant.

6. RESULTS

A total of 66 patients enrolled, out of which 60 completed the study. Biosocial and demographic characteristics of patients were comparable (**Table 1**). Baseline bodyweight, pulse rate, blood pressure and biochemical parameters in both the groups were matching. On completion of treatment there were reduction in mean body weight in both groups which was statistically significant ($P < 0.05$). In FLX group bodyweight reduced from 59.27 ± 1.93 kg to 58.84 ± 1.84 kg (p value < 0.05) while in DLX group body weight reduced from 57.37 ± 1.53 kg to 56.77 ± 1.52 kg after 16 weeks. In DLX group blood sugar levels also reduced significantly (p value < 0.05) from 104.60 ± 2.33 mg/dl to 102.73 ± 2.03 mg/dl while there was statistically significant rise in hemoglobin at end of the study. The mean values of other parameters after16 week were comparable within as well as between the both groups.

7. CLINICAL RESPONSE (EFFICACY)

Baseline HAMD-24 scores in FLX and DLX groups were 17.46 ± 0.13 and 17.70 ± 0.08 respectively (**Table 2**). In FLX group the HAMD-24 scores reduced signifycantly from 17.46 ± 0.13 to 16.16 ± 0.19 (p value < 0.05) after first week followed by 16.3 ± 0.19, 14.8 ± 0.19, 12 ± 0.15 and 9.9 ± 0.14 HAMD-24 scores respectively at 2 weeks, 4 week, 8 week and 16 week. Similarly in DLX group the score reduced significantly from 17.70 ± 0.08 to 16.80 ± 0.21 after first week, there after 15.96 ± 0.16, 14.86 ± 0.20, 11.10 ± 0.24 and 8.06 ± 0.15 HAMD-24

Table 1. Comparison of biosocial characteristics of study patients.

Characteristics	FLX	DLX
No. of patients	30	30
Age (years) Mean ± SEM	34.06 ± 1.76	32.50 ± 2.01
Sex [n]		
Male (n = 18)	12 (40.0%)	6 (20.0%)
Female (n = 42)	18 (60.0%)	24 (80.0%)
Marital status [n (%)]		
Married	20 (66.66%)	20 (66.66%)
Unmarried	5 (16.6%)	8 (26.6%)
Divorce	5 (16.6%)	2 (06.6%)

Table 2. Mean fall in HAMD-24 scores with time in both groups (Mean ± SEM).

Time	FLX	DLX
Baseline (0 week)	17.46 ± 0.13	17.70 ± 0.08
1 week	16.16 ± 0.19	16.80 ± 0.21
2week	16.30 ± 0.19	15.96 ± 0.16
4 week	14.83 ± 0.19	14.86 ± 0.20
8 week	12.06 ± 0.15	11.10 ± 0.24*
16 week	9.96 ± 0.14	8.06 ± 0.15***

*P < 0.05 (Student's t-test), *** P < 0.01(Student's t-test).

scores were recorded at 2 week, 4 week, 8 week and 16 week respectively.

On comparing FLX and DLX groups the mean fall in HAMD-24 scores were comparable till 4 weeks. Thereafter, at 8 weeks the mean fall in HAMD score was significantly greater in DLX group *i.e.* 11.10 ± 0.24 ($p < 0.05$). At 16 weeks the mean fall was highly significant (p value < 0.01) in DLX group vs. the FLX group (**Table 2**).

8. REMISSION AND RELAPSE

In FLX group there was no remission till 16 weeks of treatment, where as in DLX group 9 patients remitted with in 16 weeks. None of the patient, in any group, reported relapse of any sign or symptoms of MDD during study period.

9. ADVERSE DRUG EVENTS

A total of 99 adverse events were recorded in FLX group as compared to 37 adverse events in DLX group. Nausea was frequent adverse events in FLX group as compared to DLX. Insomnia, sweating and diarrhoea were the other frequent adverse events observed in FLX group. Nausea, dry mouth, diarrhoea and fatigue constituted the frequent adverse events in DLX group. These adverse effects were mild to moderate and there were no serious adverse event in both the groups (**Table 3**).

10. DISCUSSION

After one week of treatment, overall fall in HAMD-24 score was greater in DLX group than FLX group. In DLX (20 mg twice daily) group, reduction in HAMD-24 score at 8 and 16 weeks from baseline value was –6.6 and –9.64 whereas in FLX (20 mg once daily) fall was –5.4 and –7.5. Similarly, Goldstein *et al.* (2002) reported a higher fall in HAMD scores with duloxetine

Table 3. Adverse drug events observed in the two treatment groups.

Adverse Event	FLX (n-99)	DLX (n-37)
Nausea	17 (17.1%)	8 (21.6%)
Dry mouth	7 (7%)	5 (13.5%)
Constipation	5 (5%)	4 (10.8%)
Diarrhoea	13 (13.1%)	4 (10.8%)
Vomiting	7 (7%)	2 (5.4%)
Anorexia	12 (12.1%)	2 (5.4%)
Fatigue	1 (1%)	3 (8.1%)
Dizziness	2 (2%)	3 (8.1%)
Somnolence	0	2 (5.4%)
Sweating	14 (14.1%)	3 (8.1%)
Insomnia	15 (15.1%)	1 (2.7%)
Anxiety	3 (3%)	0
Ejaculation delayed	3 (3%)	0

(n- Number of total adverse events)

(–9.73) than with fluoxetine (–7.75) or placebo (–6.6) in an 8 week study [7]. Hudson *et al.* (2007) reported higher reduction in HAMD-24 scores at 12 weeks with duloxetine (60 mg/day) [1]. The early and higher response rate may be due to higher dose of duloxetine. In present 16 week study and previous short period studies, duloxetine shown to have better efficacy than SSRIs, which may be due to its combined effects on both the serotonin and norepinephrine systems. This dual mechanism makes duloxetine potent (4.5 times) and to act faster than SSRIs [1,13]. Also, when compared with other dual action antidepressants like venlafaxine, duloxetine appears to be more balanced inhibitor of serotonin and norepinephrine reuptake. Further, experimental data predicted that dual serotonin and norepinephrine exert analgesic effects via descending pain pathways; therefore duloxetine is more suitable in depression with somatic symptoms [1].

In this study response rate from one week onward was 100% in both the groups and remission rate was 33% and 0% in DLX and FLX group respectively at end of 16 weeks. Goldstein D.J. *et al.* (2002) also reported response and remission rate of 64% and 56% respectively for du- loxetine (20 mg twice daily), compared with 52% and 30% for fluoxetine (20 mg once daily) and 48% and 32% for placebo, indicates superior efficacy of duloxetine over Fluoxetine [7]. In another 8 weeks trial, Goldstein DJ (2004) showed response and remission rates were 54% and 37% for duloxetine (40 mg once daily), 60% and 58% for duloxetine (80 mg) in comparison to

50% and 34% for paroxetine (20 mg once daily) and 30% and 28% for placebo [14]. In a metanalysis by Jennifer *et al*. (2006) the remission rate was found to be superior for duloxetine as compared to SSRIs (43% vs. 38%). Also when alternative definition of remission (MADRS score $\leq$ 12) was used, duloxetine was found to be sig- nificantly better than SSRIs [9].

In both groups there was no relapse till 16 weeks. Although the study duration of 4 months is not sufficient to comment upon relapse rate but as per definition of relapse there was no reoccurrence of sign and symptoms after response has been establish in both groups. Relapse rate for the fluoxetine group was found to be 35.2% after 6 month and 45.9% after 1 year of treatment [15]. In randomized double blind, multicenter, placebo controlled, parallel group study, David G.P. *et al.* (2006) showed that duloxetine (60 mg once daily) was effective in the prevention of relapse of MDD during continuation of treatment [16]. Geddes *et al.* (2003) had concluded on systemic review of 31 antidepressant trials that continuing with antidepressant reduced the odds of depressive relapse by 70% compared to placebo. This suggests continued antidepressant treatment has ability to reduce the chances of depressive relapse [17].

Duloxetine has better adverse effect profile. In DLX group 30% of patients experienced adverse events in which nausea (26.7%) and dry mouth (16.7%) were principally reported. Other adverse events were diarrhea, fatigue, dizziness and vomiting. Sharma A. *et al.* (2000) have also reported similar adverse effect profile for duloxetine [18]. As a reuptake inhibitor of norepinephrine, duloxetine has been found to be associated with mean increase in heart rate as well as blood pressure (less than 2 mmHg) [8]. No such cardiovascular adverse effect was observed in this study .In FLX group, patients mainly reported nausea (56.6%) and insomnia (50%) followed by other adverse events which includes sweating, diarrhea, anorexia, vomiting, dry mouth and anxiety. In a study of 6 months, Zajecka *et al*. (1999) have reported similar adverse events [19].

Ejaculation delayed was reported by 3 male patients (10%) in FLX group. Similarly Harman J.B. *et al.* (1990) also reported frequent sexual dysfunction with Fluoxetine [20]. Comparatively in DLX group, there was no sexual dysfunction occurred in any patients although it has been reported by Detke M.J. *et al.* (2002) [21].

All the adverse effects during study period were mild to moderate severity. On the WHO casualty assessment scale they were classified as possible. Favorable adverse effect profile of duloxetine may be because of its less affinity for dopaminergic, adrenergic, cholinergic, histaminergic, opioid, glutamate and GABA receptors [1].

11. CONCLUSIONS

In this comparative study duloxetine was found to be more efficacious than fluoxetine in reducing HAMD-24 score in 16 weeks as well as found to have better adverse effect profile than fluoxetine. This suggests that for treatment of MDD duloxetine is better option over fluoxetine.

REFERENCES

[1] Hudson, J.I., Perahia, D.G., Gilaberte, I., Wang, F., Watkin, J.G. and Detke, M.J. (2007) Duloxetine in the treatment of major depressive disorder: An open-label study. *BMC Psychiatry*, **7(1)**, 43. http://www.biomedcentral.com

[2] Williams, R.H.M. (2005) Duloxetine (Cymbalta): a new treatment option in depression. *Prescriber*, **16(12)**, 49-50. http://www.escriber.com

[3] McIntyre, R.S., Muller, A., Mancini, D.A. and Silver, E.S. (2003) What to do it an initial antidepressant fails. *Canadian Family Physician*, **49(4)**, 449-457.

[4] Gartlehner, G., Hansen, R.A., Kahwati, L., *et al*. (2006) Drug class review on second generation antidepressants Final Report March. Oregan Evidance based Practice Centre, Oregan Health & Science University, Portland.

[5] Detke, M.J., Lu, Y., Goldstein, D.J., Hayes, J.R. and Demitrack, M.A. (2002) Duloxetine, 60 mg once daily for major depressive disorder: a randomized double blind placebo controlled trial. *Journal of Clinical Psychiatry*, **63(4)**, 308-315.

[6] Detke, M.J., Lu, Y., Goldstein, D.J., McNamara, R.K. and Demitrack, M.A. (2002) Duloxetine, 60 mg once daily dosing versus placebo in the acute treatment of major depression. *Journal of Psychiatric Research*, **36(6)**, 383-390.

[7] Goldstein, D.J., Mallinckrodt, C., Lu, Y., Demitrack and M.A. (2002) Duloxetine in the treatment of major depressive disorder: A double blind clinical trial. *Journal of Clinical Psychiatry*, **63(3)**, 225-231.

[8] Raskin, J., Goldstein, D.J., Mallinckrodt, C.H. and Ferguson, M.B. (2003) Duloxetine in the long term treatment of major depressive disorder. *Journal of Clinical Psychiatry*, **64(10)**, 1237-1244.

