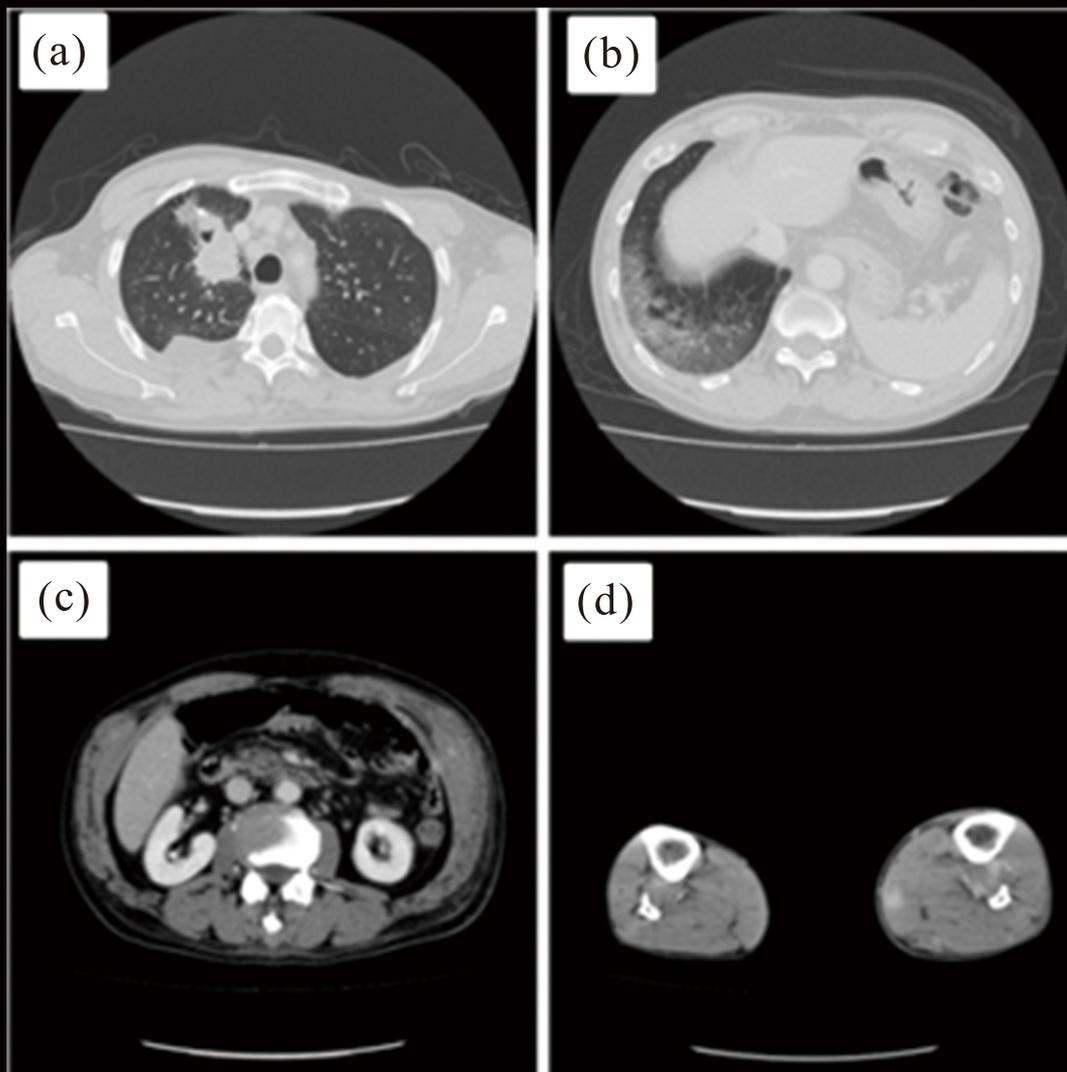


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Polycations. 23. Antimicrobial Surfaces for Prevention of Pathogen Transmission*

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Abstract

The continued evolution of bacterial and fungal species poses a significant difficulty for the treatment of disease of microbial origin. Given this situation, the prevention of transmission of such microbial diseases becomes of increasing importance. Efforts of this laboratory have been directed toward the destruction of microbial species on environmental surfaces as a prophylaxis toward infection, and we herein report on the efficacy of a system that demonstrates activity against both Gram-positive and Gram-negative bacteria, as well as fungi. We report specifically herein on the use of fabric materials so activated for the destruction of these microbial species, useful for a variety of surfaces within hospital and related settings wherein transmission of microbial disease is a major problem, while these approaches are also applicable for a variety of other types of surfaces.

Keywords

Antimicrobial, Polycationic Organic Salts, Cell Disruption, Fabric, Environmental Surfaces, Prophylaxis

1. Introduction

The evolution of bacterial species to generate resistance to classical antibiotics has become a major problem for the treatment of diseases of microbial origin [2]. As a result, *prevention of transmission* of these microbial diseases is of increased importance, particularly in a health service setting [3]. Thus, our laboratory has searched for approaches to reduce the threat of resistant bacteria through development of environmental surfaces that destroy the microbe in a passive manner (upon contact with the treated sur-

*Please see reference [1].

face). Our emphasis has been particularly on fabric surfaces. Such modified fabric surfaces used in health care settings (modified linens, gowns, clothing, etc.) are critically important as they are active against a broad range of bacterial and fungal species, can be used multiple times (with proper laundering), and are relatively inexpensive to produce.

Specifically, we have developed several approaches for the permanent association of cationic lipids with fabric surfaces that would destroy a wide variety of microbial species that might come into contact with them. Additionally, these fabrics are antimicrobial to those microbial species that have undergone evolutionary mutations to generate resistance to classical antibiotic treatment. A method to destroy microbes before they have the opportunity infect the patient was the focus of our research. To this end we have developed and reported on several approaches toward the permanent attachment of cationic (polycationic) lipid species to fabric surfaces of several types resulting in antibacterial activity [4]-[11]. In addition to the antibacterial activity previously reported, we demonstrate an additional range of bacteria important in the healthcare section.

2. Results and Discussion

The fabrics investigated in this effort all bore functionalities that were capable of being modified by a two-step process of activation and functionalization, incorporating cationic lipid species as covalently attached entities. These fabrics specifically were carbohydrate based—**A** 100% cotton; **B** 50% cotton/50% nylon—or proteinaceous—**C** 100% silk; **D** 100% wool. With the carbohydrate fabrics activation of the pendant primary hydroxyl groups of the glucose residues was achieved by tosylation using tosyl chloride in the presence of sodium bicarbonate in aqueous/2-propanol medium, while the proteinaceous fabrics were similarly activated at the pendant primary hydroxyl groups of serine residues. Following activation, covalent attachment of the cationic lipid units were accomplished by nucleophilic displacement of the tosylate groups by reaction with the appropriate cationic lipid species, 1-alkyl-1-azonia-4-azabicyclo [2.2.2] octane halides. For a carbohydrate residue so activated and functionalized, the reaction scheme is shown in **Figure 1**.

Upon drying in air, the resultant modified surfaces were suitable for testing for antimicrobial activity. All modified fabric surfaces were investigated using a standard procedure. This procedure involved:

Bactericidal activity was observed for *S. aureus*, as well as the microorganisms in **Table 1**. After treated fabrics were incubated with bacteria or fungi and a lack of growth observed, a sample of the incubation medium was transferred to fresh growth medium and re-incubated (along with that from untreated samples as a control) to observe any residual growth of bacteria/fungi that were not killed by the treatment. The second incubations were found not to exhibit any noticeable growth of microbes, indicating that these fabrics were bactericidal.

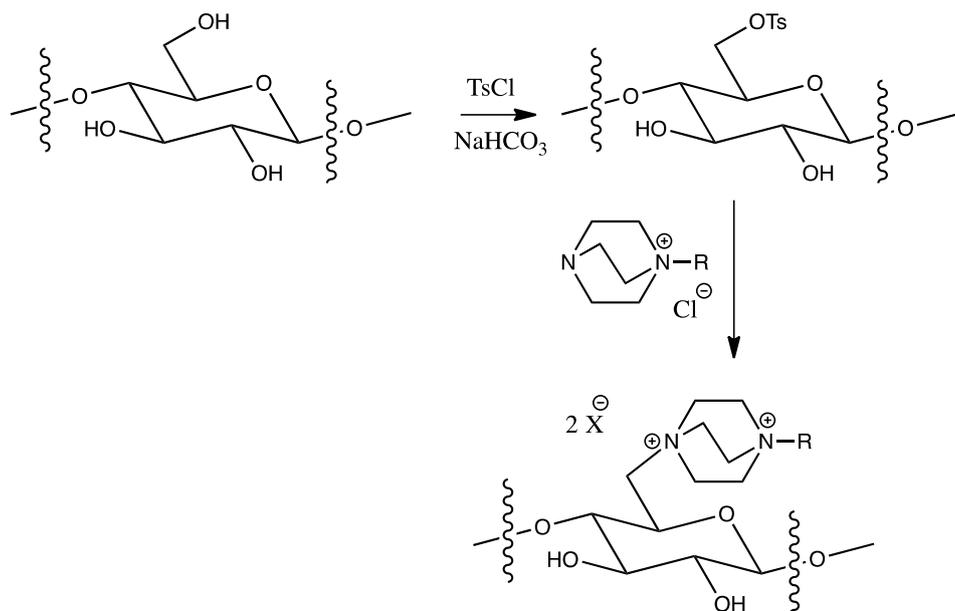


Figure 1. Activation and functionalization of a carbohydrate-based fabric surface after washing with salt solution for association of desired anions.

Table 1. Microbial species killed by impinging on the modified surfaces—“Effective Modifications” indicate the length of carbon chains that provided kill of microbes.

Name	Gram Type	Effective Modifications	Source
<i>Staphylococcus aureus</i> (ATCC 35218)	Positive	C12-18	This work
<i>Staphylococcus aureus</i> (MRSA)	Positive	C12-16	[12]
<i>Staphylococcus aureus</i> (MSSA)	Positive	C12-16	[12]
<i>Bacillus anthracis</i> (spores)	Positive	C12-16	[13] [14]
<i>Bacillus cereus</i>	Positive	C12-18	This work
<i>Clostridium difficile</i>	Positive	C12-16	This work
<i>Burkholder cepacia</i>	Negative	C16	[12]
<i>Acinetobacter baumannii</i>	Negative	C16	[15]
<i>Klebsiella pneumonia</i>	Negative	C16	[12]
<i>Proteus mirabilis</i>	Negative	C16	[12]
<i>Proteus vulgaris</i>	Negative	C12-18	This work
<i>Pseudomonas aeruginosa</i>	Negative	C12 C12	This work
		C12/14 mix C12/16 mix C12 & C16	
<i>Escherichia coli</i>	Negative	C12/14 mix C12/16 mix C14/16 mix	This work
<i>Chaetomium globosum</i>	Fungus	C12-18	This work
<i>Saccharomyces cerevisiae</i>	Fungus	C12-18	This work
<i>Aspergillus niger</i>	Fungus	C12-18	This work
<i>Candida albicans</i>	Fungus	C12-18	This work

It was determined that the kill for all bacterial species investigated occurred in a very short period of time (<5 min) after addition to the modified surface. It was not possible to perform the experiments rapidly enough to make an exact measurement of time of kill.

The variety of bacterial and fungal species observed to be killed by contact with these surfaces is noted in **Table 1**. Except for those so noted, these observations were made in this laboratory.

The kill of bacterial and fungal species is viewed as occurring rapidly by disruption of the cell. This was demonstrated using the Gram-positive bacterium *S. aureus* that had been treated with Gram stain after placement on the modified surface. Instead of retaining the crystal violet dye, it was released from the cell, destained and counterstained indicating disruption of the cell membrane. Similarly, this type of test was performed with *Saccharomyces cerevisiae* and shown below. Normal *S. cerevisiae* cells are Gram stained purple, as shown in **Figure 2** below.

In **Figure 3** are shown two examples of the cells after contact with a surface modified by attachment of a cationic lipid bearing a C16 chain. Cells have been destained and counterstained with the uptake of safranin, indicative of membrane disruption (intact cells would remain purple).

The modified fabric surfaces maintain their activity through multiple washings. Due to the physical nature of the kill, it is necessary to remove the build-up of dead cell debris from the treated surfaces for continued cell interactions with the active surface. Fabric samples have been washed under standardized conditions up to fifty times with an aqueous solution of non-ionic detergent and maintained full activity. The use of anionic detergents results in the detergent remaining associated with the surface and thereby inhibiting bactericidal activity.