[9] Kirwin, J.L. and Gören, J.L. (2005) Duloxetine: a dual serotonin-norepinephrine reuptake inhibitor for treatment of major depressive disorder. *Pharmacotherapy*, **25(3)**, 396-410.

[10] Depressive Episode F-32 (1992) The ICD-10 classification of mental and behavioural disorders, diagnostic criteria for research. World Health Organization, Geneva. 81-85.

[11] Blacker, D. (2005) Psychiatric rating scales. In. Kaplan, H.I. and Sadock, B.J. Eds. *Comprehensive textbook of Psychiatry*, 8th Edition, Williams and Wilkins, Baltimore.

[12] Segal, Z.V., Pearson, J.L. and Thase, M.E. (2003) Challenges in preventing relapse in major depression: Report of a National Institute of Mental Health Workshop on state of the science of relapse prevention in major de-

pression. *Journal of Affective Disorders*, **77(2)**, 97-108.

[13] Schweitzer, I., Burrows, G. and Tuckwell, V., *et al*. (2001) Sustained response to open-label venlafaxine in drug-resistant major depression. *Journal of Clinical Psychopharmacology*, **21(2)**, 185-189.

[14] Goldstein, D.J., Lu, Y., Detke, M.J., Wilste, C., Mallinckrodt, C. and Demitrack, M.A. (2004) Duloxetine in the treatment of depression: A double blind placebo controlled comparison with paroxetine. *Journal of Clinical Psychopharmacology*, **24(4)**, 389-399.

[15] McGrath, P.J., Stewart, J.W., Quitkin, F.M., *et al*. (2006) Predictors of Relapse in a Prospective Study of Fluoxetine Treatment of Major Depression. *American Journal of Psychiatry*, **163 (9)**, 1542-1548.

[16] Perahia, D.G., Gilaberte, I., Wang, F., *et al*. (2006) Duloxetine in the prevention of relapse of major depressive disorder: a double blind placebo controlled study. *British Journal of Psychiatry*, **188**, 346-353.

[17] Geddes, J.R., Carney, S.M., Davis, C., *et al*. (2003) Relapse prevention with antidepressant drug treatment in depressive disorders: A systemic review. *Lancet*, **361 (9358)**, 653-661.

[18] Sharma, A., Goldberg, M.J. and Cerimele, B.J. (2000) Pharmacokinetics and safety of duloxetine, a dual serotonin and norepinephrine reuptake inhibitor. *Journal Clinical Pharmacology*, **40(2)**, 161-167.

[19] Zajecka, J., Amsterdam, J.D., Quitkin, F.M., *et al*. (1999) Changes in adverse events reported by patients in six months of fluoxetine therapy. *Journal of Clinical Psychiatry*, **60(6)**, 389-394.

[20] Harman, J.B., Brotman, A.W., Pollock, M.H., Falk, W.E., Biederman, J. and Rosenbaum, J.F. (1990) Flouxetine induced sexual dysfunction. *Journal of Clinical Psychiatry*, **51(1)**, 25-27.

[21] Detke, M.J., Lu, Y., Goldstein, D.J., Hayes, J.R. and Demitrack, M.A. (2002) Duloxetine, 60 mg once daily for major depressive disorder: A randomized double blind placebo controlled trial. *Journal of Clinical Psychiatry*, **63(4)**, 308-315.

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Antibiotic sensitivity pattern of common bacterial pathogens in NICU and neonatal ward in Hamedan province of Iran

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ABSTRACT

Bacterial pathogens and drug resistance are different in hospitals of each country. In this study we determined bacterial path- ogens and drug sensitivity in the neonatal ward and neonatal intensive care unit (NICU) in Ekbatan hospital in Hamedan. This cross-sectional descriptive study was done on 1150 hospitalized neonates in neonatal and NICU wards of Ekbatan hospital of the Hamadan university of medical sciences from September 2004 to September 2006. Blood, cerebrospinal fluid (CSF), urine, stool, eye excretion, synovial fluid, umbilical secretion and ascitic fluid were evaluated. Positive cultures were evaluated for antibiotic resistance with disk diffusion test methed. All of the data in questionnaires was analyzed with SPSS 13. Cultures including blood, urine, CSF , stool, eye excretion, synovial fluid, umbilical secretion and ascitic fluid was done in 417 neonates (833 cultures). These cultures were including: urine, 323 cases (38.8%) blood 293 cases (35.2%), CSF 180 cases (21.6%) , stool 17 cases (2%), eye secretion 16 cases (1.9%) and other secretions (synovial, umbilical, etc) 4 cases (0.5%). The cultures were positive in 105 cases (25.2%). 60 male neonates (57.1%) and 45 female neonates (42.9%) were culture positive. The most common microorganisms were E coli 66.7% (70 cases), Klebsiella 10.5% (11 cases). Drug resistance was high in these microorganisms. The most common microorganisms were Ecoli and klebsiella. Drug resistance was high in the isolated microorganisms.

Keywords: Drug Resistance; Neonate; Bacterial Infections

1. INTRODUCTION

There has been high incidence of antimicrobial resistance in bacterial infections in different parts of a country and also in any hospital [1-3]. Different mechanisms are involved in drug resistance of microorganisms. It may be genetic or non genetic in origin [4]. Widespread use of broadspectrum antibiotics is the most important factor, so antibiotic control policy has great importance in drug resistance control [5]. The incidence of infections in hospitalized neonates is very high. After widespread use of antibiotics in agriculture industry and medicine from 1950, antibiotics resistance in common pathogenic agents had increasingly emerged [6]. In developed countries constant investigation in this field had done for identifying antibiotic resistance pattern. Recently, the spectrum and resistance of the pathogenic bacteria have constantly changed year after year because of wide application of antimicrobial drugs [5-9]. It is necessary to treat neonatal infections by empirical use of antimicrobial drugs as soon as possible to reduce the mortality of them. It is based on the knowledge of epidemiology of bacterial susceptibility pattern in each area [6-15]. This study had done for identifying antimicrobial susceptibility pattern in a western province of Iran in neonatal ward to help the treatment of infected neonates.

2. MATERIAL AND METHOD

This cross-sectional descriptive study was done on 1150 hospitalized neonates in neonatal and neonatal intensive care unit (NICU) wards of Ekbatan hospital of the Hamadan University of Medical Sciences from September 2004 to September 2006. Approval from Hamadan University of Medical Sciences was obtained prior the study. All of the samples that had sent for bacteriological analysis entered the study. They included blood, cerebro-spinal fluid (CSF), urine, stool, eye excretion, syno-

vial fluid, umbilical excretion and peritoneal fluid.

Urine samples in neonates had obtained by suprapubic method. In case of bacterial growth, identification of the bacteria had done and antibiogram by disc diffusion method had performed (Karbibauer method, Padtan Teb antibiotic disc Company).

The quality control modalities had done on the National Reference Laboratory of Health standards. The antibiotics susceptibility test results had entered the questionnaires. The data had analyzed by SPSS software (version 13).

3. RESULTS

A total of 1150 hospitalized neonates entered the study. There were 417 cultured samples, 239 cases (56.1%) were male and 183 cases (43.9%) were female.

The mean age of neonates was 11.3 ± 9.3 days, (1 to 30 days).

We found 833 cultures in 417 cases, they included: urine culture 322 (38.7%), blood culture 293 (35.2%), CSF culture 180 (21.6%), stool culture 17 (2%), eye excretion 16 (1.9%), others (synovial fluid, cord excretion …) 5 (0.6%).

In this study 25.5% of the cultures were positive and 74.8% were negative. 57.1% (60 neonates) of positive cultures were male and remaining 42.9% (45 neonates) were female.

Eye excretion was the most positive culture (81.2%). (**Table 1**) **Table 1** shows the results of cultures.

The most frequent isolated microorganisms were E.coli 66.7% (70 cases) and Klebciella 10.5% (11 cases) in decreasing order (**Figure 1**).

Table 2 shows the frequency of isolated bacteria in the culture.

Table 3 shows the sensitivity and resistance pattern of bacteria cultured in urine culture. **Tables 4** and **5** reveal of the bacterial susceptibility tests in the neonates.

4. DISCUSSION

Bacterial infection is still prevalent in newborns and it is a major medical problem [1-3]. According to the european countries reports, its prevalence had not changes in previous half decade, whereas bacterial etiologic agents had changed. From 1928 to 1932 beta hemolytic streptococci with 38% prevalence and staphylococcus aurous with 28% were the most frequent causes of septicemia, although the former is not so prevalent yet [13-14]. In the present study Escherichia coli (Ecoli) was 66.7% and Klebciella was 10.5%, they were the more frequent bacteria isolated from urine, blood and eye excretion cul tures. Ecoli was the most frequent isolated bacteria from urine, eye excretion and blood cultures in an order of

Table 1. Abundance distribution of positive cultures according to the source of culture in hospitalized neonates.

Source of culture	Culture results	
	Negative (%) NO	Positive (%) NO
Urine (N = 323)	253 (78.3)	70 (21.7)
Blood (N = 293)	278 (94.9)	15 (5.1)
CSF (N = 180)	180 (100)	-
Stool (N = 17)	14(82.4)	3 (17.6)
Eye secretions (N = 16)	3(18.8)	13(81.2)
Other body fluid (N = 5)	1(20)	4(80)

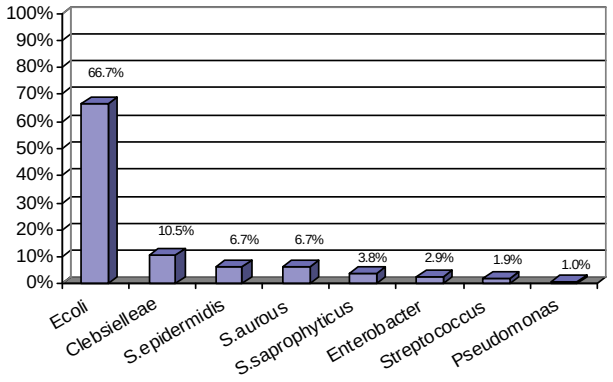

Figure 1. Abundance distribution of isolated microorganisms in culture positive newborns.

Table 2. Abundance distribution of isolated microorganisms according to the source of culture in hospitalized neonates.

Isolated microorganisms	Source of culture				
	Urine (N = 70)	Blood (N = 15)	Stool (N = 3)	Eye secretions (N = 13)	Other body fluid (N=4)
E.coli	(%77.1)54	(%53.3)8	-	(46.2%)6	(50%)2
Klebsiella	(%12.9)9	(%6.7)1	(33.3)1	-	-
S.Saprophyticus	(%5.7)4	-	-	-	-
Enterobacter	(%1.4)1	(%6.7)1	(33.3)1		-
S.Aurous	(%2.9)2	(%6.7)1	-	(%23.1)3	(%25)1
S.Epidermidis	-	(%26.7)4	-	(%23.1)3	-
pseudomonas	-	-	(33.3)1		-
Streptocucus	-	-	-	(%7.7)1	(%25)1

77.1%, 46.2% and 53.6% respectively. These findings are consistent with the results of Eghbalian *et al*. study [15]. Yalaz *et al*. in Turkey studied 909 newborns, 9.1% of them had sepsis, the isolated bacteria were: coagulase negative staphylococcus 31.3%, fungi 19.2%, staphylococcus aurous 13%, Klebsiella Pneumonia 10.5% in decreasing order [11].

Another study of Aurangzeb *et al*. at 2003 revealed 112 hospitalized newborns as sepsis, 67% had positive blood culture and E.coli was the most frequent cause with 77.1% frequency [12]. Bacterial meningitis has various ethologic factors and majority of authors believe that its agents varies according to age and geographic distribution [1,3,15,16]. In present study we did not found any positive cerebrospinal fluid culture in the newborns. In our study E.coli, the most prevalent isolated microorganism, shows high degree resistance to cephalosporins. It showed 75.9% resistance in urine culture to Ceftriaxone, and 60% to Ceftizoxime (**Table 3**).

When we separated the E.coli from the blood and eye excretion cultures, most of them were resistant to Cephalosporins specially to Ceftriaxone. Also E.coli was resistant to majority of aminoglycozid antibiotics including Amikacin, Gentamycin and Tobramycin. (**Tables 4, 5**) In our study the resistance of E.coli to the Ciprofloxacin, Nalidixic acid, Nitrofurantoin and Cotrimoxazol were in a decreasing order (**Tables 3, 4, 5**) .These findings are consistent with the results of a similar study [15]. Aurangzeb *et al*. in a similar study showed high degree resistance of gram negative bacteria to conventional antibiotics: 79.3% to Ampicillin, 74.6% to Amoxicillin, 71.6% to Ceftazidime, 50.2% to Cefotaxime, 43.2% to Gentamycin, 34.3 to Tobramycin, 23.6% to Imipenem, 22.3% to Amikacin and 11.9% to Ciprofloxacin [12]. Also staphylococcus aurous showed 75% resistance to Ampicillin [12]. The present study revealed high percentage resistance of staphylococcus aurous to cephalosporin and other broad spectrum antibiotics.

Table 3. Percentage of sensitivity and resistance of isolated bacteria from positive urine culture in hospitalized neonates.

Microorganisms										Antibiotic
S.Aurous		Enterobacter		S.Saprophyticus		Clebsiellea		E.coli		
Resistance	Sencetive	Resistance	Sencetive	Resistance	Sencetive	Resistance	Sencetive	Resistance	Sensitive	
---	---	---	---	---	---	6 (%100)	0	22 (%9/75)	7 (%1/24)	**Ceftriaxon**
1 (%100)	0	0	1 (%100)	3 (%100)	0	4 (%7/66)	2 (%3/33)	28 (%1/65)	15 (%9/34)	**Amikacin**
---	---	0	1 (%100)	0	1 (%100)	0	6 (%100)	6 (%2/18)	27 (%8/81)	**Ciprofloxacin**
0	1 (%100)	1 (%100)	0	3 (%100)	0	6 (%75)	2 (%25)	32 (%4/74)	11 (%6/25)	**Jentamycin**
---	---	---	---	---	---	1 (%100)	0	12 (%7/85)	2 (%3/14)	**Tobramycin**
---	---	0	1 (%100)	1 (%3/33)	2 (%7/88)	1 (%1/11)	8 (%9/88)	11 (%25)	33 (%75)	**Nalidixic acid**
---	---	---	---	---	---	2 (%100)	0	9 (%60)	6 (%40)	**Trimetoprim**
0	1 (%100)	0	1 (%100)	1 (%100)	0	6 (%100)	0	21 (%60)	14 (%40)	**Ceftixocim**
1 (%100)	0	0	1 (%100)	2 (%7/66)	1 (%3/33)	5 (%4/71)	2 (%6/28)	13 (%3/33)	26 (%7/66)	**Nitrofurantoin**
---	---	---	---	0	2 (%100)	2 (%3/33)	4 (%7/66)	9 (%36)	16 (%64)	**Cotrimoxazol**
---	---	1 (%100)	0	1 (%50)	1)50(%	4 (%100)	0	15 (%60)	10 (%40)	**Cefotacxim**
---	---	---	---	2 (%100)	0	---	---	---	---	**Vancomycine**
1 (%100)	0	---	---	2 (%100)	0	1 (%100)	0	5 (%50)	5 (%50)	**Cephalexin**
---	---	---	---	0	1 (%100)	---	---	4 (%100)	0	**Cephtazidim**
---	---	1 (%100)	0	---	---	---	---	3 (%60)	2 (%40)	**Ampicillin**
---	---	---	---	---	---	---	---	0	4 (%100)	**Cephalotin**

Table 4. Percentage of sensitivity and resistance of isolated bacteria from positive blood culture in hospitalized neonates.

Microorganisms										Antibiotic
S.Aurous		Enterobacter		S.Epidermidis		klebsiellea		E.coli		
Resistance	Sencetive	Resistance	Sencetive	Resistance	Sencetive	Resistance	Sencetive	Resistance	Sensitive	
---	---	---	---	0	1 (%100)	---	---	4 (%100)	0	**Ceftriaxon**
---	---	0	1 (%100)	0	1 (%100)	1 (%100)	0	3 (%50)	3 (%50)	**Amikacin**
---	---	0	1 (%100)	0	1 (%100)	---	---	0	5 (%100)	**Ciprofloxacin**
1 (%100)	0	0	1 (%100)	0	1 (%100)	1 (%100)	0	3 (%60)	2 (%40)	**Gentamycin**
---	---	0	1 (%100)	---	---	0	1 (%100)	1 (%25)	3 (%75)	**Nalidixic acid**
---	---	---	---	1 (%50)	1 (%50)	0	1 (%100)	2 (%50)	2 (%50)	**Ceftizoxime**
---	---	0	1 (%100)	---	---	1 (%100)	0	2 (%28/6)	5 (%71/4)	**Tetracyclin**
---	---	1 (%100)	0	0	1 (%100)	1 (%100)	0	3 (%42/9)	4 (%57/1)	**Nitrofurantoin**
---	---	1 (%100)	0	---	---	0	1 (%100)	2 (%50)	2 (%50)	**Cotrimoxazol**
---	---	---	---	3 (%100)	0	---	---	---	---	**Cloxacillin**
1 (%100)	0	---	---	0	1 (%100)	1 (%100)	0	3 (%6)	2 (%40)	**Cefotacxim**
---	---	---	---	0	4 (%100)	---	---	---	---	**Vancomycine**

Table 5. Percentage of sensitivity and resistance of isolated bacteria from positive eye excretions culture in hospitalized neonates.

Microorganisms								Antibiotic
Streptocucus		S.Epidermidis		S.Aurous		E.coli		
Resistance	Sencetive	Resistance	Sencetive	Resistance	Sencetive	Resistance	Sencetive	
---	---	---	---	(%100) 1	0	(%100) 4	0	**Ceftriaxon**
(%100) 1	0	(%100) 1	0	(%50) 1	(%50) 1	(%40) 2	(%60) 3	**Amikacin**
---	---	(%100) 1	0	---	---	(%66/7) 2	(%33/3) 1	**Ciprofloxacin**
(%100) 1	0	(%100) 1	0	0	(%100) 1	(%66/7) 2	(%33/3) 1	**Gentamycin**
---	---	(%50) 1	(%50) 1	(%100) 1	0	(%75) 3	(%25) 1	**Ceftixocim**
---	---	---	---	---	---	0	(%100) 3	**Tetracyclin**
---	---	0	(%100) 1	(%100) 1	0	---	---	**Amoxicillin**
---	---	(%100) 2	0	(%100) 1	0	0	(%100) 1	**Cloxacillin**
---	---	(%50) 1	(%50) 1	(%100) 2	0	---	---	**Erythromycin**
0	(%100) 1	(%100) 1	0	(%50) 1	(%50) 1	(%75) 3	(%25) 1	**Cefotacxim**
0	(%100) 1	0	(%100) 3	0	(%100) 1	---	---	**vancomycin**
---	---	0	(%100) 1	(%50) 1	(%50) 1	(%100) 1	0	**Cephalexin**
---	---	(%50) 1		(%100) 1	0	(%100) 2	0	**Ampicillin**
---	---	---	---	0	(%100) 1	---	---	**Chloramphenicol**

5. CONCLUSIONS

Our experience showed that gram-negative bacteria were the most prevalent cause of infections in neonates in our hospital. E.coli and Klebsiella were the most frequent bacteria and majority of them were resistant to broad spectrum antibiotics. Drug resistance to conventional antibiotics is a common problem and it grows readily. Antimicrobials must be administered conservatively according to epidemiologic studies in the region, with confirmed indications, and based on the results of susceptibility tests.

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REFERENCES

[1] Behrman, R.E., Kliegman, R.M. and Jenson, H.B. (2007) Nelson textbook of pediatrics. 17th Edition, Saunders, Philadelphia.

[2] Feigin, R.D., Chery, J.D., Demmier, G.J. and Kapian, S.L. (2004) Textbook of pediatric infectious diseases. 5th Edition, Saunders, Philadelphia.

[3] Helio, S., Sader, A., Gales, C., *et al*. (2001) Pathogen frequency and resistance patterns in Brazilian hospitals: Summary of results from three years of the SENTRY antimicrobial surveillance program. *The Brazilian Journal of Infectious Diseases*, **5(4)**, 200-214.

[4] Brooks, G., Jawet, E., Butel, J., Morse, S., *et al*. (2001) Adelberg's medical microbiology. 22nd Edition, McGraw-Hill, New York.

[5] Zaleznik, D.F. (2005) Hospital-acquired and intravascular device-related infections. In. Kasper, D.L., Braunwald, E., Fauci, A.S., Hauser, S.L., Longo, D.L. and Jameson, J.L., *Harrison's principles of internal medicine*, 16th Edition, McGraw-Hill, New York.

[6] Hawkes, C.A. (2000) Antibiotic resistance: A clinicians perspective. *Mil Medicine*, **165 (7)**, 43-45.

[7] Steven, M.O. (2000) Mechanisms of bacterial antibiotic resistance. In. Mandel, G.L., *Principles and practice of infectious disease*, 5th Edition, Churchill Livingstone, London.

[8] Klugman, K.P. and Mahdi, S.A. (1999) Emergence of drug resistance. *Infectious Disease Clinics of North America*, **13(3)**, 637-646.

[9] Pavia, M., Nobile, C. and Salpietro, L. (2000) Vancomycin resistance and antibiotic susceptibility of enterococci in raw meat. *Journal of Food Protection*, **63(7)**, 912-915.

[10] Binsztein, N., Picandet, A. and Notario, R. (1999) Antimicrobial resistance among species of salmonella, shigella, Escherichia, and aeromonas isolated from children with diarrhea in 7 Argentinean centers. *Revista Latinoamericana de Microbiología*, **41(3)**, 121-126.

[11] Yalaz, M., Cetin, H., Akisu, M., Aydemir, S. and Tunger, A. (2006) Neonatal nosocomial sepsis in a level-III NICU: Evaluation of the causative agents and antimicrobial susceptibilities. *Turkish Journal of Pediatrics*, **48(1)**, 13-18.

[12] Aurangzeb, B. and Hameed, A. (2003) Neonatal sepsis in hospital- born babies: bacterial isolates and antibiotic susceptibility patterns. *Journal of College of Physicians and Surgeons Pakistan*, **13(11)**, 629-632.

[13] Collee, J., Duguide, J. and Fraser, A. (1989) Practical medical microbiology. 13th Edition, Churchill Livingstone, New York.

[14] Bedford, H., Louvois, J., Halket, S., *et al*. (2001) Meningitis in infancy in England and Wales: follow up at age 5 years. *British Medical Journal*, **323(7312)**, 533-536.

[15] Eghbalian, F. and Yousefi, R. (2005) Determining the frequency of the bacterial agents in urinary tract infection in hospitalized patients under 18 years old. *Journal of Army university of medical sciences of the I.R.Iran*, **3(3)**, 636-639.