Figure 2. *S. cerevisiae* cells stained purple with Gram stain—bright-field microscope, 1000× magnification.

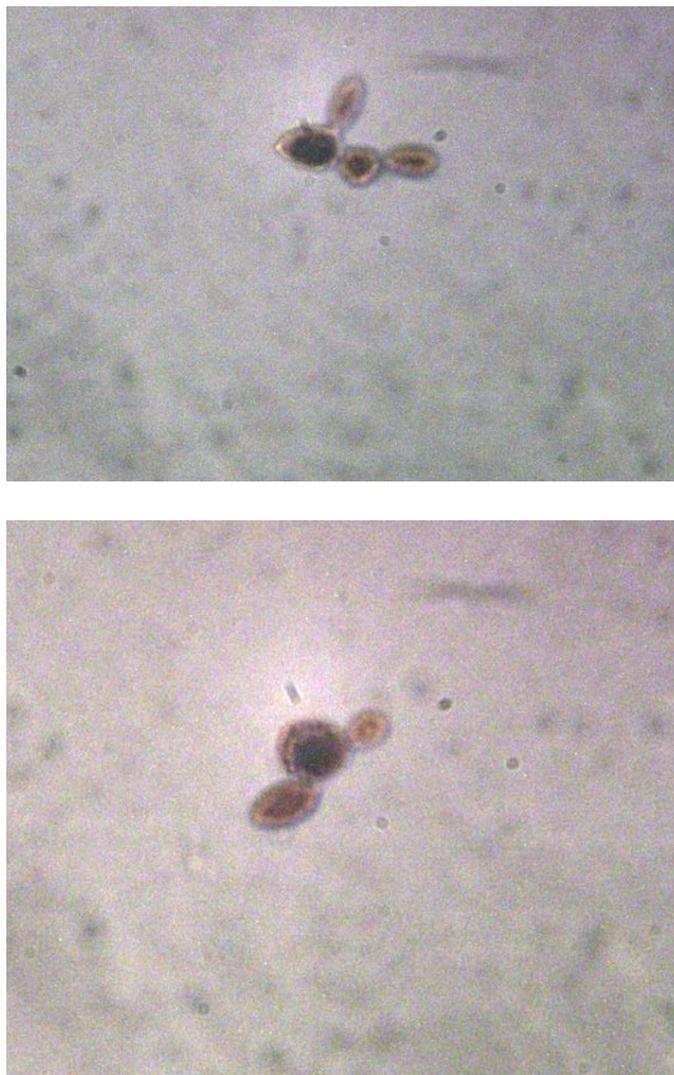


Figure 3. Change in staining (safranin) of cells after contact with a surface modified by attachment of a cationic lipid with a C16 chain—bright-field microscope, 1000× magnification.

3. Materials and Methods

Modified fabric materials were prepared as has been previously reported [4] [5]. Fundamentally, strips of fabric were weighed and subjected to submersion in a 1/1 water/2-propanol solution (100 mL per gram of fabric used) containing an amount of *p*-toluenesulfonyl chloride and sodium bicarbonate, each equivalent to 10% of the mass of the fabric used. After immersion for 24 hours at ambient temperature ($27^{\circ}\text{C} \pm 2^{\circ}\text{C}$) with constant agitation of the solution (by a magnetic stirrer—185 rpm) the fabric strips were removed, washed with ice/water, and reimmersed in a solution (1/1 water/2-propanol; 100 mL per g fabric used) containing 1-alkyl-1-azonia-4-azabicyclo [2.2.2] octane chloride (1 g per 10 g fabric). The solution was agitated by magnetic stirrer for 24 hours after which the fabric was removed, washed with ice/water, and allowed to dry

in the air. The modifications of the fabric samples were performed using salts bearing linear saturated alkyl groups of twelve, fourteen, and sixteen carbon atoms.

The 1-alkyl-1-azonia-4-azabicyclo [2.2.2] octane chloride salts were prepared by our previously reported method [4] [5] with linear saturated alkyl groups of twelve, fourteen, and sixteen carbon atoms, as illustrated in **Figure 4**. This process involved the reaction of 1 equivalent amount each of the appropriate 1-chloroalkane and 1,4-diazabicyclo [2.2.2] octane in ethyl acetate solution, 10 mL of ethyl acetate used for each gram of 1,4-diazabicyclo [2.2.2] octane used. After stirring at ambient temperature for 24 hours, the resultant precipitate was recovered by suction filtration, washed with ethyl acetate, and dried in air. As previously reported [4] [5] these materials exhibited ^1H and ^{13}C NMR spectra in accord with their proposed structures as well as quantitative elemental analyses.

Treated and untreated fabrics (1 cm \times 1 cm) were incubated with 1×10^5 bacteria (*S. aureus*) and the others shown in **Table 1** in 4 mL of Tryptic Soy Broth overnight at 37°C with shaking (100 rpm). The next day, 100 μL of each sample were transferred to 4 mL of fresh growth medium and incubated overnight at 37°C with shaking. Samples were observed visually for growth.

The mechanism of bacterial (and fungal) kill is understood to be invasion of the outer membrane or cell wall by the lipid portion of the cationic lipid covalently attached to the surface through the cationic end, lipid being taken up until the cationic site impinges on the wall or membrane and causes an electrostatic disruption of that wall or membrane. A hole thus being generated in the bacterium (fungus) resulted in the cell contents being capable of being ejected. While a fully functionalized cotton surface would provide $\sim 100,000$ such possible penetrations on a normal sized *E. coli* cell lying on such a surface, reductions in the amount of surface sites activated indicate that no more than 10 such invasions are necessary to kill such a cell. Thus, the method is quite efficient.

4. Conclusion

The *prevention* of bacterial infection can go a long way toward ridding our health care facilities of diseases. Our approach is one that has been demonstrated to prevent the casual transmission of bacterial diseases among patients and health-care workers in such settings. Treatment of the surfaces on which bacteria (and fungi) can be transmitted from infected patient to those uninfected, using covalently attached cationic lipids, has been shown to kill bacteria and fungi on those surfaces within minutes. In this manner, the transmission of disease can be significantly prevented and a major source

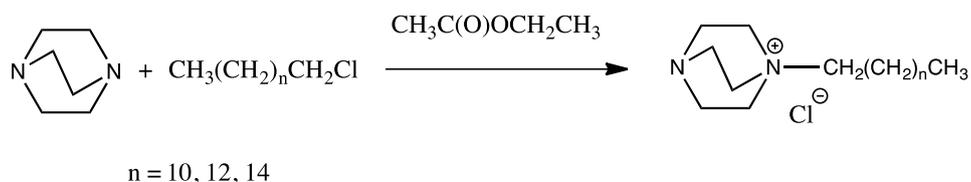


Figure 4. Synthesis of 1-alkyl-1-azonia-4-azabicyclo [2.2.2] octane chlorides.

of disease eliminated.

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The Number of Lymph Nodes and Relationship with Presence of Thyroiditis and Thymic Tissue in the Central Neck Dissection Materials for Thyroid Papillary Carcinoma: Pathologic Analysis

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Abstract

Background: Central lymph node dissection (CLND) for papillary thyroid carcinoma (PTC) allows correct pathologic staging of lymph nodes and planning of postoperative management. The purpose of this study was to determine the number of the lymph nodes in the CLND and the relationship to presence of chronic lymphocytic thyroiditis (CLT) and thymic tissue (TT). **Methods:** Total thyroidectomy and CLND materials from 153 patients with PTC were included in this study. Two histopathologic features (presence of CLT and TT) were evaluated for their value in adequacy of CLND. **Results:** Histopathologic examination revealed CLT and TT in CLND materials in 70 (46%) and 63 (41%) patients, respectively. Total number of lymph nodes in CLND materials was significantly higher in CLT (+) and TT (+) groups ($p < 0.001$), independently. LN metastases in central compartment were found in 40% of patients ($n = 61$). Of these, 30 patients had underlying CLT. The metastatic LN ratio (metastatic LN/total LN) was significantly lower in CLT (+) group, comparing to CLT (-) group ($16.8\% \pm 12.2\%$ vs $46.4\% \pm 27.5\%$; $p < 0.001$). **Conclusions:** Our study demonstrates that presence of CLT in thyroid gland has been associated with higher number of central lymph nodes mainly due to increased number of benign hyperplastic lymph nodes. It may be possible to conclude that upper limit of lymph nodes for satisfactory CLND would be higher to correctly evaluate central lymph node status in existing staging systems if specimens have CLT. Results of this study also show that the presence of TT in surgical materials may represent the adequacy of CLND.

Keywords

Thyroid Papillary Carcinoma, Central Lymph Node Dissection, Thyroiditis, Thymus

1. Introduction

Regional lymph node (LN) metastasis is a common finding at first diagnosis in papillary thyroid carcinoma (PTC) occurring in 20% to 90% of cases depending on the surgical and histological techniques [1]. The LNs in central compartment of the neck (level VI) are involved most frequently [2]. While compartment oriented LN dissection is the favored surgical approach for preoperatively proven lateral cervical and central LN metastases, controversy exist for clinically silent nodal disease. It has generally been accepted that LN metastases at presentation increase the risk of locoregional tumor recurrence, but do not adversely affect survival in patients with PTC. However, the findings of a large population-based study show significantly higher mortality rates for PTC involving lymph nodes [3]. Similarly, recent studies shows that central LN metastasis is also an important prognostic factor in patients with PTC and routine central lymph node dissection (CLND) reduces recurrent/persistent disease and postoperative thyroglobulin levels [2] [4].

Since, preoperative physical examination and ultrasonography, and intraoperative palpation do not seem sensitive enough; routine CLND remains the only reliable method to identify the status of central LNs [5]. Despite potential increase in perioperative complication rates, mainly permanent hypoparathyroidism and recurrent laryngeal nerve dysfunction, routine CLND has also potential to lessen the need for adjuvant radioiodine therapy by providing possible surgical cure and accurate staging.

The extent of CLND and macroscopic examination of dissection materials should be adequate for an accurate nodal staging of central compartment. Additionally, the total number of nodes found is critical since it reflects both the extent of the node dissection and the care with which the pathologic examination was carried out [6]. Several clinicopathologic parameters including surgeon experience, surgical techniques and length of fresh CLND specimen have been already studied as factors for adequacy of CLND materials [7]-[10]. However, impacts of underlying chronic lymphocytic thyroiditis (CLT) as well as existence of thymic tissue (TT) in CLND material on adequacy of central dissection are unknown. Current study aims to define effects of CLT and existence of TT on adequacy of central dissection under standardized surgical and pathological approach.

2. Material and Methods

This study was approved by the Institutional Ethical and Scientific Committee of Guven Hospital. Data were retrieved from retrospectively maintained surgical and pathology databases.

2.1. Selection of Patients

Patients included in the study had proven PTC in all sizes. The diagnosis of PTC was made by fine-needle aspiration biopsy and reconfirmed by the surgical pathology according to characteristics nuclear features and diagnostic structural patterns in all patients. Preoperative LN status in lateral cervical chain was determined by routine neck

ultrasonography and all recorded lymph nodes with suspicious sonographic features were further investigated accordingly. Standard approach to PTC without documented lateral cervical LN metastasis in our hospital is total thyroidectomy and CLND regardless of preoperative clinical and ultrasonographic findings. Lateral cervical LN dissection was added to standard surgical procedure for patients with proven LN metastasis in lateral compartment (23 patients), though results of lateral LN dissection were not taken into account.

From January 2012 to September 2014, a total of 153 consecutive patients, diagnosed as PTC and underwent total thyroidectomy plus CLND, were included in this study. Patients were excluded from the study if they had thyroid/parathyroid surgeries previously. All operations were performed by the same surgical team headed by one of our senior endocrine surgeons (SK, SO).

2.2. Surgery

Total thyroidectomy was performed by extracapsular dissection and the operation also included bilateral level VI LN clearance. Lymph nodes in central compartment, including all perithyroidal and paratracheal soft tissue and LNs with borders extending superiorly to the hyoid bone, inferiorly to the innominate artery, and laterally to the common carotid arteries, were dissected following total thyroidectomy. This compartment includes the pre- and paratracheal nodes, precricoid (Delphian) node, and the perithyroidal nodes, including the LNs along the recurrent laryngeal nerve. The prelaryngeal LNs, sitting directly anteriorly to the cricothyroid membrane between the cricothyroid muscles, were dissected at the time of mobilization of the thyroid pyramidal lobe and isthmus. In each patient, the recurrent laryngeal nerves (RLN) were exposed. We prefer to identify the RLN just caudal to the point where it crosses the inferior thyroid artery and to dissect it in both directions: caudally to the mediastinum and cranially to the cricothyroid junction. Intraoperative electrophysiological nerve monitoring was also employed in most of the patients. All vessels were ligated close to the thyroid gland especially the branches of the inferior thyroid artery. The parathyroid glands were dissected meticulously from the thyroid gland, and an effort was made to identify all four parathyroids and preserve as many as possible in situ. Any parathyroid gland that could not be preserved or devascularized during dissection was reimplanted into the sternocleidomastoid muscle. Bilateral thymectomy (upper poles of thymus gland) included into the dissection material when (1) central LN metastases documented preoperatively or (2) both superior and at least one of the inferior parathyroid glands ideally preserved along with their vascular integrity.

2.3. Pathologic Analysis

Macroscopic examinations were done by same experienced pathologist. Lymph nodes were dissected by manual palpation in the CLND specimen and rest of the fibro-adipose tissue submitted for pathological process for possible microscopic lymph nodes. All specimens were fixed in 10% formalin solution and were preserved in paraffin

blocks, which were cut serially in sections of 3 µm thickness and deparaffinized sections were stained with hematoxylin-eosin. Lymph nodes were assessed by routine histological examination and no additional techniques were used. The number of microscopic lymph nodes was assessed according to previous report by Parkash *et al.* [11]. Organized collection of lymphocytes and lymphoid follicles with well defined capsule were counted as a lymph node. If multiple nodes were submitted in a single cassette and there were 2 lymph nodes in a single fragment of fat, we counted this as 2 nodes. If the gross description said a single node was determined and microscopic observing showed 1 grossly remarkable node and 1 minor lymph node, we counted this as 2 nodes. If gross examination revealed as 2 or more cm diameters aggregate of adipose tissue which completely submitted, and the slide shows multiple lymph nodes, we counted this as 1 lymph node [11].

Each patient's clinicopathologic information, including age, gender, number of total LNs and metastatic LNs, presence of TT in dissection material and existence of underlying CLT were obtained from pathology reports. CLT was diagnosed as pathological specimens regardless of preoperative anti-thyroid antibodies and ultrasonographic findings. The diagnosis of CLT was defined as the presence of extensive lymphocytic infiltrations in the thyroid parenchyma, with well-developed germinal centers and presence of oxyphilic cells metaplasia in thyroid follicular cells. Assessment of the clinical stage of PTC was based on the American Joint Committee on Cancer (AJCC) TNM system [12].

Patients divided into three different LN score according to total number of LNs in CLND material as follows: low LN score (1 - 5 lymph nodes), intermediate LN score (6 - 10 lymph nodes), and high LN score (>10 lymph nodes).

2.4. Statistical Analyses

SPSS 12.0 for windows (SPSS Inc., Chicago, IL) was used for statistical analysis. The wilcoxon test, were used to analyze the statistical relationship between total lymph node number in CLND material and existence of CLT and TT. A two-tailed *p* less than 0.05 was considered statistically significant.

3. Results

A total of 153 patients (mean age 41 ± 11 yr, range 15 - 78) with PTC were evaluated in this study. The clinical and pathologic characteristics of the study group are reported in **Table 1**. Histopathologic examination revealed CLT and TT in CLND materials in 70 and 63 patients, respectively. Distribution of patients according to presence of CLT and TT in dissection material was summarized in **Table 2**.

The mean number of LNs removed from central compartment was mean 11 ± 5.7 (2 - 49). LN numbers in CLND materials and statistical analysis results for CLT and TT groups as well as subgroups were given in **Table 3**. Total number of LNs in CLND materials was significantly higher in CLT (+) and TT (+) groups ($p < 0.001$), independently. Existence of CLT associated with significantly higher number of central LNs in both TT

Table 1. Demographic and pathologic characteristics of study group.

Characteristics	<i>n</i>	%
Gender		
Male	29	19
Female	124	81
Age at diagnosis (years)		
<45	101	66
≥45	52	34
Histopathologic type		
Well differentiated type	140	91.5
Poor prognostic types*	13	8.5
T stage		
T1a	77	50
T1b	58	38
T2	10	6.5
T3	8	5.5
Central LN metastasis (+/-)	61/92	40/60
Lateral LN metastasis (+/-)	23/130	15/85
Presence of CLT (+/-)	70/83	46/54
Existence of TT in CLND material (+/-)	63/90	41/59

LN: lymph node; CLND: central lymph node dissection; CLT: chronic lymphocytic thyroiditis; TT: thymic tissue, *AJCC TNM system 2010 [12].

Table 2. The distribution of patients according to co-occurrence of chronic lymphocytic thyroiditis and presence of thymic tissue in central lymph node dissection material.

	CLT (-)	CLT (+)	<i>n</i>
TT (-)	50	40	90
TT (+)	33	30	63
<i>n</i>	83	70	

CLT: chronic lymphocytic thyroiditis; TT: thymic tissue.

Table 3. The relationship between the mean number of central lymph node and presence of chronic lymphocytic thyroiditis and thymic tissue in all groups and subgroups.

	CLT (-)	CLT (+)	<i>p</i> (subgroups)	TT Group	<i>p</i> (TT group)
TT (-)	6 (3 - 12)	11.0 (5.5 - 15)	0.007	8 (4 - 14)	<0.001
TT (+)	9.5 (7 - 13.25)	14 (9 - 28.5)	0.006	11 (8 - 16)	
<i>p</i> (subgroups)	0.006	0.024			
CLT Group	7.5 (4 - 13)	12 (8 - 16.25)			
<i>p</i> (CLT group)	<0.001				

CLT: chronic lymphocytic thyroiditis; TT: thymic tissue.

(+) and TT (-) subgroups. On the other hand, LN numbers did not differ statistically between CLT (+) and TT (-) subgroups in existence of TT.

Both existence of CLT and TT associated with more patients with intermediate and

high LN scores. LN scores according to total number of LNs in CLND material were given in **Table 4**.

LN metastases in central compartment were found in 40% of patients (n = 61). Of these, 30 patients had underlying CLT. Though it was not one of the main objectives of this study, metastatic LN ratio (metastatic LN/total LN) was significantly lower in CLT (+) group, comparing to CLT (-) group (16.8% ± 12.2% vs 46.4% ± 27.5%; p < 0.001). Demographic and pathologic characteristics of study group according to co-existence of CLT were summarized in **Table 5**. No thymic metastases were found in central dissection materials.

Table 4. Lymph node scores according to total number of lymph nodes in central lymph node dissection material in all groups and subgroups.

	Low LN score	Intermediate LN score	High LN score	<i>p</i>
CLT (+)	8	17	45	0.016
CLT (-)	22	32	29	
TT (+)	5	23	35	0.017
TT (-)	26	26	38	

CLT: chronic lymphocytic thyroiditis; TT: thymic tissue.

Table 5. Demographic and pathologic characteristics of study group according to co-existence of CLT.

Characteristics	n (CLT+)	n (CLT-)	<i>p</i> value
Gender			
Male	7	24	
Female	63	59	
Age at diagnosis (years)			
<45	46	52	
≥45	24	31	
Histopathologic type			
Well differentiated types	64	76	
Poor prognostic types*	6	7	
T stage			
T1a	33	44	
T1b	30	28	
T2	2	8	
T3	3	5	
N stage			
N0	35	57	
N1a	23	38	
N1b	9	14	
Number of LN in CLND material (mean ± SD)	12 (8 - 16.25)	7.5 (4 - 13)	<0.001
Metastatic LN ratio (%) (mean ± SD)	16.8 ± 12.2	46.4 ± 27.5	<0.001

LN: lymph node; CLND: central lymph node dissection; CLT: chronic lymphocytic thyroiditis; TT: thymic tissue, *AJCC TNM system 2010 [12].

4. Discussion

LN metastases are often detected at the time of initial diagnosis or during follow-up of patients with PTC. Efforts were concentrated on the ability to timely identify the presence of cervical LN metastasis prior first surgery for PTC, to justify an additional and more radical surgical procedure. Nevertheless, it remains difficult to assess central compartment nodal status preoperatively with high precision [13] [14]. Despite normal preoperative physical examination and ultrasonography, the incidence of LN metastasis in central compartment is high (29.4%) even in microcarcinomas [4]. Rates similar to this and higher have been described by some others [15] [16]. However, routine prophylactic CLND with total thyroidectomy in clinically node negative patients still subject of an ongoing debate mostly due to increased rates of transient and permanent hypoparathyroidism. Though re-operations associate with a higher complication rates comparing to initial surgery, revision surgery still represents the main treatment for known recurrent/persistent disease in central compartment [17]. Being aware of higher complication rates of re-operations [18], it has been advocated that efforts should be made to avoid central compartment reoperation by performing a definitive and more radical initial surgical treatment. Beside providing objective information about LN status, CLND has also potential to reduce postoperative Tg levels and local recurrence rates [4].

The LN involvement in central compartment is one of the main determinants of accurate staging in PTC patients [19]. The N has traditionally been based on the presence or absence of positive LNs, unrelated to the number of positive LNs and to the total number of LNs detected in existing staging systems [12]. Recent studies have introduced, however, that the number and size of central LN metastases as well as extranodal involvement may also be correlated with both lateral LN metastases and central neck recurrences [7] [20]-[22]. Clinically no apparent detectable metastatic lymph nodes, so called cN0, for PTC actually declares Nx status according to TNM classification system of the AJCC. Therefore, correct staging of central compartment in the absence of gross nodal involvement requires pathologic assessment.

To accurately determine the nodal status in central compartment, the extent of central lymph node dissection and macroscopic examination of dissection materials should be adequate. The number of central compartment lymph nodes should also be known to decide on adequacy of central dissection materials. Although the adequate number of lymph nodes yet to be defined for CLND, the number of central compartment LNs and adequacy criteria have been previously described in several studies [20] [21] [23]. Tavares *et al* studied the anatomic distribution of the lymph nodes in the central compartment of the neck by dissection of 30 fresh cadavers. They reported that the number of central lymph nodes varied from 2 to 42 [24]. According to the protocol of the College of American Pathologist, histological examination of a selective neck dissection specimen includes 6 or more lymph nodes. However, this number represents total LNs in dissection material and adequacy criteria for central LN number are not specified separately [25].

The variability in the number of LNs in central compartment might be explained by several factors including experience of surgeon, surgical technique used, pathologic process and interobserver variability among pathologist as well as nonneoplastic thyroid pathologies. The total number of nodes found is important since it reflects both the completeness of the node dissection or sampling and the care with which the pathologic examination was carried out.

The number of LNs in central compartment varied from 2 to 49 (mean 11 ± 5.7) with standardized surgical and pathologic approach in our study group.

With regard to the number of LNs, there were significant differences ($p < 0.001$) between CLT (+) and CLT (-) groups. Mean numbers of central LNs in CLT (+) and CLT (-) groups were 12 (8 - 16.25) and 7.5 (4 - 13), respectively. Previous reports also introduced that more central LNs were removed in PTC with CLT mainly due to increased number of benign hyperplastic cervical LNs [26]-[29]. Kim *et al.* have also reported a negative association between presence of CLT and central LN metastasis suggesting that coexistence of PTC with CLT might point out less aggressive features [27] [28]. In our study group, the mean number of central metastatic LNs ($p = 0.306$) and metastatic LNs ratio was also lower in CLT (+) group comparing to CLT (-) one ($p < 0.001$). It is not clear that lower metastatic rates in CLT (+) group is simply due to increased number of benign hyperplastic LNs in central compartment and hence sampling error or truly protective effect of CLT. But, data from our study group as well as previous reports shows that existence of CLT consistently associate higher central LNs in dissection material. Therefore, it may be possible to conclude that upper limit of adequacy for LN numbers would be also higher (>10) in PTC patients with CLT for optimal definition of central compartment LN status.

The other determinant for adequacy of CLND that has been analyzed in our study was the presence of TT. Since thymus constitutes the inferior boundaries of central compartment, thymic tissue frequently seen in central dissection materials. TT was seen in central dissection materials of 63 patients in our series. The LN numbers in central compartment were significantly higher in TT (+) group ($p < 0.001$) indicating more effective dissection. Mean numbers of central LNs in TT (+) and TT (-) groups were 8 (4 - 14) and 11 (8 - 16), respectively. This result indicated that the presence of TT in CLND materials might reflect the extent and adequacy of CLND and, consequently leads to correct pathologic staging. Highest mean LN numbers reached in study group was 14 (9 - 28.5) and found in CLT (+) and TT (+) subgroup, as expected.