[16] Wenger, J., Hightower, A., Facklam, R., *et al*. (1990) Bacterial meningitis in the United States. *Journal of Infectious Diseases*, **162(6)**, 1316-1323.

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Modifying action of heavy metal salts on anti-inflammatory aspirin action

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ABSTRACT

Nowadays pollution of the environment is one of the major problems of mankind. Moreover, studying of the effect of different kinds of medicine on selected, specially bred, non-exposed to external pollutants animals is becoming distant from reality. Thus in this work we have investigated the modifying action of heavy metals on anti-inflammatory effect of aspirin. The investigation were carried out on rats which were injected during 7 days intraperitoneally by $PbCl_3$, $HgCl_2$, $CdCl_2$ in concentration of 100, 20, 1 mg/kg accordingly, modulating accumulation of the metals in the organism tissues. On the 8 days inflammation was invoked by formalin. As anti-inflammatory medicine was use aspirin. Judging by obtained results the conclusion can be drawn that cadmium in concentration 1 mg/kg significantly increases anti-inflammatory aspirin activity. The observed outcome can be explained in the following way. It is generally known that zinc in a certain concentration demonstrates anti-inflammatory properties. Being an element of the same group cadmium has similar properties and also can have anti-inflammatory action. Lead and mercury suppressed anti-inflammatory aspirin activity. Obvious inhibitory action of mercury and lead salts on aspirin action related to the fact that these elements by themselves were inflammation factors. From the obtained results the following conclusion can be drawn: a definite dose of anti-inflammatory medicine (aspirin) which is sufficient in normal conditions became less effective against the background of accumulation of ions of some heavy metals in an organism.

Keywords: Heavy Metals; Lead; Mercury; Cadmium; Acetylsalicylic Acid; Prostaglandin; Inflammation; Anti-Inflammatory Activity

1. INTRODUCTION

Nowadays pollution of the environment is one of the major problems of mankind. Long-term observations show that pollution by heavy metals occurs not only in anthropogenic areas but also in the distance from the sources of pollution [1]. Exhausts, sewers, emissions of the factories pollute cities and this is just a small part of what influences our environment. As lots of agricultural lands are subjected to anthropogenic influence foodstuff often contains a maximum permissible dose of heavy metals. In its turn heavy metals which enter an organism with food are especially dangerous because they are in conjunction with biologically-active substances and rather easily penetrate through natural barriers thus violating normal organism functioning [2-4]. A characteristic feature of all heavy metals that increases danger is their cumulation and very slow excretion. So even if the elements come to an organism in small doses which are within the bounds of the norm their concentrations will increase to a harmful level with a lapse of time. We may say that heavy metals accumulate in any human organism living in the condition of high anthropogenic development [5,6]. Their toxic effect both on separate organs and on physiological and mental state of an organism is well known [5,7]. But along with this fact such high concentration of heavy metals significantly modifies not only physiological state of an organism but also its reaction to the influence of various chemical substances including pharmaceutical ones. Moreover, studying of the effect of different kinds of medicine on selected, specially bred, non-exposed to external pollutants animals is becoming distant from reality. Thus in this work we have investigated the modifying action of heavy metals on anti-inflammatory effect of aspirin.

2. MATERIALS AND METHODS

The investigations were carried out on white outbred male rats weighing between 140-180 grams. The group

of rats was divided into 4 subgroups 10 rats in each group. One of the subgroups served as a control one. These rats were not subjected to the influence of heavy metals. For the estimation of the modifying action of heavy metals the 3 subgroups of rats were injected during seven days intraperitoneally by $PbCl_3$, $HgCl_2$, $CdCl_2$ in concentration of 100, 20, 1 mg/kg accordingly, modulating accumulation of the metals in the organism tissues. On the eighth day inflammation of soft tissues of hind legs was invoked in all the 4 subgroups of the rats. The inflammatory process was stimulated by subcutaneous introduction of 0.2 ml of 1% solution of formalin into the rat hind leg–the so called "formalin test". Then aspirin was injected to the subgroups intraperitoneally in a form of solution at a rate of 40 mg/kg. Measurements of size of a swollen limb began at the thirtieth minute after the injection. 8 measurements were done with the interval of 12 minutes. In all the solutions physiological solution was a dissolvent. The results of the experiments were calculated statistically using Mann-Whitney U test. During the experiments all the ethic norms were observed.

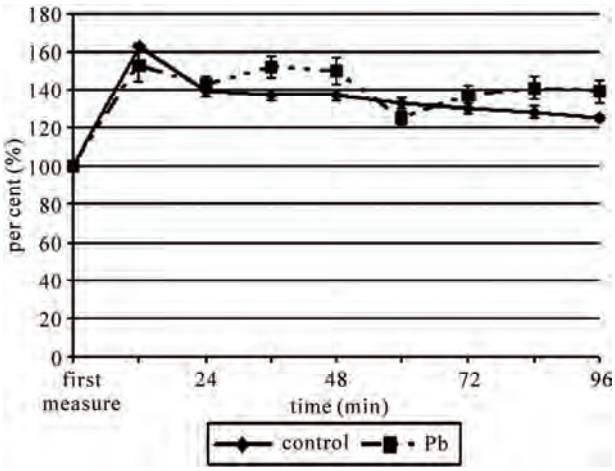
3. RESULTS OF THE EXPERIMENTS

3.1. Modifying Action of Lead on Anti-Inflammatory Aspirin Action

The analysis of the dynamics of the change of aspirin anti-inflammatory activity against the background of increased lead concentration revealed some degradation of therapeutic activity of acetylsalicylic acid. But analysis didn't show essential differences in comparison with the control subgroup as significant differences ($p < 0.05$) are marked just only for one measuring position—the sixtieth minute from the beginning of the measurement. Values of the dimensions of inflamed rat leg in the control subgroup and the experimental subgroup did not have significant differences in all the rest time intervals. But despite this fact kinetics of rat leg dimensions of the control subgroup had practically linear dependence and went in lesser values in comparison with the same parameter in the subgroup of rats that was subjected to lead influence. The picture of change of inflamed limb color was also important: intensity of red color significantly decreased in control subgroup by the end of the experiments and on the contrary it remained lilac-red in the experimental subgroup. Summarizing all received results one could conclude that lead showed antagonistic interaction with anti-inflammatory aspirin activity.

3.2. Modifying Action of Mercury

Comparative analysis of time dynamics of swollen limb dimensions in a control subgroup with the subgroup of rats that was injected with mercury allowed to reveals the following: significant differences of swollen limb dimensions were marked starting from the sixtieth minute of the measurements (level of significance is shown in the table). If take a look at the graph (**Figure 2(a)**) can see clear differences between two subgroups that point undoubtedly to an inhibitory mercury action on anti-inflammatory aspirin activity. At the same time the difference of swollen limb dimension of the rats that were subjected to mercury action and the rats from the control subgroup happened to exceed 30%. Judging by obtained results the conclusion can be drawn that mercury ions significantly suppressed anti-inflammatory aspirin activity.

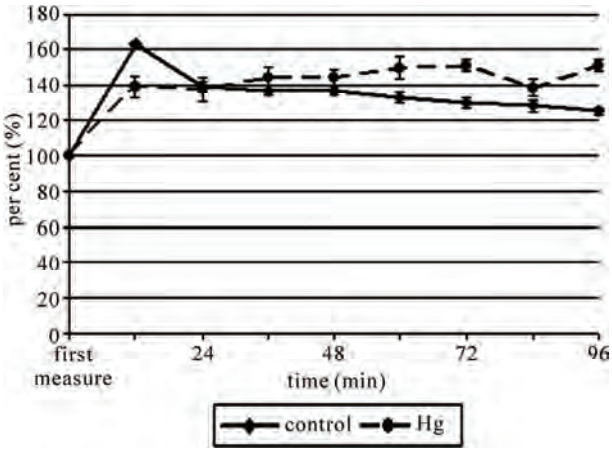


(a)

		aspirin	Aspirin + lead	p-level
Time intervals	*First Measure*	5 ± 0.25	4.7 ± 0.1	
	12	8.2 ± 0.1	7.2 ± 0.65	0.12
	24	7 ± 0.2	6.7 ± 0.3	0.51
	36	6.9 ± 0.2	7.1 ± 0.4	0.51
	48	6.9 ± 0.2	7 ± 0.5	0.74
	60	6.7 ± 0.2	5.8 ± 0.2	0.002
	72	6.5 ± 0.2	6.4 ± 0.3	0.86
	84	6.4 ± 0.2	6.6 ± 0.4	1
	96	6.3 ± 0.1	6.5 ± 0.4	0.41

(b)

Figure 1. Modifying action of lead on anti-inflammatory aspirin action. (a) The changing of mean value of rat's leg dimensions during measuring expressed in per cents (%); (b) Table of mean values of leg dimensions in millimeter (mm)).

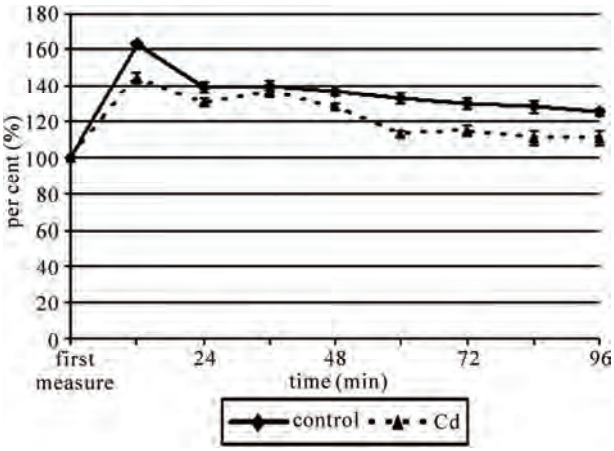


(a)

		aspirin	Aspirin + mercury	p-level
Time intervals	First Measure	5 ± 0.25	5.3 ± 0.2	
	12	8.2 ± 0.1	7.4 ± 0.45	0.17
	24	7 ± 0.2	7.3 ± 0.5	0.5
	36	6.9 ± 0.2	7.7 ± 0.5	0.1
	48	6.9 ± 0.2	7.7 ± 0.4	0.1
	60	6.7 ± 0.2	8 ± 0.5	0.05
	72	6.5 ± 0.2	8.1 ± 0.3	0.005
	84	6.4 ± 0.2	7.8 ± 0.4	0.003
	96	6.3 ± 0.1	8.1 ± 0.3	0.003

(b)

Figure 2. Modifying action of mercury on anti-inflammatory aspirin action. (a) The changing of mean value of rat's leg dimensions during measuring expressed in per cents (%); (b) Table of mean values of leg dimensions in millimeter (mm)).



(a)

		aspirin	Aspirin + cadmium	p-level
Time intervals	First Measure	5 ± 0.25	4.5 ± 0.2	
	12	8.2 ± 0.1	6.5 ± 0.2	0.003
	24	7 ± 0.2	5.9 ± 0.1	0.005
	36	6.9 ± 0.2	6.2 ± 0.2	0.01
	48	6.9 ± 0.2	5.8 ± 0.1	0.009
	60	6.7 ± 0.2	5.1 ± 0.1	0.002
	72	6.5 ± 0.2	5.2 ± 0.2	0.005
	84	6.4 ± 0.2	5 ± 0.2	0.005
	96	6.3 ± 0.1	5 ± 0.2	0.003

(b)

Figure 3. Modifying action of cadmium on anti-inflammatory aspirin action. (a) The changing of mean value of rat's leg dimensions during measuring expressed in per cents (%); (b) Table of mean values of leg dimensions in millimeter (mm)).