5. Conclusion

In conclusion, co-existence of PTC with CLT is quite common in routine clinical practice and results in higher LN number in central dissection material if performed, mainly due to benign hyperplastic LNs. This feature should be taken into consideration during the pathologic examination of the central dissection materials for PTC, as well as interpretation of results. The LN numbers in central compartment materials that would have been ideal otherwise might be suboptimal in the presence of CLT. Therefore,

adequacy criteria in assessing central compartment nodal status would be different in patients with CLT. We recommend that the cut-off number for adequacy of central compartment LN numbers would be at least 10, which is almost double than value suggested by the existing staging systems, if specimens have CLT. The other main result of this paper is that the existence of thymus in central compartment dissection material might be an important factor suggesting sufficient LN resection.

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Author Disclosure Statement

The authors declare that there is no conflict of interest.

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Scurvy in the Postoperative Oropharyngeal Cancer Patient

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Abstract

Scurvy is now an almost forgotten disease, but it hasn't yet disappeared entirely. Here, we report the case of a patient with scurvy who presented with multiple hemorrhages about 5 years after undergoing pharyngeal surgery and radiochemotherapy for oropharyngeal cancer. This 60-year-old man was admitted to our hospital because of sudden onset of dyspnea and purpura. A physical examination and computed tomography revealed multifocal hemorrhages, namely, purpuras and petechiae on the legs and intramuscular and alveolar hemorrhages. Coagulation tests indicated a normal bleeding time and mild extension of the activated partial thromboplastin time. The coagulation factor activities were not low enough to account for such severe hemorrhages. No new hemorrhages were observed after admission. On questioning about his past eating habits, the patient reported having long had an extremely unbalanced diet, namely, a diet that was composed largely of carbohydrates with few fresh fruits and vegetables. This was due to the development of mechanical dysphagia after the operation. Since his vitamin C level was 0.5 µg/mL (normal range: 5.5 - 16.8 µg/mL), a diagnosis of scurvy was established. Thus, scurvy can still occur in modern times due to poor eating habits that arise from unusual settings. We must keep in mind that scurvy may be the cause when a patient presents with an apparently inexplicable tendency to hemorrhage.

Keywords

Scurvy, Vitamin C, Oropharyngeal Cancer

1. Introduction

Scurvy is a disease that was extremely prevalent among sailors during the Age of Dis-

covery between the 15th and 18th centuries. It was not clear until the 19th century that scurvy was the result of severe vitamin C deficiency [1]. Since vitamin C is an enzyme cofactor that is needed for collagen synthesis, long-term vitamin C deficiency induces connective tissue fragility and multiple organs become prone to hemorrhages after even very slight physical pressure or damage [2]. Our thorough understanding of the importance of vitamin C consumption has led in modern times to supplementation of commercial foods with vitamin C. Consequently, although scurvy still occurs in developing countries, it is an almost forgotten disease in industrialized countries. This is particularly true for Japan because it has a culture of eating raw food. We report here the case of a patient with scurvy who presented with multiple hemorrhages about 5 years after undergoing pharyngeal surgery for oropharyngeal cancer.

2. Case Presentation

A 60-year-old man was admitted to our hospital in January 2016 because of sudden onset of dyspnea and purpura. The patient had felt as usual until 1 month before admission, at which point he noticed multifocal petechiae on his legs. Over the next few days, the lesions increased and his left calf swelled painfully. Several days before admission, the patient became aware of purpura on the left side of his abdomen, which was causing intermittent pain. On the day of admission, he developed dyspnea. The history of the patient included surgery for oropharyngeal cancer in 2007. The cancer relapsed in 2010 and the patient underwent a second operation that was then followed by radiochemotherapy. Thereafter, relapse was not detected. The operations meant that the patient had to keep using an artificial saliva aerosol. The patient did not have a tendency towards hemorrhage as a child and there was no familial history of coagulation disorders. At the time of the most recent presentation, the patient had retired from his occupation as a fisherman and lived alone. Starting at the age of 20 years, the patient had consumed alcohol socially and smoked two packs of cigarettes a day. However, the patient stopped smoking at the age of 51 years, when he was first admitted for oropharyngeal cancer.

On examination, the patient was awake and alert. His temperature was 36.2°C, his blood pressure was 106/68 mmHg, his pulse was 52 beats per minute, and his oxygen saturation while breathing ambient air was 88%. His palpebral conjunctivae were anemic and no adventitious lung sounds were heard. Purpuras were observed along with tender swellings on the left lower side of his abdomen, his left calf, and his left ankle. There were also purpuras on the periungual areas of his toes and petechiae on his legs (**Figure 1**).

Hematological tests revealed pancytopenia: the white blood cell count was $3.4 \times 10^9/L$ and consisted of 82% neutrophils, 2.4% eosinophils, 0% basophils, 3.6% monocytes, and 12.5% lymphocytes. Moreover, the hemoglobin concentration was 10.6 g/dL, and the platelet count was $137 \times 10^9/L$. The bleeding time was normal and blood coagulation tests revealed mild extension of activated partial thromboplastin time (APTT) without extension of prothrombin time (PT) and a mild increase in the levels of



Figure 1. Physical manifestations. The patient had multiple purpuras and petechiae, which indicates an obvious tendency to hemorrhage. (a) Purpura of the abdominal wall; (b) petechiae of the legs; (c) swelling of the left calf; (d) purpuras on the periungual areas of the toes.

fibrinogen, fibrin degradation products, and D-dimer. Serological and biochemical tests showed that the C-reactive protein, indirect bilirubin (id-BIL), and lactate dehydrogenase (LDH) levels were high at 7.5 g/dL, 1.44 mg/dL, and 300 U/L, respectively. In terms of electrolytes, the sodium level was low (128 mmol/L), but the potassium, calcium, and chloride levels were normal. The blood levels of free T4 and thyroid-stimulating hormone (TSH) were normal. The laboratory test results of the patient at admission are shown in **Table 1**.

Chest radiography showed a mass shadow on the right upper lobe. Contrast-enhanced computed tomography (CT) of the chest found that this shadow was a solitary mass that had a cavity inside it. CT also detected ground-glass opacities on the right inferior lobe that could be alveolar hemorrhages. CT of the trunk and legs detected multiple intramuscular hemorrhages on the patient's left abdominal oblique muscle and the muscles of both legs (**Figure 2**).

Curiously, the patient did not develop further hemorrhages after admission. Initially, we wondered whether the patient might have some kind of acquired coagulation disorder because of the sudden onset of the hemorrhages and the presence of lung mass, which could indicate the presence of a malignant cancer. However, the activities of VIII and IX coagulation factors were sufficient. Moreover, although the XI, XII, and XIII levels were overall slightly under the lower limit of normal, the weak decrease was considered to be unlikely to account for the severity of the patient's hemorrhages.

We also speculated whether the patient had a collagen disease that could induce

Table 1. Laboratory data on admission.

Complete blood count			Biochemistry			Coagulation		
WBC	3350	/ μ L	TP	6.3	g/dL	PT	13.1	sec
Neu	82	%	Alb	3.3	g/dL	PT	69	%
Lym	12.5	%	T-Bil	1.6	mg/dL	APTT	43.4	sec
Mono	3.6	%	D-Bil	0.16	mg/dL	Fib	437	mg/dL
Eosino	2.4	%	AST	17	U/L	FDP	13.6	μ g/mL
Baso	0	%	ALT	9	U/L	D-dimer	4.7	μ g/mL
RBC	340×10^4	/ μ L	LDH	300	U/L			
Hb	10.6	g/dL	rGTP	30	U/L			
Ret	26	%	ALP	272	U/L			
Ht	31	%	CK	154	U/L			
PLT	13.7×10^4	/ μ L	BUN	13	mg/dL			
			Cr	0.86	mg/dL			
			Na	128	mmol/L			
			K	4.8	mmol/L			
			Cl	96	mmol/L			
			CRP	7.5	mg/dL			
			TSH	56	μ IU/mL			
			F-T4	0.8	μ IU/mL			

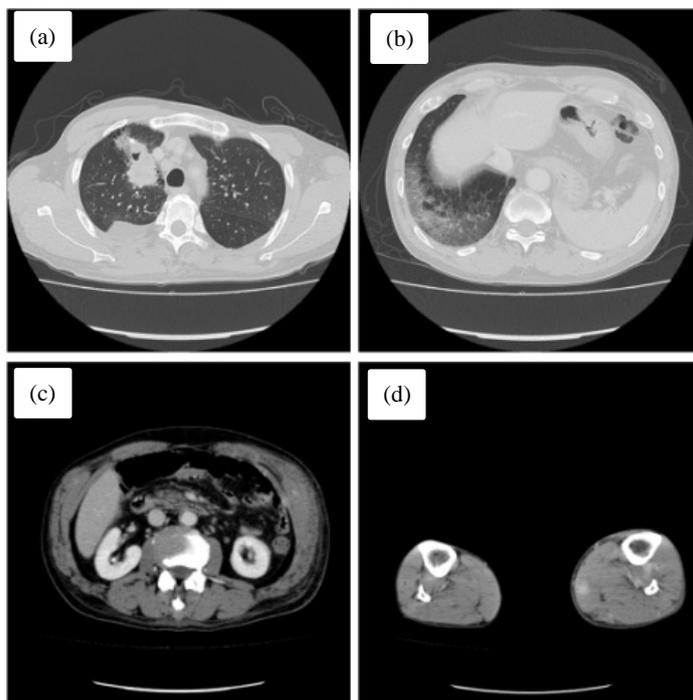


Figure 2. Contrast-enhanced computed tomography findings. (a) Lung mass on the right upper lobe; (b) ground-glass opacities on the right inferior lobe that may indicate alveolar hemorrhages; (c) intramuscular hemorrhage of the left abdominal oblique muscles; (d) intramuscular hemorrhage of the bilateral calves.

microangiopathy of the small vessels, such as antineutrophil cytoplasmic antibody (ANCA)-related vasculitis, systemic lupus erythematosus, or malignant rheumatoid arthritis. We obtained a skin biopsy from the purpura on the patient's left calf: histopathology revealed extravasation of red blood cells in the superficial to middle dermis but no infiltrating inflammatory cells around the small vessels. Thus, vasculitis was not indicated. The histopathological diagnosis was a purpura compatible. Thus, none of these tests led to an explanation for the cause of the multiple hemorrhages of the patient.

Another puzzling finding was the laboratory result of pancytopenia. However, this together with the slight increase in LDH and id-BIL led us to suspect hemolysis with an ineffective hematopoiesis such as myelodysplastic syndrome and megaloblastic anemia due to vitamin B12 and/or folic acid deficiency. Thus, further laboratory tests were requested. The test results are shown in **Table 2**.

The tests showed low levels of vitamin B12 and folic acid level despite the fact that the patient did not have a history of gastrectomy. This led to suspicion of a poor diet and thus a careful history of the patient's daily diet was taken. Surprisingly, the diet of the patient had become extremely unbalanced ever since he underwent the second operation for the oropharyngeal cancer in 2010: In the 5 years before admission, the patient had only eaten rice or tofu for breakfast and Japanese or Chinese-style noodles for lunch and dinner. Thus, the intake of fresh fruits, vegetables, and meat by the patient had been very limited for several years. This explained the vitamin B12 and folic acid deficiencies and was suggestive of other vitamin deficiencies, especially vitamin C deficiency. Indeed, testing for vitamin C 11 days after admission revealed a level of 0.5 µg/mL (normal range: 5.5 - 16.8 µg/mL). Thus, a diagnosis of scurvy was established.

The patient was started on 1000 mg of vitamin C per day for the first 3 days, after which 400 mg was given per day for 2 months. In addition, a multivitamin supplement that included vitamin B12 and folic acid was administered. The purpuras and petechiae and the pancytopenia of the patient disappeared gradually. Since the patient's tendency to hemorrhage has been resolved, he is currently scheduled to undergo a bronchoscopy to examine the lung mass.

Table 2. Additional laboratory data obtained a few days after admission.