3.3. Modifying Action of Cadmium

Evaluating the results obtained in series of experiments with the rat subgroup that was receiving injections of cadmium salt an opposite effect was revealed as to two the previous metals. Dynamics of change of swollen limb dimension in the experimental subgroup demonstrated its agonistic action to aspirin. Analyzing the values of swollen limb dimensions displayed in **Figure 3**, it got obvious that with cadmium injections they were less than in the control subgroup. In spite of relatively small differences they were statistically significant beginning with the first measurement and significantly lesser in comparison with the control subgroup. Given results was an evidence that cadmium improves anti-inflammatory properties of acetylsalicylic acid and the chosen concentration increases the effectiveness of this medicine.

4. DISCUSSION

Thus the results of the experiments explicitly demonstrated that heavy metals noticeably changed therapeutic aspirin activity. Obvious inhibitory action of mercury and lead salts on aspirin action related to the fact that these elements by themselves are inflammation factors [8,9]. From the obtained results the following conclusion can be drawn: a definite dose of anti-inflammatory medicine (aspirin) which is sufficient in normal conditions became less effective against the background of accumulation of ions of some heavy metals in an organism.

The pattern of modifying action of cadmium salt had another direction. Acetylsalicylic acid showed much stronger anti-inflammatory action against the background

of cadmium accumulation in a rat's organism. The observed outcome can be explained in the following way. It is generally known that zinc in a certain concentration demonstrates anti-inflammatory properties. Being an element of the same group cadmium has similar properties and also can have anti-inflammatory action [10-13].

Besides, the observed properties of the metals can be explained through the mechanism of salicylate and aspirin action. As its generally known salicylate action is associated with the inhibition of the synthesis of prostaglandins of various classes that are responsible for penetrability of vessels, edema, chemotaxis. Prostaglandins occur in tissues in trace amount but their concentration increases sharply under the influence of toxic substances and some hormones [14]. First of all inhibition of prostaglandin synthesis under salicylate action is associated with ferment inhibition videlicet cyclooxygenase (COG). The latter leads to synthesis reduction from arachidonic acid of anti-inflammatory prostaglandins potentiating the activity of inflammation mediators—histamine, serotonin, bradykinin. As is known prostaglandins invoke hyperalgesia *i.e.* improve nociceptor sensitivity to chemical and mechanical stimuli. Prostaglandin synthesis inhibition stanch/remove pain, reduce inflammatory reaction and feverish body temperature as well. So a basic anti-inflammatory mechanism of salicylates is COG inhibition but at the same time mercury and lead are toxic substances that invoke additional inflow of prostaglandins to the place of inflammation and that determinates their property to intensify inflammatory process. Regarding cadmium anti-inflammatory effect the following can be added: as is generally known heavy metals can violate ferment interaction due to inhibiting some enzymes. Therefore it is quite possible that cadmium inhibits the synthesis of ferments which participate in the formation of the mediator of inflammation and it determined anti-inflammatory influence of the metal and improves aspirin effectiveness.

5. CONCLUSIONS

1) It is revealed that lead, mercury and cadmium cumulation significantly modified inflammation process flow. Degree and character of inflammation depended on the metal that is accumulated.

2) A tendency of inhibition of acetylsalicylic acid action was traced for lead. Mercury clearly demonstrated pro-inflammatory effect. On the contrary, cadmium improved anti-inflammation action of acetylsalicylic acid.

3) Taking into consideration the obtained results a correction of a therapeutic dose with the adjustment to the level of heavy metals content in an organism becomes actual.

REFERENCES

[1] Duruibe, J.O., Ogwuegbu, M.O. and Egwurugwu, J.N. (2007) Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*, **2(5),** 112-118.

[2] Duffus, J.H. (2002) Chemistry and human health division clinical chemistry section, commission on toxicology. *Applied Chemistry*, **74(5)**, 793-807.

[3] Kumar, A., Dey, P.K., Singla, P.N., Ambasht, R.S. and Upadhyay, S.K. (1998) Blood lead levels in children with neurological disorders. *Journal of Tropical Pediatrics*, **44 (6)**, 320-322.

[4] Miranda, M., López-Alonso, M., Castillo, C., Hernández, J. and Benedito, J.L. (2005) Effects of moderate pollution on toxic and trace metal levels in calves from a polluted area of northern Spain. *Environment International*, **31(4)**, 543-548.

[5] Banks, E.C., Ferretti, L.E. and Shucard, D.W. (1997) Effects of low level lead exposure on cognitive function in children: a review of behavioral, neuropsychological and biological evidence. *Neurotoxicology*, **18(1)**, 237-281.

[6] Brito, G., Díaz, C., Galindo, L., Hardisson, A., Santiago, D. and Montelongo G.F. (2005) Levels of metals in canned meat products: Intermetallic correlations. *Bulletin of Environmental Contamination and Toxicology*, **44(2)**, 309-316.

[7] Walkowiak, J., Altmann, L., Krämer, U., Sveinsson, K., Turfeld, M., Weishoff-Houben, M. and Winneke, G. (1998) Cognitive and sensorimotor functions in 6-year-old children in relation to lead and mercury levels: adjustment for intelligence and contrast sensitivity in computerized testing. *Neurotoxicology and Teratology*, **20(5)**, 511-521.

[8] Mameli, O., Caria, M.A., Metis, F., Solinas, A., Tavera, C., Ibba, A., Tocco, M., Flore, C. and Randaccio, S.F. (2001) Neurotoxic effect of lead at low concentrations. *Brain Research Bulletin*, **55(2)**, 269-275.

[9] Vane, J.R. and Botting, R.M. (1992) Aspirin and Other Salicylates. Chapman & Hall Medical, London.

[10] Fosmire, G.J. (1990) Zinc toxicity. *American Journal of Clinical Nutrition*, **51(2)**, 225-227.

[11] Abou-Mohamed, G., El-Kashef, H.A., Salem, H.A. and Elmazaf, M.M. (1995) Effect of Zinc on the Anti-Inflammatory and Ulcerogenic Activities of Indometacin and Diclofenac. *Pharmacology*, **50(4)**, 266-272.

[12] Sokolik, J., Tumova, I., Blahova, M. and Svajlenova, O. (2006) Anti-inflammatory activity of copper (II) and zinc (II) 3, 6-dimethylsalicylates and their equimolar mixture. *Acta Facultatis Pharmaceuticae Universitatis Comenianae*, **53**, 224-228.

[13] Sokolik, J., Tumova, I., Blahova, M., Bernatova, M. and Svec, P. (2002) Anti-inflammatory activities of (o-cresotato) copper (II) and zinc (II) aqua complexes. *Ceska Slov Farm*, **51**, 205-207.

[14] Eliasson, R. (1963) Prostaglandin: properties, actions and significance. *Biochemical Pharmacology*, **12**, 405-412.

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Rokcall score versus forrest classification in endoscopic management of bleeding peptic ulcer

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ABSTRACT

Acute upper gastrointestinal bleeding (UGIB) remains an important emergency situation. In the last two decades, major developments took place influencing incidence, etiology and outcome of patients with acute UGIB. Peptic ulcer bleeding is the most significant complication of ulcer disease being responsible for 50% of all cases mortality. *Aim of the study*: To compare between endoscopic clip application versus argon plasma coagulation in management of bleeding peptic ulcer (BPU). *Patients and Methods*: Sixty patients suffering from acute UGIB were randomly divided into two groups: group I included 30 patients treated with endoscopic clip application and Group II included 30 patients subjected to endoscopic APC. All patients were classified according to Forrest classification and the clinical Rockall score. *Results:* There were significant differences between the two groups as regard Forrest classification (P < 0.05) there were insignificant difference between the two groups as regard rockall score, site of the ulcer and re bleeding (P > 0.05). Re bleeding was significant with higher Rockall score in group I (P < 0.05) but it was insignificant in group II (P > 0.05). *Conclusion:* Endoscopic application of hemoclips have a less re bleeding rate than Argon plasma coagulation for treatment of bleeding peptic ulcer, although this was statistically insignificant, meanwhile APC is still less cost and easy. Clinical and endoscopic assessment (through Rokcall score and Forrest classification) could help in making best choice for endoscopic management.

Keywords: Endoscope; Bleeding Peptic Ulcer; Argon Plasma Coagulation; Clip Applications

1. INTRODUCTION

Since the late 1980s, endoscopic haemostatic therapy has been widely accepted as the first-line therapy for upper-gastrointestinal bleeding. Most clinical trials demonstrated a reduction in both recurrent bleeding and the need for surgical intervention when endoscopic hemostasis was used [1]. Endoscopic therapy can be broadly categorized into injection therapy, thermal coagulation, and mechanical hemostasis. When analyzed separately, injection therapy, thermal-contact devices, and mechanical treatment all decrease the frequency of recurrent bleeding and rate of surgical intervention [2]. Argon plasma coagulation (APC) is a non contact type of coagulation that is easier to target to bleeding sites. A high-frequency current is transmitted by the ionized, electrically conductive argon gas. The argon gas flows onto the target surface, even if approached tangentially. APC has been used successfully to obtain hemostasis during open surgery. The use of APC in digestive tract endoscopy was first described in 1994. It is being applied more and more widely in the treatment of different GI pathologic disorders, hemorrhagic lesions in particular [3]. The only mechanical therapies widely available are endoscopically placed clips and band ligation devices. Endoscopic clips usually are placed over a bleeding site (e.g. visible vessel) and left in place [4]. This consisted of a stainless steel clip (of size approximately 6 mm long and 1.2 mm wide at the prongs) with a metal deployment device (that could be used to insert the clip into the endoscopic camera, and deployed outside the camera) enclosed in a plastic sheath. These clips were

initially reloadable [5]. Numerous prognostic scores have been devised to aid the gastroenterologist in the management of upper gastro-intestinal bleeding, stratifying individual patients by risk of re-bleeding and death. These scores range from the simple, endoscopy-based analysis of ulcer appearance described by Forrest *et al.* [6], through pre-endoscopic clinical scores such as the 'clinical' Rockall scores [7], to combined clinical and endoscopic evaluation, best exemplified by the classical Rockall [8]. Such a scheme should aid in making clinical decisions, as to both the need for urgent intervention and the prediction of continued or recurrent bleeding in the context of endoscopic therapy [9].

The purpose of this study was designed to compare between endoscopic clip applications versus argon plasma coagulation in management of bleeding peptic ulcer.

2. PATIENTS AND METHODS

This study was conducted on 60 patients (30 males with mean age 50.8 ± 8.9 and 30 females with mean age 49.7 ± 9.8) between January 2007 and August 2008, all patients admitted to Ain-Shams University Hospital, presenting with hematemesis. After fluid resuscitation, the patients underwent endoscopy of the upper gastrointestinal tract within 12 hours of admission. Those with duodenal, gastric, or stomal ulcers and stigmata of recent hemorrhage were enrolled in the study. The patients were selected according to Forrest classification between groups IA (spurting bleeding) to IIB (non bleeding ulcer with an adherent clot). A score was calculated to them according to Rockall's score.

Patients were excluded from the study if they had severe terminal illness that made endoscopic examination hazardous or undesirable; profuse hemorrhage accompanied by persistent shock, during which the upper gastrointestinal tract was filled with fresh blood, limiting visibility through the endoscope and necessitating emergency surgery as a life-saving procedure; or bleeding from a Mallory—Weiss tear, varices, erosions, tumors, or an unknown source.

All patients gave informed consent and the study was approved by the Institutional Ethical Committee.