Coagulation factors			Biochemistry		
VIII	152.5 (60 - 140)	%	Fe	63	µg/dL
IX	78.5 (60 - 140)	%	Ferritin	177	ng/mL
XI	66 (75 - 145)	%	Vitamin B12	116	pg/mL
XII	42 (50 - 150)	%	Folic acid	3.9	ng/mL
XIII	59 (70 - 140)	%	LA	1.05	
vWF antigen	150 (50 - 155)	%	ANA	<40	
vWF activity	178 (60 - 170)	%	RF	0	IU/mL
			P-ANCA	<1.0	U/mL
			C-ANCA	<1.0	U/mL

3. Discussion

Scurvy was first described by the Portuguese sailor Vasco da Gama during his voyage from Lisbon to India via Africa in 1498. Most of his crew became afflicted by this mysterious and frightening disease, which was characterized by poor wound healing, generalized edema, and life-threatening hemorrhages. In 1753, Sir James Lind, a ship's surgeon in the British Navy, found that if ships carried citrus fruits such as limes, oranges, and lemons, their sailors were less likely to develop scurvy. He reported on the basis of a controlled clinical trial (the first ever to be reported) that eating limes would protect the sailors from scurvy. It was only in 1932 that Szent-Györgyi in Hungary and C.G. King in the United States of America proved that vitamin C is the key substance that prevents scurvy. Vitamin C was then given the name "ascorbic acid", meaning a substance effective against scurvy [2].

Today, scurvy is an almost forgotten disease and most physicians in industrialized countries will never encounter a patient with it. However, it is still occasionally reported. In 2006, Olmed *et al.* reported 12 cases of scurvy that had been diagnosed at the Mayo Clinic between 1976 and 2002. Ten of these cases associated with alcohol abuse, illicit drug use, and severe mental disorders (e.g., depression or dementia). Moreover, five patients had food faddism or avoided products that contained vitamin C because of an alleged intolerance or allergy [3]. Today, patients with these factors remain at risk of developing scurvy. Indeed, our literature search revealed three reports of cases of scurvy in Japan in the last 20 years [4]-[6]. In all three reports, the patients had the risk factors described by Olmed *et al.* In our case, the patient developed scurvy because he had mechanical dysphagia after undergoing surgery for oropharyngeal cancer. This caused the patient to prefer eating soft foods that were easy to swallow. Thus, the diet of our patient for several years before admission consisted largely of carbohydrates; vitamin C-containing products were seldom ingested and thus the patient developed scurvy.

Vitamin C plays an essential role in the synthesis of collagens, which are major constituents of the extracellular matrix in the connective tissues. These collagens are thus important components of the physical barrier against external pressure and are responsible for maintaining organ form. Vitamin C is an enzyme cofactor for collagen prolyl hydroxylases, which catalyze the formation of intra- and inter-chain disulfide bonds during collagen synthesis [7] [8]. Thus, vitamin C deficiency inhibits collagen synthesis and results in tissue fragility, especially in the skin, gums, mucus membranes, and blood vessels.

Scurvy associates with a wide variety of cutaneous manifestations, including xerosis, ecchymosis, edema, poor wound healing, and gingival hemorrhage. "Perifollicular hemorrhage" and "corkscrew hair" are particularly characteristic manifestations of scurvy; their detection should arouse suspicion of the disease. Perifollicular hemorrhage is hemorrhage from a perifollicular site, especially on the legs, where hydrostatic pressure and hair movement due to mechanical irritation rupture the fragile perifollicular capillaries. Corkscrew hair is characterized by coiled body hairs that have a swan-neck

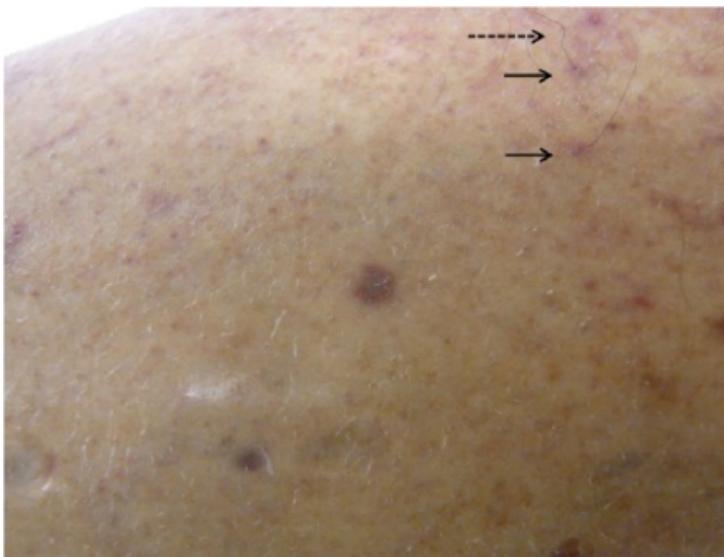


Figure 3. Enlarged photo of the left femoral skin, “perifollicular hemorrhages” (arrows) and “corkscrew hair” (dotted arrow) were observed after the patient was inspected carefully.

shape. This reflects the inhibition of disulphide bonding during hair formation that is caused by vitamin C deficiency [9] [10]. On closer examination of our patient after the diagnosis, we found both perifollicular hemorrhages and corkscrew hairs (**Figure 3**).

To develop scurvy, multiple years of vitamin C deficiency are needed. Although today such a situation is very unlikely to occur, our case shows clearly that scurvy can still arise in even the 21st century. The culprit is a prolonged unbalanced diet that was adopted for various reasons. Thus, when we encounter a patient with an inexplicable tendency to bleed, vitamin C deficiency should be included in the differential diagnosis. Thus, such patients should be asked about their dietary history and carefully inspected for the characteristic skin manifestations of scurvy.

Conflicts of Interest

The authors declare that they have no conflicts of interest to declare.

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Biermer Disease: Initial Presentation and Follow-Up of 66 Patients in Internal Medicine Department in Senegal

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Abstract

Pernicious anemia in black people, is little known. Through this study we assess its diagnostic and evolutive aspects, and compare vitamin therapy B12 intramuscular and oral. Sixty six Biermer disease patients followed (January 2000-June 2014) at Internal Medicine Department of Aristide Le Dantec University Teaching Hospital (Senegal) are included. They were 26 men and 46 women (gender ratio: 0.65), who had a mean age of 47.84 years \pm 15.25 years. Patients consulted for anemia (65 cases), acquired melanoderma (36 cases), gastrointestinal symptoms (30 cases), peripheral neuropathy (27 cases), venous thrombosis (2 cases), acute depression (1 case). Macrocytosis was observed in 52 cases. The mean hemoglobin in the vitamin B12 intramuscular group (52 patients) or oral group (14 patients) was the inclusion: 6.55 g/dl \pm 3.12 g/dl vs 6.52 g/dl \pm 2.18 g/dl ($p = 0.04$); and at day 8 treatment: 8.69 g/dl \pm 2.49 g/dl vs 8.85 g/dl \pm 1.9 g/dl ($p = 0.43$). Neurological and vascular presentations are unusual in contrast to macrocytic anemia. Oral administration of vitamin B12, simple and effective should be recommended in country with limited resources.

Keywords

Pernicious Anemia, Intramuscular Vitamin B12, Oral Vitamin B12, Senegal

1. Introduction

Biermer disease or pernicious anemia is a chronic auto-immune disease responsible for a chronic gastritis and a vitamin B12 deficiency, reversible under vitamin B12 therapy which oral administration is validated [1]. It is considered rare in black people and re-

lated literature in sub-Saharan Africa is limited to few case reports [2]-[5]. To our knowledge no publications about practice of oral vitamin therapy B12, is available in sub-Saharan Africa. With this series we propose to describe diagnostic and progression aspects, and to compare outcome on vitamin therapy B12, intramuscular and oral.

2. Patients and Methods

It is a retrospective, descriptive study with analytical outlook conducted upon comprehensive enrollment of medical files of Biermer disease patients followed from 1st January 2000 to 30th June 2014. The study held at Internal Medicine Department of superior referral hospital of Aristide Le Dantec University Teaching Hospital (Senegal) which has consultation, hospitalization and research activities. Our study included 66 files of Biermer disease, onto an annual average of 685 inpatients and 14,871 outpatients at Internal Medicine Department.

The diagnosis of Biermer disease was made in the presence of positive anti-intrinsic factor or anti-parietal cells antibodies, associated or not with vitamin B12 deficiency and atrophic gastritis [1]. Epidemiological, clinical and paraclinical data were analyzed. Bone marrow aspiration and analysis provided precision on the existence of megaloblastosis. Vitamin B12 deficiency was defined by measurements below normal values between 187 and 883 pg/ml.

Positive anti-intrinsic factor and anti-parietal cells antibodies were defined by measurements higher than 1.53 and 40 AU/ml respectively. Upper gastro-intestinal-tract (GIT) endoscopy with systematic antrum and fundusbiopsies and histology revealed gastric atrophy, metaplasia and helicobacter pylori (HP).

Cyanocobalamine is administered intramuscularly (1000 µg once a day for the 1st week, then once a week for a month and once a month for life) or orally (2000 µg per day for 10 days, followed by the same dose once a week for 4 weeks and then once a month for life). Indications for oral administration were: Ongoing anticoagulation, thrombocytopenia below 50,000/mm³ and difficult access to intramuscular injections. The median follow-up of patients treated with vitamin B12 (oral or intramuscular) was 1135.72, days [8 - 4886 days].

Statistic tests (medium, standard deviation, Student test) were done using Statistical Package for Social Sciences 20 software.

3. Results

The files of 40 women and 26 men (gender ratio: 0.65), with mean age of 47.84 years ± 15.25 were included.

Comorbidities were metrorrhagia (2 cases), hemorrhagic cystitis (1 case) and partial gastrectomy for a benign tumour (1 case), vitiligo (5 cases), type 2 diabetes mellitus, Hashimoto thyroiditis (2 cases) and multiple auto immune disease syndrome (2 cases).

In 28 medical files blood group was specified and it was O (12 cases), A (7 cases), B (7 cases) and AB (2 cases)

Mean diagnostic time was 16 months (6 - 48 months). Presenting symptoms (**Table 1**) were anemia signs (65 cases), palmo-plantar acquired diffuse melanoderma (36

Table 1. Clinical and paraclinical characteristics of the patients of our study.

<u>Clinical signs on diagnosis</u>	n/N = 66	(%)
Anemia manifestations		
Anemic syndrome	40	(60.6)
Hemolytic anemia	13	(19.7)
Anemic heart disease	7	(10.6)
Isolated conjunctiva pallor	5	(7.6)
GIT manifestations		
Epigastric pain	30	(45.5)
glossitis	21	(31.8)
diarrhea	6	(9)
constipation	4	(6)
dysphagia	3	(4.5)
Dermatologic manifestations		
melanodermia	36	(54.5)
Neuropsychiatric manifestations		
polyneuropathy	27	(40.9)
posterior cord syndrom	1	(1.5)
Acute depression	1	(1.5)
Venous manifestations		
Saphenous and femoral thrombophlebitis	1	(1.5)
Portal vein thrombosis incidentally discovered	1	(1.5)
<u>Paraclinical signs</u>		
	n/N	(%)
Anemia	65/66	(98.5)
Thrombocytoenia	33/66	(50)
Leukocytopenia	29/66	(43.9)
Pancytopenia	25/66	(37.9)
Bicytopenia	18/66	(27.3)
Thrombocytosis	2/66	(3)
Hyperleukocytosis	1/66	(1.5)
Vitamin B12 deficiency	59/66	(89.39)
Central megaloblastosis	37/47	(88.1)
Positive anti-intrinsic factor antibodies	50/51	(98.03)
Positive anti-parietal cells antibodies	25/36	(69.44)
Atrophic gastritis	34/37	(91.89)
Antrum and pyloric metaplasia	10/37	(27.02)
Helicobacter pylori	2/37	(5.4)

N: Total number of patients who did the test; n: Number of patients with abnormalities; %: Percentage.

cases), GIT signs including Hunter glossitis (21), polyneuropathy (27 cases) which one posterior cord syndrom, acute transient depression (1 case) and deep venous thrombosis (2 cases). The discovery of the disease was incidental in one patient who only had macrocytosis with no anemia (1).

Blood count (BC) (**Table 1** & **Table 2**) revealed anemia (65 cases), macrocytosis (52

Table 2. Blood Count and reticulocyte count: At the beginning of the study and progression according to the administration route of vitamin B12.

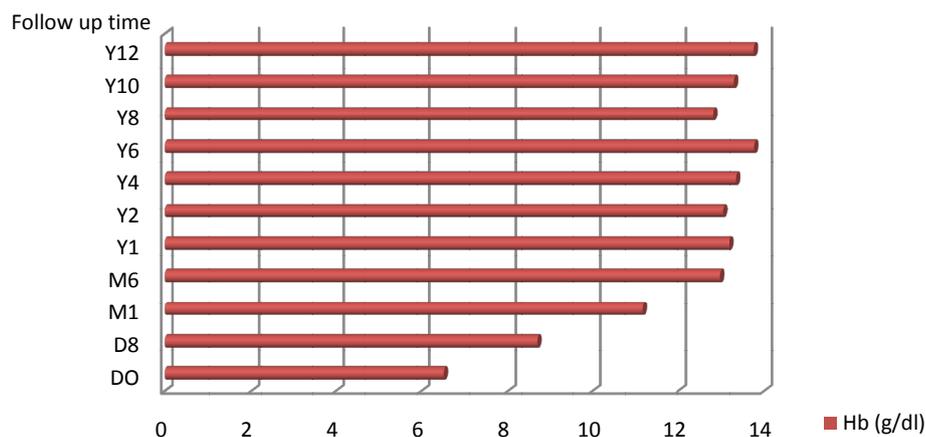
Parameters	Beginning of the study	Mean	SD	Extreme	
D0: Hb (g/dl)	6.52	2.93		[1.3 - 15.2]	
MCV (fl)	107.04	11.36		[81 - 131]	
MCTH	34.4	5.04		[22 - 42.06]	
PLT (G/L)	148.31	487.44		[170 - 488]	
WBC (G/L)	4.39	2.17		[1.5 - 15.10]	
Ret. count (G/L)	30.89	29.24		[2 - 98]	
According to the route of administration of vitamin B12					p [‡]
	Intramuscular (N = 52)	(n) Mean SD	Oral (N = 14)	(n) Mean SD	
D0: Hb (g/dl)	(52) 6.55	3.12	(14) 6.52	2.18	0.43
MCV (fl)	106.06	11.76	110.66	9.21	0.09
Ret. count (G/L)	55.26	29.78	49.29	28.74	0.37
D8: Hb (g/dl)	(52) 8.69	2.49	(14) 8.85	1.9	0.83
MCV (fl)	100.03	10.72	99.53	8.51	0.89
Ret. count(G/L)	104.65	102.78	145.52	87.39	0.27
M1: Hb (g/dl)	(41) 10.83	2.23	(12) 11.81	1.16	0.16
MCV (fl)	94.0	13.34	90.91	6.81	0.18
Ret. count(G/L)	149.58	130.70	212.21	79.71	0.22
M6: Hb (g/dl)	(23) 12.71	1.89	(12) 13.33	1.02	0.96
MCV (fl)	86.02	8.60	86.14	8.28	0.97
Ret count (G/L)	213.76	102.32	297.00	54.76	0.04

N: total number of patients treated, n: number of patients explored; Hb: hemoglobin; MCV: Mean Corpuscular Volume; Ret Count: reticulocyte count; SD: Standard deviation; Mean: Mean value; D0: Day 0 on admission; D8: Day 8; M1: 1st month; M6: 6th month; p[‡]: Student test.

cases) and hypochromia (4 cases). One patient had isolated macrocytosis at 115fl with hemoglobin of 15.2 g/dl. The mean hemoglobin was 6.52 g/dl \pm 2.93 g/dl was below 6 g/dl in 47.7% of patients. BC (**Table 1**) also revealed thrombocytopenia (39 cases) out of which 7 were below 50 G/L without bleeding, leukocytopenia (28 cases), thrombocytosis at 460 G/L reactive to iron deficiency, and thrombocytosis at 488 G/L associated with leukocytosis at 15.1 G/L in the context of pyelonephritis. There were cytopenias in form of pancytopenia (25 cases) and bicytopenia (18 cases). Reticulocyte count was low in all patients (**Table 2**). Traces of hemolysis were noted in 18 patients who had mean LDH at 522IU/l [451 - 612 UI/l] and direct serum bilirubin at 21 mg/l [13 - 32 mg/l].

In addition to BC abnormalities, we found megaloblastosis (88.7%), positive anti intrinsic factor (98.03%) and anti parietal cells antibodies (69.40%), atrophic gastritis (91.89%) and HP infection (5.4%) (**Table 1**).

Overall evolution after intramuscular (52 cases) or oral (14 cases) vitamin therapy B12 is favorable. On day eighth, is observed a mean reticulocytosis crisis of 119.63 G/L \pm 97.94 G/L [36 to 466.46 G/L]. Hemoglobin levels is rising during vitamin B12 administration (**Figure 1**). Hemoglobin levels increased after 6 months in 83.9% of patients



DO : day on admission ;D8: day eight; M1 : 1st month ; M6: 6th ; month; Y1: 1st year; y2: 2nd year ; Y4: 4th year; Y6: 6th year ; Y8: 8th year; Y10: 10th year ; Y12 : 12th year
Hb : mean of hemoglobin ;

Figure 1. Evolution of the average rate of hemoglobin.

and after 1 year the improvement was at 91.7% of patients.

In analytical study the mean hemoglobin level on vitamin B12 supplements in the intramuscular group versus oral, was on day eight 8.69 ± 2.5 g/dl Vs 8.85 ± 1.9 g/dl ($p = 0.43$) and after 1 month it was 10.83 ± 2.2 g/dl Vs 11.81 ± 1.1 g/dl ($p = 0.16$) (**Table 2**). In oral group the reticulocyte count which was at 49.29 G/L initially rose to 297 G/L after 6 months (**Table 2**).

4. Discussion

The available literature on Biermer disease in sub-Saharan Africa is made of limited series.

In 2003, Segbenaet al [2] reported 4 observations and in 2013, Diopet al [3] collected 28 cases over 6 years. To our knowledge, our study is the largest series carried out in Senegal (66 cases) and is the 1st describing oral vitamin B12 therapy.

In our study as well as in other african publications [2]-[5], Biermer disease is common in women in their fifties. Predominant signs on presentation in our study were anemia signs, which also were almost present in de Segbenaet al [2] and Ndiaye *et al.* [4] with respective prevalences of 100% and 80%.

In our study, anemia signs are predominantly made of anemic syndromefar ahead of hemolytic anemia and anemic heart disease. Heart failure as described in our patients is a chronic complication of cardiovascular manifestations common to all vitamin B12 deficiencies as described in almost 50% of cases by Nafil *et al.* [6].

Acquired melanoderma, second presenting sign in our study is also frequently reported in African publications [3] [4]. It is a diffuse homogenous melanoderma with buccal and palmo-plantar predominance secondary to disturbed tyrosine synthesis, this being a melanin precursor [7].

The third diagnostic condition in our study was GIT signs with atypical epigastric

pain, followed by Hunter glossitis. This one is more specific of Biermer disease and was noted in 78.57% of patients in Diop *et al.* series [3].

The least observed manifestation in our patients was deep venous thrombosis. As a comparison Zulfiquar *et al.* [8] and Diop *et al.* [3] respectively reported 10 and 2 observations.

Beside thrombosis, acute depression and combined sclerosis of the bone marrow are rare in our study (1 case) like in the literature [4] [8] [9].

Main BC abnormalities were macrocytic anemia followed by thrombocytopenia and neutropenia both in our patients and in Song and al series [10] who reported 65% of thrombocytopenia and 45.5% of leucopenia. However some authors [11] didn't note any difference in the prevalence of leukocytopenia and thrombocytopenia. These cytopenias are more combined into pancytopenia orbicytopenia [3] [4] [8]. Early diagnosis at the macrocytosis stage before cytopenia is rare [5], we only had one observation. Beside the frequent macrocytosis, normocytosis and hypochromia are not exceptional [3] [8]. They are due to additional factors like iron deficiency in bleeding of our patients for example or other comorbidities like association with beta thalassemia and Plummer Vinson [3].

Characteristic bone marrow aspirate findings in our study were the high frequency of megaloblastosis, coinciding with results of de Segbena *et al.* [2] (100%) and Wun Chan *et al.* [9] (95%).

Positive anti-intrinsic factor antibody which is more specific of the disease [3] [9], was almost constantly present in our study.

Prevalence of atrophic gastritis was high unlike HP surinfection which was rare. Similar findings in this regard were also obtained by Wun Chun *et al.* [9] who published cases of HP surinfection par HP in 109 Biermer disease patients who underwent upper GIT endoscopy with biopsies.

On therapeutic aspect, hematologic abnormalities are reversible with vitamin B12 treatment, more frequently administered intramuscularly in our study and in the literature [1] [4]. Song *et al.* [10] noted that 94.3% of anemia regressed after 3 months treatment with vitamin B12.

Oral route of administration is effective and is indicated in case of severe thrombocytopenia or anticoagulation treatment [1]. In addition therapeutic compliance is better obtained with oral administration in patients with difficult access to health care. In analytical study we noted that oral vitamin B12 allowed cytopenia regression by day eight and reticulocytosis was statistically significant after 6 months. The absence of statistical significance of some of our tests was a bias due to the limited number our series enrolled.

5. Conclusion

Macrocytic anemia and acquired melanoderma are frequently revealing signs of Biermer disease, in contrast to neuropsychiatric and vascular manifestations. Oral route of vitamin B12 administration, simple and equally effective should be strongly promoted

in sub-Saharan Africa regions with limited resources.

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Retiform Sertoli-Leydig Cell Tumor of the Ovary

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Abstract

Sertoli-Leydig cell tumor of the ovary is a kind of sex cord-stromal tumor, which occurs between teens and twenties with symptoms including abdominal pain and swelling. The incidence rate is infinitely rare comprising less than 0.5% of all ovarian tumor. The average age of “retiform Sertoli-Leydig cell tumor” is 17 years as compared to 25 years for Sertoli-Leydig cell tumors as a group. We have experienced this rare case of retiform Sertoli-Leydig cell tumor in a 25-year-old foreign patient with the complaint of palpable mass on the right lower quadrant and an irregular menstrual period. The patient underwent right salpingo-oophorectomy and tumor stage was FIGO stage 1A. We report with a brief review of literature.

Keywords

Sertoli-Leydig Cell Tumor, Retiform Pattern, Sex Cord-Stromal Tumor, Ovary

1. Introduction

Sertoli-Leydig cell tumor is a very rare tumor originating from sex cord-stromal tumor. The incidence rate is infinitely rare comprising less than 0.5% of all ovarian tumor [1]. In 1905, Pick had found a microscopically odd ovary, which was comparable with male genital duct [2]. Meyer found that an odd ovary was extremely analogous to ductli efferents and defined it as Sertoli-Leydig cell tumor [3]. Among these Sertoli-Leydig cell tumors, Kanter and Klawans have first sorted out and reported retiform pattern in 1940 [4]. Sertoli-Leydig cell tumor is highly related with normal testis embryologically. World Health Organization (WHO) uses the terminology of the Sertoli-Leydig cell tumor as synonym of Androblastoma [5]-[7]. Whatever terms, Sertoli-Leydig cell tumor is considered as a clinically malignant tumor. And this tumor is usually occurred in young age female. The average age of ‘retiform Sertoli-Leydig cell tumor’ is 17 years as compared to 25 years for Sertoli-Leydig cell tumors as a group [8]-[11].

Young and Scully and WHO had classified the Sertoli-Leydig cell tumor into six patterns: 1) Well differentiated; 2) intermediate differentiation; 3) poorly differentiated; 4) retiform; 5) heterologous elements; and 6) mixed. A typical microscopic feature of the retiform pattern shows irregular anastomosing tubules made by cuboidal or columnar cells [7] [9] [12]. The objective of our paper is to present this rare case of retiform Sertoli-Leydig cell tumor in a 25-year-old woman and review of relevant literatures.

2. Case Report

A 25-year-old woman visited a local Obstetrics and Gynecology clinic because of amenorrhea for six months and right lower quadrant pain occasionally. Computed tomography (CT) scan revealed a tumor of the right ovary, measuring 5 cm in diameter. After then she was referred to the Department of Obstetrics and Gynecology in Dankook University Medical Center for further management.