All participants were subjected to:

Resuscitation including IV fluids, packed RBC transfusion until becomes hemodynamically stable. Routine laboratory investigations: complete blood count, liver function and kidney function tests, prothrombin time, partial thromboplastin time.

Upper GIT endoscopy was done. Patients with selection criteria of bleeding ulcer were divided into two groups: Group I: Consisting of 30 patients subjected to clip application using a metallic clips (Hemoclip), Group II: Consisting of 30 patients in whom Argon Plasma Coagulation (APC) was done using an argon plasma coagulator unit.

Clip application device: clip application was done using a metallic clips (Hemoclip; Olympus America, rotational clip fixing device HX'6UR'1 through flexible endoscopes). The clip fixing device length is 23 mm and maximum insertion portion diameter is 2.8 mm with processing port. Clips are loaded onto the fixing device and drawn into a sheath. At the target lesion, the clip is advanced out of the sheath, oriented with the rotational handle, and then deployed. The mechanism of hemostasis is mechanical compression [10].

Argon Plasma Coagulation (APC) was done using an argon plasma coagulator unit (TERNO ABC TOM 201, Germany). Spray mode was used with 2 power/gas settings (respectively, 40 and 70 W and 1.5 to 3 L/min). Probe of 2.3 mm was used with endoscopes with corresponding channel diameters (2.8 mm diameter accessory channels). The maximum coagulation depth achieved by APC is 3 to 4 mm, which minimizes the risk of perforation. Continuous suction was applied to remove smoke and prevent over inflation of the GIT [3].

Follow up: After endoscopy, all patients were closely monitored clinically for one weak looking for symptoms and signs of bleeding. All patients received the same proton pump inhibitor, and Blood transfusion was given to maintain the hemoglobin level above 8 g/dL. Clinical recurrent bleeding was defined as signs of bleeding: vomiting of fresh blood, passage of melena with pulse rate higher than 100beat/min, decrease in systolic blood pressure exceeding 30 mmHg, after the early stabilization of pulse, blood pressure, and or decrease in hemoglobin concentration by at least 2 g/dL over a 24-hour period.

In case of re bleeding endoscopy was repeated as an emergency procedure and the same primary endoscopic management was used. Indications for surgery ; where failed endoscopic treatment on second endoscopy, recurrence of bleeding after a second therapeutic endoscopy, or a total blood transfusion requirement of greater than 8 units to maintain a hemoglobin level of 10 g/dL.

2.1. Statistical Analysis

Data were statistically described in terms of mean ± standard deviation (± SD).

Chi–square test of significance was used in order to compare proportions between two categorical variables. For comparing between two means, t-test of significance was done and one way analysis of variance ANOVA was used when comparing between more than two means. When data were not normally distributed, nonparametric Mann-Whitney test was used for comparing between

two means. A probability value (p value) less than 0.05 was considered statistically significant.

All statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA), version 16, statistical program.

2.2. Results

The characteristic stigmata of bleeding ulcers according to their appearance at endoscopy were listed in **Table 1**, there was insignificant difference between the 2 groups regarding the presence of ulcer with visible or with oozing vessel (P > 0.05).

Table 2 shows significant difference between the 2 groups regarding Forrest's classification (P < 0.05).

Table 3 shows insignificant difference between the 2 groups regarding the Rockall'S Score (P > 0.05).

Table 4 shows the characteristic stigmata of bleeding ulcers according to their site at endoscopy, there was no significant difference between the 2 groups regarding presence of gastric or duodenal ulcer (P > 0.05).

There was a highly significant difference in re bleeding incidence in different Forrest's classes in group I (P < 0.01), while there was insignificant difference in re bleeding incidence in different Forrest's classes in group II (P > 0.05) as shown in **Table 5**.

Also there was a significant occurrence of re bleeding with higher Rockall's Score in group I. (P < 0.05), while there was insignificant occurrence of re bleeding with higher Rockall's Score in group II (P > 0.05) as shown in **Table 6**.

Table 7 shows insignificant relation between < 5 or _5 Rockall's score and the occurrence of re bleeding in the whole patient population (P > 0.05). There was also insignificant difference in the 2 groups regarding occurrence of re bleeding in relation to the site of ulcer, gastric ulcer (GU) or duodenal ulcer (DU) (P > 0.05).

Table 1. Characteristic stigmata of bleeding ulcers according to their appearance at endoscopy.

		Ulcer with visible vessel			Ulcer with oozing vessel		
		Negative	Positive	Total	Negative	Positive	Total
Group I	N	26	4	30	16	14	30
	%	86.67	13.33	100.00	53.33	46.67	100.00
Group II	N	24	6	30	22	8	30
	%	80.00	20.00	100.00	73.33	26.67	100.00
Chi-square	X^2		0.240			1.292	
	P-value		> 0.05 (N.S)			> 0.05 (N.S)	

N.S: non significant; S: significant

Table 2. Forrest's classification for the patient groups.

		Forrest's Classification				
		IA	IIA	IB	IIB	Total
Group I	N	0	4	14	12	30
	%	0.00	13.33	46.67	40.00	100.00
Group II	N	10	6	8	6	30
	%	33.33	20.00	26.67	20.00	100.00
Chi-square	X^2			8.981		
	P-value			< 0.05 (S)		

Table 3. Rockall's Score for the patient groups.

Group	Rockall's Score				T-test	
	Range		Mean	SD	t	P-value
Group I	3.000	8.000	4.933	1.668	–0.585	> 0.05(N.S)
Group II	3.000	9.000	5.333	2.059		

Table 4. Characteristic stigmata of bleeding ulcers according to their site at endoscopy.

		GASTRIC OR DUODENAL		
		DU	GU	Total
Group I	N	14	16	30
	%	46.67	53.33	100.00
Group II	N	20	10	30
	%	66.67	33.33	100.00
Chi-square	X^2		1.222	
	P-value		> 0.05 (N.S)	

Table 5. Incidence of rebleeding in relation to Forrest's classification.

Group	Rebleeding		Forrest's Classification					Chi-square	
			IA	IIA	IB	IIB	Total	X^2	P-value
Group I	Negative	N	0	0	12	12	24	9.643	< 0.01 (H.S)
		%	0.00	0.00	40.00	40.00	80.00		
	Positive	N	0	4	2	0	6		
		%	0.00	13.33	6.67	0.00	20. 0		
Group II	Negative	N	6	6	4	4	20	2.100	> 0.05 (N.S)
		%	20.00	20.00	13.33	13.33	66.67		
	Positive	N	4	0	4	2	10		
		%	13.33	0.00	13.33	6.67	33.33		

Table 6. Incidence of rebleeding in relation to Rockall's Score.

Rockall's Score	Rebleeding					
	Negative		Positive		T-test	
	Mean	SD	Mean	SD	t	P-value
Group I	4.417	1.240	7.000	1.732	–3.014	< 0.05 (S)
Group II	5.900	2.234	4.200	1.095	1.587	> 0.05 (N.S)

Table 7. Correlation between high risk Rockall's score and rebleeding.

Rockall's Score		Rebleeding		
		Negative	Positive	Total
< 5	N	20	4	24
	%	33.33	6.67	40.00
≥ 5	N	24	12	36
	%	40.00	20. 00	60.00
Chi-square	X^2	1.02		
	P-value	> 0.05 (N.S)		

3. DISCUSSION

Peptic ulcer bleeding is the most common cause of upper gastrointestinal bleeding, responsible for about 50% of all cases. Mortality is increasing with increasing age and is significantly higher in patients who are already admitted in hospital for co-morbidity [3].

Risk factors for peptic ulcer bleeding are non-steroid anti-inflammatory drugs (NSAIDs) use and Helicobacter Pylori (HP) infection [8].

In patients with ulcers presenting with ongoing bleeding or high risk features (Forrest I, IIA, IIB); surgery was frequently required in the past to solve the situation. However, endoscopic therapy has been well documented to treat these ulcers [11].

The timing of the initial endoscopy has been debated. In general, red hematemesis indicates emergency upper endoscopy while black hematemesis and/or melena without haemodynamic instability can wait until normal working hours. However, from a logistic point of view early endoscopy has been advocated to ensure optimal utilization of resources [11] .In this study there was no significant difference in both groups regarding age, shock, presence of co morbid illness or liver cell failure, ulcer size, rockall score and site of ulcer; factors known to affect prognosis in many previous studies.

Our study showed that the rate of re bleeding was slightly higher in APC group despite of being statistically insignificant. Also there was no significant relation between the rates of re bleeding and the size of the ulcer. Few reports have concerned the indication for and efficacy of each hemostatic therapy according to location, depth and size of ulcer and bleeding activity of the exposed vessel as if the ulcer is large or deep, the possibility of complications including further ulceration, recurrence of bleeding and perforation is high [12].

A great care is required in performing the procedure if the bleeding ulcer is located on the posterior wall or lesser curvature of the gastric body or on the posterior wall of the duodenal bulb, the hemostatic rate is lower than for other therapies because of the technical difficulty of approaching the lesion [13].

In the present study although there was no statistical significance difference in re bleeding incidence in both groups there was highly significant difference in re bleeding incidence in relation to different Forrest's classes in group I ($P < 0.01$), while there was insignificant difference in re bleeding incidence in different Forrest's classes in group II. Also, the rate of surgical interference of both groups was 0%.

In recent years, the Rockall score has been used to select patients with a low risk of rebleeding for early discharge. Almost all patients in this low risk group belong to patients without any stigmata of recent hemorrhage (SRH). However, patients with a SRH are a high-risk group for further re-bleeding and also mortality. It is therefore important to determine whether the Rockall score could be useful in patients who have undergone endoscopic therapy for UGIB to identify high-risk patients and thus improve their management and outcome [14].

In the present study we assessed correlation between high risk Rockall's score (> 5) and occurrence of re bleeding which re bleeding was 6.8 % in low risk Rockall's score (< 5) while re bleeding was 20% in high risk Rockall's score (> 5). However, this was statistically non significant, but incidence of re bleeding in relation to high risk Rockall's score was significant in group I. This did not go in agreement with others [12], who concluded that the Rockall scoring system accurately identifies patients at high risk of death but not of re bleeding [12].

In spite that our study partially goes with others, who observe good correlation between the Rockall score and both the probability of re bleeding and mortality in patients undergoing endoscopic therapy for peptic ulcer hemorrhage [15,16].

In the present study the mortality rates between the two groups were the same which was 0% in the two groups despite of significantly higher need for surgery in group II.

This goes with others, who concluded that there was

no difference in all-cause mortality irrespective of the modalities of endoscopic treatment [13,16].

Sung *et al*. in a meta-analysis of 15 studies reported that regardless of improvements in sustaining hemostasis by clipping leading to less re bleeding and fewer interventions with surgery, mortality has not been reduced and there is no indication of a reduction in the death rate [17]. Nevertheless, it is a mystery that despite successful control of hemorrhage in many studies using various combinations of endoscopic and pharmacological therapies the mortality rate remains unchanged.

4. CONCLUSIONS

Endoscopic application of hemoclips have a less re bleeding rate than Argon plasma coagulation for treatment of bleeding peptic ulcer, although this was statistically insignificant meanwhile APC is still less cost and easy. Clinical and endoscopic assessment (through Rockall score and Forrest classification) could help in making best choice for endoscopic management.

REFERENCES

[1] Barkun, A., *et al*. (2003) Consensus for managing patients with non variceal upper gastrointestinal bleeding. *Annals of Internal Medicine*, **139(10)**, 843-857.