There was no evidence of hirsutism, baldness, or deepening of the voice. On physical examination, a boggy feeling mass was palpated with tenderness on the right lower quadrant. Cervix showed normal appearance and no motion tenderness. Complete blood count, blood coagulation test, liver function test, renal function test, electrolyte, lipid test, urine test, chest X-ray, and electrocardiography were all within normal ranges. Papanicolaou smear test showed atrophy with inflammation. Serum levels of α -FP was 4.4 ng/mL, CA-125 was 23.9 U/mL, and SCC was 0.84 ng/mL.

Transvaginal ultrasonography showed an approximately 6.1×4.8 cm sized multilocular mass including the solid portion on the right ovary. There were no abnormal findings on the uterus. Magnetic resonance image (MRI) revealed the suspected diagnosis of cystadenofibroma or sex cord-stromal tumor originating from the right ovary (Figure 1).

Pelviscopic operation was performed. Diagnostic pelviscopy showed a large mass originating from the right ovary and no adhesion in the pelvic cavity. Right salpingo-oophorectomy was performed. Specimen and abdominal fluid for cytology were sent to the pathology. Pathologic classification was read according to the International histologic classification of tumors modified by Roth, *et al.* Grossly, the tumor was yellow tan solid mass with partly cystic area, measuring $6 \times 5 \times 3$ cm. Microscopically, the tumor showed solid cords, slit-like tubules, and irregularly branching cystic spaces with some papillae lined by cuboidal cells with round to ovoid nuclei (Figure 2). Tumor cells showed no significant nuclear atypia, and mitotic figures were rare. Immunohistochemical staining results were positive for inhibin but negative for vimentin, Wilm's tumor protein (WT-1) and p53. Cytology in abdominal fluid revealed no tumor cell. According to this histologic finding and immunohistochemical staining results, the tumor was found to be Sertoli-Leydig cell tumor with retiform pattern.

The patient was discharged on the 4th post-operative day without any other complication. On the 9th post-operative day, she was re-admitted for staging work-up of ovarian cancer. Tumor markers and hormone tests were performed (LH 14.24 mIU/mL, FSH 4.18 mIU/mL, estradiol 52.5 pg/mL, testosterone 0.50 ng/mL, progesterone

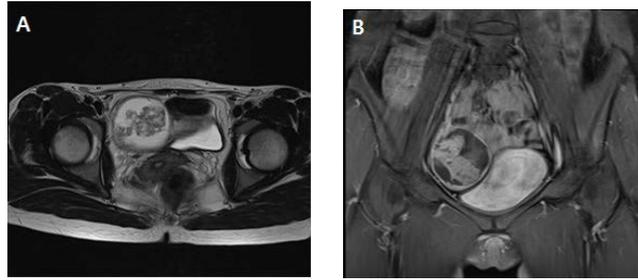


Figure 1. Pelvis Magnetic Resonance Imaging image shows a large multi-cystic mass measuring approximately 5.8×5.5×5.5 cm in size, with an enhancing irregular thick wall and an amorphous enhancing solid portion with low signal intensity in the right pelvic cavity. (A) Coronal view and (B) axial view.

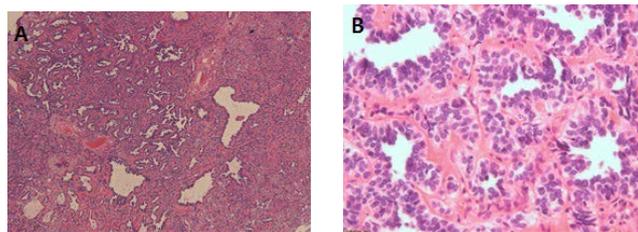


Figure 2. Microscopic finding of the Retiform Sertoli-Leydig cell tumor. The tumor showed irregular network of slit-like tubules and elongated spaces, typifying retiform Sertoli-Leydig cell tumor (A; H&E stain, ×40). The tubules are lined by Sertoli cells with round to ovoid nuclei that exhibit varying degrees of stratification (B; H&E stain, ×400).

0.45 ng/mL, 5 α -dihydrotestosterone 0.11 ng/mL, prolactin 3.66 ng/mL, CA-125 28.2 U/mL, α -FP 2.2 ng/mL, CA19-9 25.6 U/mL, SCC 0.75 ng/mL). All of them were within normal limit. MRI, positron emission tomography (PET)-CT, intravenous pyelography (IVP), cystoscopy, sigmoidofibroscope, and colon studies showed no abnormal findings. Therefore we concluded that any other adjuvant management would not be necessary. She is followed up every 3 months, and shows no evidence of disease till now for 25 months after the surgical treatment. Follow-up MRI examination revealed no evidence of local recurrence or distant metastasis. And now she is happily pregnant state at 29 weeks of gestational age.

3. Discussion

Sertoli-Leydig cell tumor, papillary serous cystadenocarcinoma, malignant mixed müllerian tumor and yolk sac tumor cannot easily be distinguished and diagnosed. Sertoli-Leydig cell tumor should be differentiated from these tumors with some key points. Clinical features and average onset age of papillary serous cystadenocarcinoma and malignant mixed müllerian tumor differ from those of Sertoli-Leydig cell tumor. These tumors tend to occur in the older age group. Between yolk sac tumor and Sertoli-Leydig cell tumors, yolk sac tumor is more pathologically primitive and includes ab-

undant glycogen. Also yolk sac tumor shows positive sign for α -FP staining [11].

In 2002, Young and Scully classified Sertoli-Leydig cell tumor according to six subtypes and incidence rates were: well differentiated (11%), intermediate differentiation (54%), poorly differentiated (13%), retiform pattern (15%), heterologous elements, and mixed type. Virilization was observed in only 33% of Sertoli-Leydig cell tumor patients [9] [10] [12].

Retiform patterned Sertoli-Leydig cell tumor is only found to be intermediate differentiation and poorly differentiated type [4]. In general, retiform pattern occurs in the younger age group and with less virilization than other types [10]. When a patient visits hospital for the first time, the chief complaint is usually unilateral abdominal pain due to a huge abdominal mass. Young and Scully reported on 24 retiform pattern cases of unilateral mass in 25 patients. With visual inspection, the retiform pattern consisted of a solid or cystic portion with a yellowish liquid portion and measured 8 cm to 22 cm, which was larger than other Sertoli-Leydig cell tumors [9] [13]. Some retiform Sertoli-Leydig cell tumor showing papillary pattern may mimic serous adenocarcinoma. In this case, as described above, the tumor showed no prominent cytologic atypia and negative WT-1 and p53 staining results. These findings were not consistent with serous adenocarcinoma. Furthermore, positive inhibin staining result indicated this case was retiform Sertoli-Leydig cell tumor rather than serous adenocarcinoma.

According to a report by Young and Scully, malignancy potential is 0% in the well differentiated, 11% in the intermediate differentiation, 59% in the poorly differentiated, and 19% in the heterologous elements type. Distance metastasis rate was 10% to 30% and recurrence rate was 66% after the first treatment trial. The prognosis was worse in cases where Sertoli-Leydig cell tumor recurred within one year from treatment [10] [11] [13].

The standard treatment for Sertoli-Leydig cell tumor is surgical treatment. Especially in early stage of Sertoli-Leydig cell tumor (FIGO stage 1A), standard treatment is unilateral salpingo-oophorectomy. In the elderly group or patients with advanced stage, debulking surgery could be attempted, however, there are controversies regarding the benefits and prognosis [14]. PEB (cisplatin, etoposide, and bleomycin) and PAC (cisplatin, dactinomycin, and cyclophosphamide) chemotherapy could be performed for intermediate differentiation or poorly differentiated type. But there is no evidence of preventing recurrence or increasing therapeutic efficacy [15].

For young patients, fertility may be affected by debulking surgery and chemotherapy. Some surgeons including Telerman reported that only unilateral salpingo-oophorectomy had shown a cure rate of 88.9% in FIGO stage 1A to Sertoli-Leydig cell tumor retiform pattern patients [13].

Both treatment and fertility preservation are the key points in management of Sertoli-Leydig cell tumor, especially for young women. In prudent consideration of FIGO stage 1A and fertility preservation unilateral salpingo-oophorectomy was performed. The final pathologic diagnosis was Retiform Sertoli-Leydig cell tumor of intermediate differentiation type, which had less malignant potential than poorly differentiated type.

And so post-operative adjuvant chemotherapy was not attempted. The patient has been in disease-free state for 25 months after the surgical treatment. And now she is happily pregnant state at 29 weeks of gestational age.

4. Conclusion

We presented the above rare case of retiform Sertoli-Leydig cell tumor in a 25-year-old foreign patient. The patient underwent right salpingo-oophorectomy and tumor stage was FIGO stage 1A. Sertoli-Leydig cell tumor shows a variety of subtypes. It occurs relatively at a young age and has a malignant potential. However, there are no standard treatment guidelines for patients who want to preserve fertility. Because of the low incidence of Sertoli-Leydig cell tumor, only a few small scale and large-scale long term studies have been conducted in light of fertility preservation and recurrence. Future large-scale studies including meta-analysis are necessary.

Conflict of Interest

No potential conflict of interest relevant of this article was reported.

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Investigating the Extent of CCL4 and CCL5 Chemokine as Well as IL17 and IL23 Cytokine Gene Expression in the Patients Afflicted with Multiple Sclerosis

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Abstract

MS is a chronic inflammatory disease of central nervous system in which T cells enter central nervous system and create an inflammatory cascade that leads to applying the other blood cells. Pre-inflammatory Cytokines are representative of the process of inflammatory diseases like MS. In addition, the increase of such cytokines has been observed in this kind of anomalies. On the other hand, helping T cells of 17 (TH17) contributes to the secretion of interleukin 17 that is a pre-inflammatory cytokine and an increase in TH17 cells is induced by interleukin 23 (IL23) which in turn causes absorption of neutrophils in the site of inflammation. Chemokine also has a determining function in the immune system including Ccl4 protein sit, the function of which is chemical absorption for calling natural fatal cells and monocytes as well as the other immune system cells; additionally, Ccl5 causes chemical absorption to absorb Eosinophil and Basophil and has an active role in applying Leukocyte-sin the site of inflammation. As a result, these 4 factors can hurt nervous cell through increasing its expression and calling leukocytes. The expression of these 4 genes, corticosteroid treatment and investigating its impact on changing the expression of such genes are the aims of this study. In this study 50 samples of blood of people suffer from multiple sclerosis (according to the diagnosis of specialist) (new case), 25 samples of blood of people suffer from MS (according to the diagnosis of specialist) using corticosteroid and 50 blood samples of healthy people without any symptoms were compared. To investigate the extent of expression of IL23, IL-17, CCL5 and CCL4 genes, first, the patients' blood is taken, RNA was extracted and Real Time PCR was carried out. According to the results obtained from Real Time PCR of patients and healthy people it was specified that the amount of expression of chemokine and cytokines is increased in the people suffering from MS and has a direct relationship

with MS. In comparing the two groups of patients (new case and people taking medicine) it was revealed that the amount of expression of IL17, IL23, CCL4 and CCL5 genes is decreased in people who take medicine. An increase in the expression of IL17, IL23, CCL4 and CCL5 genes is the symptom of MS disease and contributes to its creation and progression. The difference in the expression of chemokine and cytokine genes can be used as an identifying maker in this disease. On the other hand, corticosteroid medicine can have a determining role in the increase of expression as well as the destructive function of immune system.