[2] Park, C.H., *et al*. (2004) Optimal volume for epinephrene for endoscpic prevention of recurrent peptic bleeding. *Gastrointestinal Endoscopy*, **60(6)**, 875-880.

[3] Canard, J.M. and Vedrenne, B. (2001) Clinical application of argon plasma coagulation in gastrointestinal endoscopy: has the time come to replace the laser? *Endoscopy*, **33(4)**, 353-357.

[4] Church, N.I., Dallal, H.J., Masson, J., *et al*. (2003) A randomized trial comparing heater probe plus thrombin with heater probe plus placebo for bleeding peptic ulcer. *Gastroenterology*, **125(2)**, 396-403.

[5] Devereaux, C.E. and Binmoeller, K.F (1999) Endoclip: closing the surgical gap. *Gastrointestinal Endoscopy*, **50 (3)**, 440-442.

[6] Forrest, J.A.H., Finlayson, N.D.C. and Shearman, D.J.C. (1974) Endoscopy in gastrointestinal bleeding. *Lancet*, **2(7877)**, 394-397.

[7] Rockall, T.A., Logan, R.F., Devlin, H.B. and Northfield, T.C. (1995) Incidence of and mortality from acute upper gastrointestinal haemorrage in the United Kingdom. Steering Committee Gastrointestinal Haemorrhage, *British Medical Journal*, **311(6999)**, 222-226.

[8] Leerdam, V. (2008) Epidemiology of acute upper gastrointestinal bleeding. *Best Practice & Research Clinical Gastroenterology*, **22(2)**, 209-224.

[9] Sung, J. (2006) Current manegement of peptic ulcer bleeding. *Nature Clinical Practice Gastroenterology & Hepatology*, **3**, 24-32.

[10] Chuttani, R., Barkun, A., Carpenter, S., *et al*. (2006) Endoscopic clip application devices. *American Society for Gastrointestinal Endoscopy*, **63(6)**, 16-51.

[11] Aabakken, L. (2008) Current endoscopic and pharmacological therapy of peptic ulcer bleeding. *Best Practice & Research Clinical Gastroenterology*, **22(2)**, 243-259.

[12] Saperas, E., Sebastian, V., Carolina, B., Joan, D., Jose, R. and Juan, R.M. (2008) Applicability of the Rockall scoring system and prediction of rebleeding and mortality after combined pharmacologic and endoscopic treatment of high-risk bleeding peptic ulcers. *Gastrointestinal Endoscopy*, **67(5)**, AB255.

[13] Chung, I.K., Ham, J.S., Kim, H.S., *et al*. (1999) Comparison of the hemostatic efficacy of the endoscopic hemoclip method with hypertonic saline-epinephrine injection and a combination of the two for the management of bleeding pepic ulcers. *Gastrointestinal Endoscopy*, **49(1)**, 13-18.

[14] Bessa, X., O'Callaghan, E., Ballesté, B., Nieto, M., *et al*. (2006) Applicability of the Rockall score in patients undergoing endoscopic therapy for upper gastrointestinal bleeding. *Digestive and Liver Disease*, **38(1)**, 12-17.

[15] Church, N.I. and Palmer, K.R. (2001) Relevance of the Rockall score in patients undergoing endoscopic therapy for peptic ulcer hemorrhage. *European Journal of Gastroenterology and Hepatology*, **13(10)**, 1149-1152.

[16] Chuan, C., Ming-Szu, H., Te-Fa, C., Jih-Chang, C. and Cheng-Ting, H. (2007) Risk scoring systems to predict need for clinical intervention for patients with non variceal upper gastrointestinal tract bleeding. *American Journal of Emergency Medicine*, **25(7)**, 774-779.

[17] Sung, J.J., Tosi, K.K., Lai, L.H., *et al*. (2007) Endoscopic clipping versus injection and thermocagulation in treatment of non variceal upper gastrointestinal bleeding: A meta-analysis. *Gut*, **56**, 1364-1373.

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Gender and environment: general and monthly gender distribution of newborns and cosmophysical parameters

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ABSTRACT

Recent publications have described a relationship between fluctuations in environmental physical activity and several aspects of fetal development. This study explored the possible effects of cosmophysical parameters on newborn gender, overall and by maternal age. The gender distribution of 123,368 infants born over a 189-month period (November 1993–July 2009) was analyzed against levels of solar, geomagnetic, and cosmic ray activity at the time of conception. The cohort was then divided into three groups by maternal age (≤ 28 years, 29-35 years, > 35 years) for further analysis. Pearson correlation coefficients and their probabilities were calculated, and chi-square test was applied, as necessary. The physical data were derived from space science centers in the USA, Russia, and Finland. The results showed that the male/female ratio for the whole cohort over the study period was 1.06. However, on monthly analysis, there was a significant male predominance in most months, with a male/female ratio of up to 4. Younger mothers (≤ 28 years) gave birth to significantly more boys than older mothers. The gender distribution in the three maternal age groups was partially linked to the different physical factors. These findings suggest that environmental physical activity in the month of conception may play a role in newborn gender. Further study is needed to determine the mechanism underlying this effect.

Keywords: Newborn; Gender; Solar; Geomagnetic; Cosmic Ray; Activity; Age; Mother; Pregnancy

1. INTRODUCTION

Newborn gender is a focus of human and scientific interest. In addition to the known genetic factors that affect fetal development, several studies published in the last decades have considered the potential influence of fluctuations in environmental physical activity (solar, geomagnetic, cosmic ray) on physiologic and pathologic aspects of pregnancy and fetal development [1-9].

The aim of the present study was to investigate the possible effect of fluctuations in cosmophysical parameters at conception on newborn gender distribution, overall and by maternal age.

2. METHODS

The study included all 123,368 infants born at a tertiary university hospital in Israel from November 1993 to July 2009 (189 months): 63,415 male and 59,953 female (liveborn or stillborn). Distribution by maternal age showed that 54,158 (43.86%) infants were born to mothers younger than 29 years, 47,655 (38.60%) to mothers aged 29-35 years, and 21,657 (17.54%) to mothers older than 35 years. These data were correlated against levels of the following cosmophysical parameters in the month of conception: solar activity indices— sunspot number, smoothed sunspot number, solar flux at 2800 MGH and 10.7 cm wavelength, and adjusted solar flux; geomagnetic activity (GMA) indices—Ap, Cp, and Am (planetary and regional, for middle latitudes); and cosmic ray activity (CRA)—represented by neutron activity at the Earth's surface (in imp/min). The cosmophysical data for

the study period were derived from weekly and monthly calculations published by space science institutions in the United States, Russia, and Finland that regularly monitor these parameters worldwide (National Geophysical Data Center, Space Weather Prediction Center, Moscow Neutron Monitor, Space Weather Prediction Center; Space Environment Services Center; November 2003-July 2009) [10-15].

2.1. Statistical Analysis

Pearson correlation coefficients (r) and their probabilities (p) were calculated between newborn gender distribution and the physical parameters. Chi-square test was used to analyze the likelihood of a newborn being male or female by physical parameters and maternal age group. Probabilities of 95% and higher were considered significant, probabilities of 90%-94% were considered trends toward significance. Non significant results were marked N.S.

3. RESULTS

The male/female ratio in the whole population of newborns was 1.06. Maximal monthly deviations ranged from 1.387 to 0.7399. One hundred fifty months (79.36%) were characterized by a significant prevalence of male newborns (79.36%), and 39 months (20.1%) by a significant prevalence of female newborns; in one month, the gender distribution was equal.

Table 1 presents the newborn gender relationship with cosmophysical parameters during the month of conception. **Table 2** presents the same relationship by maternal age group: 28 years or less; 29-35 years; and 35 years or more.

The results showed that mothers aged 28 years or less gave birth to significantly more male newborns than older mothers ($\chi^2 = 3.9$, $p = 0.047$). The ratio of months with more male newborns to months with more female infants in this maternal age group was 2.62. In the two older groups, this ratio was 2.16 ($p < 0.0001$).

The differences among the maternal age groups prompted our use of multifactorial analysis for each gender and each of the three maternal age groups to predict the chances of a mother of particular age giving birth to a boy or girl according to levels of the cosmophysical parameters. The findings are shown in **Tables 3-5**. Factors that failed to show a significant relationship in the total correlation study (**Table 1**) were included as significant in the subgroup of multifactorial analysis (for example, monthly GMA Cp for mothers older 35 years) (**Tables 3-5**).

The relationship of solar and neutron activity was also different among the three age groups, changing from a positive in the young mother group to an inverse relationship

Table 1. Monthly (n = 189) newborn (n = 123683) gender distribution by physcial parameters in the month of conception (Pearson correlation coefficients and their probabilities).

Parameters	Physical Activity 9 Months to Delivery		
	Male (n = 63415)	**Female (n = 59953)**	**Ratio (1.06)**
Year	0.72 p < 0.0001	0.704 p < 0.0001	NS
Month	NS	NS	NS
Solar activity			
Sunspot number	0.21 p = 0.0039	0.16 p = 0.02	NS
Smoothed sunspot number	0.24 p = 0.0012	0.2 p = 0.0056	NS
Solar flux 2800 MGH, 10.7 cm	0.3 p < 0.0001	0.26 p = 0.0003	NS
Adjusted solar flux	0.283 p < 0.0001	0.243 p = 0.0008	NS
Geomagnetic activity			
Ap	NS	NS	NS
Cp	NS	NS	NS
Am	NS	NS	NS
Cosmic ray (neutron) activity (imp/min)			
Moscow	–0.295 p < 0.0001	–0.28 p = 0.0001	NS
Oulo	–0.27 p = 0.0002	–0.25 p = 0.0005	NS

Note: 150 months, more males; 39 months, more females Chi-square = 134.8, p < 0.0001; range: 1.387-0.7399.

Table 2. Monthly (n = 189) gender distribution by environmental physical activity in month of conception and maternal age (Pearson correlation coefficients and their probabilities).

Parameter	Age ≤ 28		Age 29-35		Age > 35	
	Male (n = 27997)	**Female (n = 26161)**	**Male (n = 24352)**	**Female (n = 23303)**	**Male (n = 11168)**	**Female (n = 10489)**
Year	0.263 0.0003	0.233 0.0013	0.88 < 0.0001	0.86 < 0.0001	0.68 < 0.0001	0.735 0.0001
Month	NS	NS	NS	NS	NS	NS
Solar activity						
Sunspot number	0.46 < 0.0001	0.44 < 0.0001	NS	NS	–0.19 0.0085	–0.21 0.0031
Smoothed sunpsot number	0.51 < 0.0001	0.49 < 0.0001	NS	NS	–0.2 0.0057	–0.2 0.0055
Solar flux 2800 MGH, 10.7 cm	0.53 < 0.0001	0.51 < 0.0001	0.13 0.08	NS	–0.12 0.1	–0.13 0.07
Adjusted solar flux	0.51 0.0001	0.49 < 0.0001	0.12 0.09	NS	–0.13 0.07	–0.14 0.049
Geomagnetic activity						
Ap	NS	NS	NS	NS	–0.12 0.1	–0.17 0.018
Cp	NS	NS	–0.17 0.02	–0.16 0.03	–0.17 0.018	–0.2 0.0055
Am	0.13 0.07	NS	NS	NS	–0.14 0.056	–0.165 0.023
Cosmic ray (neutron) activity (imp/min)						
Moscow	–0.465 < 0.0001	–0.44 < 0.0001	–0.16 0.025	–0.14 0.06	0.15 0.033	0.134 0.065
Oulo	–0.4 < 0.0001	–0.34 < 0.0001	–0.17 0.02	–0.16 0.03	0.14 0.057	0.12 0.1

Table 3. Prediction model to determine likelihood of a woman older than 29 years giving birth to a male or female infant by levels of physical factors in the month of conception.

Variables	Parameter estimate	Standard error	Probability
Likelihood of male infant (n = 27897)			
Intercept	–3569	621.2	< 0.0001
Year	1.85	0.311	< 0.0001
Solar flux 2800 MGH	2.485	0.436	< 0.01
Adjusted solar flux	–2.54	0.454	< 0.0001
Smoothed sunspot number	0.4	0.123	0.0012
Likelihood of female infant (n = 26161)			
Intercept	–3097	610.4	< 0.0001
Year	1.61	0.306	< 0.0001
Solar flux 2800 MGH	2.43	0.428	< 0.0001
Adjusted solar flux	–2.5	0.446	< 0.0001
Smoothed sunspot number	0.l38	0.12	0.0016

Table 4. Prediction model to determine likelihood of a woman aged 29-35 years giving birth to a male or female infant by levels of physical factors in the month of conception.

Variables	Parameter estimate	Standard error	Probability
Likelihood of male infant (n = 24352)			
Intercept	–12310	437	< 0.0001
Year	6.21	0.218	< 0.0001
Month	0.788	0.289	0.0071
Smoothed sunspot number	0.124	0.00257	< 0.0001
Likelihood of female infant (n =23303)			
Intercept	–11071	469.76	< 0.0001
Year	5.62	0.234	< 0.0001
Month	0.54	0.311	0.08
CRA	–0.01	0.003	0.0028

CRA—cosmic ray activity

Table 5. Prediction model to determine likelihood of a woman older than 35 years giving birth to a male or female infant by levels of physical factors in the month of conception.

Variables	Parameter estimate	Standard error	Probability
Likelihood of male infant (n = 11166)			
Intercept	–4343	323	< 0.0001
Year	2.16	0.159	< 0.0001
GMA (Cp)	10.98	4.267	< 0.01
CRA	0.011	0.00258	< 0.0001
Likelihood of female infant (n = 10489)			
Intercept	–4339	288.08	< 0.0001
Year	2.167	0.14	< 0.0001
GMA (Cp)	19.98	4.267	< 0.001
CRA	0.0062	0.00158	0.0001

GMA—geomagnetic activity, CRA—cosmic ray activity

in the older than 35. In the intermediate age group (age 28-35) non significant relationship was seen (**Table 2**).

4. DISCUSSION

Newborn gender is known to be affected by genetic (chromosome X, Y interaction) and endocrine (hormonal) factors [16,17]. A series of publications has demonstrated that environmental physical parameters, such as solar, cosmic ray and geomagnetic activity, at the beginning of pregnancy may also play a role [1-9,17-19], perhaps via their effects on chromosome function (clearly shown in the case of Down syndrome) and hormone secretion [5] Accordingly, other neonatology parameters, including monthly number of infants born, newborn length and weight, preterm deliveries, and occurrence of congenital heart disease and other malformations have been linked to cosmophysical activity [1-6,9,15,17,19, 20]

The mechanism underlying this relationship is still unknown, although the physiologic and teratogenic potential of cosmophysical factors is clear. The effect of solar activity on human biological behavior is apparently due to solar corpuscular and wave energy. High levels of

cosmic rays in space leave remains of crushed atoms in the form of neutrons, and the measurement of neutron activity on the Earths' surface serves as an indirect measure of cosmic ray activity. It is assumed that neutrons, by the nature of their physical properties, connect with H^+ ions and are converted to protons, which attack cell nuclei in enzymes and other regulatory systems [19,21]. Solar and geomagnetic activities shield the Earth from cosmic rays; when they are weak, the effect of cosmic ray activity increases. One study conducted over a 216-month period found that the correlation between solar and cosmic ray activity was –0.84 ($p < 0.0001$) [22,23]. Recent study, describing the Y chromosome as a very labile structure [24], allows to see it as an object for possible physical influences.

According to the world-renowed physicist, Dr. Feinmann, "Probably the most powerful single assumption that contributes to the progress of biology is the assumption that everything from the animals to the atoms can do that are seen in the biological world are the result of the behavior of physical and chemical phenomena" [25].

Overall, we found that the number of male newborns only slightly surpassed the number of female newborns. A male/female ratio of 1.06 to 1.07 has been consistently reported in studies in various countries and regions [6]. However, males showed a considerable predominance when we compared monthly deliveries: in some months, the male/female ratio was close to 4. This trend was more apparent in younger mothers (age 28 years or less). Further analysis of gender distribution by maternal age showed that in the youngest age group, solar activity had a strong effect and cosmic ray activity a weak effect, whereas in the older groups, this relationship was reversed. Given that the youngest group was larger, our findings for the overall link of monthly gender distribution with physical factors were close to those for the youngest group.

Although we focused only on maternal age in this study, we assume a concomitant younger age of the fathers as well, which may also play a role in the physical influences at the time of conception.

The presented data constitute another chapter in the study of clinical cosmobiology and the opposing physical forces in our environment ("equilibrium paradigm") [26]. The link between the physical environmental and human homeostasis brings to mind the statement of Albert Einstein: "The human will is free only within the bounds of a determined cosmic system" [27].

5. CONCLUSIONS

Both gender monthly newborn number is linked with the level of cosmophysical activity.

Overall, there is a small male prevalence among newborns, although monthly calculation of this relationship reveals a considerable male predominance. In addition to other known factors that determine newborn gender, environmental physical activity during the month of conception may also be involved.

The relationship of the different physical factors with newborn gender varies by maternal age.

More studies are needed to further our understanding of the ways in which physical forces affect newborn gender.

REFERENCES

[1] Merlob, P., Raisner, S.H., Shimshoni, M. and Stoupel, E. (1989) Fetal growth in periods of extreme solar activity. *Journal Foetal Medicine*, **9(1-2)**, 1-4.

[2] Stoupel, E., Keret, R., Assa, S., Kaufman, H., Shimshoni, M. and Laron, Z. (1983) Secretion of growth hormone, prolactin and corticosteroids during different levels of of geomagnetic activity. *Neuroendocrinology Letters*, **5(6)**, 1-4.

[3] Stoupel, E., Silbergeld, A., Gil-Ad, I., Keret, R., Shimshoni, M. and Laron, Z. (1989) Relationship between solar activity and levels of of plasma growth hormone and prolactin. *Neuroendocrinology Letters*, **11(5)**, 1-9.

[4] Stoupel, E., Hod, M., Shimshoni, M., Fridman, S., Ovadia, J. and Keith, L. (1990) Monthly cosmic activity and pregnancy induced hypertension. *Clinical & Experimental Obstetrics & Gynecology*, **17(1)**, 7-12.

[5] Stoupel, E., Frimer, H., Appelman, Z., Ben-Neriah, Z. Dar, H., Fejgin, M., Gershuni-Baruch, R., Manor, E., Barkai, G., Shalev, S., *et al*. (2005) Chromosome aberration and environmental physical activity: Down syndrome and solar activity, Israel, 1990-2000. *International Journal of Biometeorology*, **28(2)**, 1-9.

[6] Stoupel, E., Kalediene, R., Petrauskiene, J., Gaizauskiene, A., Israelevich, P., Abramson, E. and Sulkes. J. (2006) Monthly number of newborns and environmental physical activity. *Medicina*, **42(3)**, 238-242.

[7] Stoupel, E., Monselize, Y. and Lahav, J. (2006) Changes in autoimmune markers of the anti-cardiolipin syndrome on days of extreme geomagnetic activity. *Journal of Basic and Clinical Physiology and Pharmacology*, **17(4)**, 269-278.

[8] Stoupel, E., Sirota, L., Osovsky, M., Klinger, G., Abramson, E., Israelevich, P., Sulkes, J. and Linder, N. (2007) Monthly number of preterm births and environmental physical activity. *Journal of Basic and Clinical Physiology and Pharmacology*, **18(2)**, 149-157.

[9] Stoupel, E., Birk, E., Kogan, A., Klinger, G., Abramson, E., Israelevich, P., Sulkes, J. and Linder, N. (2009) Congenital heart disease: Correlation with fluctuations in cosmophysical activity, 1995-2005. *International Journal of Cardiology*, **135(2)**, 207-210.

[10] National Geophysical Data Center (monthly) Solar Activity Bulletin. Boulder, CO: NOAA/NGSD.

[11] National Geophysical Data Center (monthly) Geomagnetic Indices Bulletin. Boulder, CO: NOAA/NGSD.

[12] Moscow Neutron Monitor. Neutron Monitor Data (daily, weekly). Izmiran Institute, Russian Academy of Sciences, Moscow.
[13] Neutron Monitoring Data. Monthly, Daily. Oulu University Neutron Monitoring Station. Oulu, Finland.
[14] Space Environment Services Center (1989) Glossary of Solar Terrestrial Terms. Boulder.
[15] Space Weather Prediction Center (weekly). Weekly Highlights and Forecasts. Boulder, CO: NOAA/NSWPC.
[16] Santolaya-Forges, J., Meyer, W.J., Burton, B. and Scommegna, A. (1999) Altered newborn gender distribution in patients with low mid-trimester maternal serum human chorionic gonadotropin. *Journal of Maternal-Fetal Medicine*, **6(2)**, 111-114.
[17] Coutandt, R., Boux de Casson, F., Douay, O., Rouleau, S., Beringue, F., Gillard, P., Limal, J.M. and Descamps, P. (2001) Relationships between placental GH concentration and maternal smoking, newborn gender and maternal leptin: possible implications for birth weight. *Journal of Clinical Endocrinology & Metabolism*, **86(10)**, 4854-4859.
[18] Archer, V.E. (1979) Anencephalus: Drinking water, geomagnetic and cosmic radiation. *American Journal of Epidemiology*, **109(1)**, 88-97.
[19] Kirby, A.J. and Hollfelder, F. (2008) Enzymes under the nanoscope. *Nature*, **456(7218),** 45-47.
[20] Fenig, E., Michaeli, M., Kalish, Y. and Lishner, M. (2001) Pregnancy and radiation. *Cancer Treatment Reviews*, **27 (1)**, 1-7.
[21] Nias, A.W.H. (1998) An Introduction to Radiotherapy. John Wiley & Sons, New York.
[22] Stoupel, E., Kalediene, R., Petrauskiene, J., Starkuviene, S., Abramson, E., Israelevich, P. and Sulkes, J. (2010) Death-optimal physical conditions. *Journal of Basic and Clinical Physiology and Pharmacology*, **21(1)**, 143-156.
[23] World Health Organization (2009) Genomic Resource Center. Gender and Genetics. http://www.who.int/genomics/gender/en/index4.html
[24] Hughes, J.F., Skaletsky, H., Pyntikova, T., Graves, T.A., Van Daalen, S.K.M., Minx, D.P., Fulton, R.S., Mcrath, S.D., Locke, D.P., Friedman, C., Trask, B.J., Mardis, E.R., Warren, W.C., Repping, S., Rozen, S., Wilkson, R.K. and Page, D.C. (2010) Chimpanzee and human Y chromosomes are remarkably divergent in structure and gene content. *Nature*, **463(7280)**, 536-539.
[25] Feinmann, R. (2003) Thoughts of a Citizen-Scientist. Perseus Books, Cambridge.
[26] Stoupel, E. (2002) The equilibrium paradigm in clinical cosmobiology. *Journal of Basic and Clinical Physiology and Pharmacology*, **13(3)**, 255-263.
[27] Einstein, A. (1932) Mein Weltbild. Europa Verlag, Zurich.

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