Keywords

Multiple Sclerosis, Chemokine, Cytokines, Real Time PCR

1. Introduction

Multiple sclerosis or MS is a chronic inflammatory disease of central nervous system which is known as an autoimmune disease. Exhaustion is one of the most important disabilities of this disease and the extent of outbreak is about 53 - 92 percent changeable depending on how to define exhaustion [1]. The exhaustion of this disease as the patients say is different from a regular one; it is along with intense weakness, leak of energy and feeling of disability. Pathogenesis of this disease is still unidentified, but generally a set of mechanisms involved in its pathogenesis are recommended including: A change in neurotransmitters, cessation in the continuous transmission of unmyelinated axons, transmission in the paths of arousal, wakefulness and alertness as well as the presence of especial amino acids, none of which are finally verified [2]-[4]. In fact, MS is known as an immunopathological disease in which T cells enter central nervous system and create an inflammatory cascade that leads to applying the other blood cells [5] [6]. However, migration of immune cells can be considered a potential cause of treatment [7]. Statistics all around the world show that women are afflicted with MS more than men, and generally 2 - 3 million Europeans and countries with Caucasian immigrants (such as America, Australia and north of Asia) are suffering from this disease [8] [9]. However, in a study the effect of geographical environment was investigated in single-egg twins by keeping them separated before puberty [10]. This disease like the other multifactorial diseases is influenced by genetic and environmental factors. Therefore, in the absence of etiological factors to be considered as the aim of prevention and cure, molecular mechanisms that are the foundation of inflammations, myelin omission and detoxification in neurons are used; this resulted in some ways of treatment and decreased progression of the disease, but it wasn't treated. Proper treatments of MS must prevent return of the disease, nervous inflammation, myelin omission and loss of neurons. But, unfortunately there is no treatment like this and treatment can reduce progression of the disease through anti-inflammatory glucocorticosteroids. Prescription of INF copolymer and the recent use of new pharmaceuticals of natalizomab and antibody monoclonal (Moab) against $\alpha 4 \beta 1$ integrin can enhance such people's life

expectancy and quality of life. However, limitations of treatment as well as the side effects of such pharmaceuticals made scientists have innovations in treatments [11]. Pre-inflammatory cytokines that are representative of inflammatory process of diseases such as MS are associated with the symptoms and exhaustion of many diseases like cancer, viral infection and exhaustion syndrome [12]-[15]. Besides, the increase of cytokines especially the increase of alpha tumor necrosis factor (TNF α) has been observed in such anomalies and caused sleepiness during the day, sudden falling asleep, sleep attack and idiopathic oversleeping [16] [17]. 17 helper T cells contribute to the secretion of interleukin 17 that is a pre-inflammatory cytokine; the increase of TH17 cells is induced by interleukin 23 and interleukin itself causes absorption of neutrophil to the site of inflammation and affects creation of inflammation [18] [19]. So, the role of such cytokines is very significant in the pathology of MS disease. On the other hand, chemokine is the factor that calls immune cells to migrate and the probability of inflammatory diseases by targeting the chemokine and its receptors have led to many researches. Chemokine is hardly fallen into two groups: First, pre-inflammatory chemokine that calls leukocytes to the site of inflammation when need be and second, homeostatic or anti-inflammatory chemokine that are regulators of continuous transmission to the site of main lymphoid. Previous studies generally focused on inflammatory chemokine. Most of the anomalies of MS disease are due to the function of B cells and patients manifest production of intrathecal immunoglobulin that has an oligo colony-band and responds specifically. The immunoglobulin oligo colony will remain in patients for a long time [20]. In the laboratory it is specified that the speed of transmission of B cells in the brain endothelium is more than T cells [21]. But, it is not specified how the chemokine that leads B cells is regulated [22]. Ccl4 or ligand chemokine is a human protein encoded by a gene under the same name, another name of which is beta MIP1 is responsible for chemical absorption for calling natural fatal cells, monocytes and the other cells of immune system [23]. Ccl5 human protein set which is copied by a gene under the same name causes chemical absorption in order to absorb eosinophil and basophil and has a determining role in applying leucocytes in the site of inflammation [24]. That's why such chemokine is essential in calling defensive cells to the site of inflammation, therefore, the change of their expression is very important in the body of patient, and thus the chemokine mentioned in the other studies were chosen. At the end, it can be stated that we will be able to diagnose patients faster and use promising treatments for them by applying new tools such as sequencing the entire genome and the related studies [25] [26] as well as investigating expressive profile and protein analysis [27] [28].

The aim of this study is to investigate the extent of change in the expression of 4 genes including 2 cytokines of IL17 and IL23 as well as 2 chemokine CCL4 and CCL5 in the patients afflicted with MS compared to healthy people, because the outcome of the 4 aforementioned genes have determining role in stimulating immune system and moving it toward inflamed nervous cells and in case of an increase in the expression, they will be the aim of treatment in the subsequent studies.

2. Materials and Methods

Molecular investigation of chemokine and cytokine genes using Real Time PCR method

Real Time PCR was used to investigate the extent of expression of IL23, IL-17, CCL4 and CCL5 genes. In this study 50 blood sample of people afflicted with Multiple Sclerosis (according to the diagnosis of specialist) was taken from Shohada Tajrish hospital (new case), 25 blood sample of people afflicted with Multiple Sclerosis (according to the diagnosis of specialist) who used corticosteroid was taken from Shohada Tajrish hospital and 50 sample of healthy people without any symptoms were compared. First, RNA was extracted with QIAamp RNA Blood Mini Kits (Qiagen, Germany) and the quality of each of them was assessed using Nanodrop. All the samples enjoy good quality and thickness and they are in the range of 1.8 - 2 (**Table 1**, **Table 2**).

Perl primer (version 20) and primer 3 software were used for primary designing of the required genome, after a proper pair of primers were selected with regard to the mentioned points, their connection was investigated on the genome through Ncbi BLAST site (National Center for Biotechnology Information) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Synthesis of cDNA

The synthesis was carried out using cDNA synthesis kit (Vivantis America) following the guidelines of the manufacturing company by adding Rnase inhibitor for removing cDNA pollution of synthesis in 5 minutes in 65 degree centigrade.

CDNA that was made using PCR method obtained proliferation, temperature and cDNA dilution and after electrophorus gel and making sure of the correction, Real Time PCR was used.

Table 1. Primers and the sequence of probes.

Gene	Primers and the sequence of probes
Beta actin (β -actin)	Forward 5-AGCCTCGCCTTTGCCGA-3 Reverse 5-CTGGTGCCTGGGGCG-3
CCL4	Forward 5-TCCTACTGCCTGCTGCTT-3 Reverse 5-GCTGGTCTCAAAGTAATCTGC-3
CCL5	Forward 5-TTCTACACCAGCAGCAAG-3 Reverse 5-TTCTACACCAGCAGCAAG-3

Table 2. Primers and the sequence of probes.

Gene	Primers and the sequence of probes
Beta actin (β -actin)	Forward 5-AGCCTCGCCTTTGCCGA-3 Reverse 5-CTGGTGCCTGGGGCG-3
Interleukin-17 (IL-17A)	Forward 5-AATCTCCACCAGCAATGAGGA-3 Reverse 5-ACGTTCCCATCAGCGTTGA-3
Interleukin{IL-23 (p19)}	Forward 5-TCAGTGCCAGCAGCTTTCAC-3 Reverse 5-TCTCTTAGATCCATGTGTCCAC-3

Real Time PCR:

After extraction of cDNA of beta actin sample as control and IL17, IL23, CCL4 and CCL5 genes were investigated for determining the percent of changes in gene expression using Real Time PCR (Polymerase Chain Reaction). Therefore, investigating the extent of expression of IL17, IL23, CCL4 and CCL5 compared to beta actin gene (control homey gene) in the same samples was estimated in the form of relative expression.

Real Time PCR reaction was carried out using Master Mix 2x (Biorad America) in final volume of 20 microliter by qiagene Real Time PCR and the data of cycle of threshold was analyzed through System software ver.2.0. For estimating the percent of changes (Fold change) in the expression of IL17, IL23, CCL4 and CCL5 genes $2^{-\Delta\Delta CT}$ formula was used in which $\Delta\Delta CT$ of the difference in the cycle of threshold of IL17, IL23, CCL4 and CCL5 genes compared to the control gene in the sample was determined. Eventually, for estimating $2^{-\Delta\Delta CT}$, excel 2013 and SPSS software were used.

In the first real time we set up the required genes so that real time is carried out under the best conditions and dilution and after that we investigate the required samples under the same conditions.

Findings

We measured and investigated the prepared cDNA using Real-time PCR, Q model from QIAGEN Company.

The following picture (**Figure 1**) shows an increase in the expression of ccl4 and ccl5 genes in patients compared to healthy people:

Figure 2 shows an increase in the expression of IL17 and IL23 genes in the patients compared to healthy people.

Figure 3 shows the difference of expression of ccl4 and ccl5 in the patients who used corticosteroid and the healthy people.

Figure 4 shows the difference of expression of IL17 and IL23 in the patients who used corticosteroid and healthy people.

The results of Real Time reveals an increase in the expression of IL17, IL23, CCL4 and CCL5 genes in the patients suffering from multiple sclerosis, such genes are higher expression in this disease. Corticosteroid treated patients had an increase in the expression of IL17, IL23, CCL4 and CCL5 genes, however this increase is not to the extent of new case people.

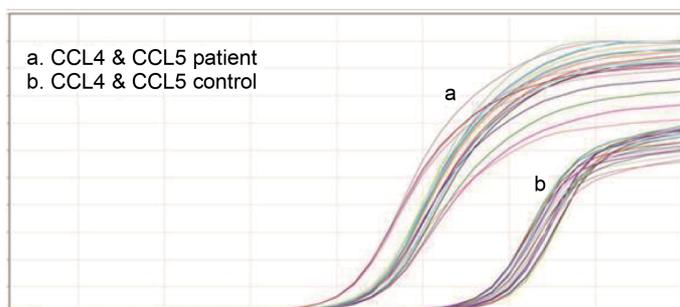


Figure 1. Increase in the expression of ccl4 and ccl5 genes in patients compared to healthy people.

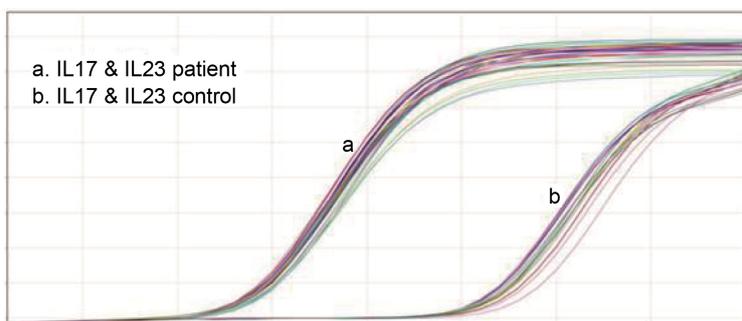


Figure 2. Increase in the expression of IL17 and IL23 genes in the patients compared to healthy people.

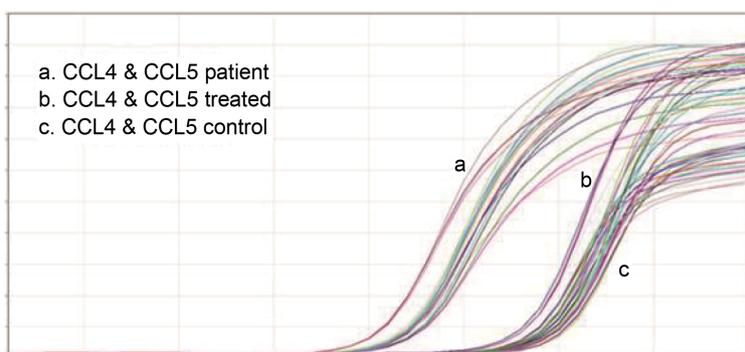


Figure 3. Difference of expression of ccl4 and ccl5 in the patients who used corticosteroid and the healthy people.

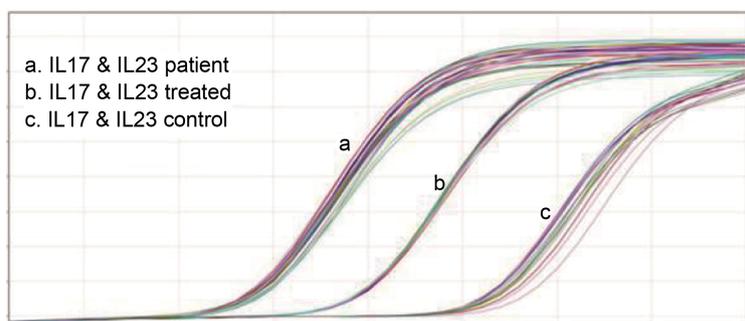


Figure 4. Difference of expression of IL17 and IL23 in the patients who used corticosteroid and healthy people.

3. Discussion

We know that keeping dynamic balanced homeostasis among the pre-inflammatory and anti-inflammatory cytokines is required. Pre-inflammatory cytokines have a key role in MS pathogenesis through activating immune system in blood and central nervous system. Anti-inflammatory cytokines containing IL-4 are effective in the recovery of MS. Pre-inflammatory cytokines include: IFN-gamma, TNF-beta, IL-12 and IL-17, the blood cells expressing mRNA, the number of TNF α and its serum concentration are increased in MS patients. IL-12, the most stimulant agent of IFN-gamma is also a

pre-inflammatory cytokine, but the new information reveal that IL-23 whose P40 chain is similar to IL-12 has more important role. Pre-inflammatory cytokines cause Oligodendrocyte and myelin harm to CSF and brain. More mononuclear cells expressing TNF α and IFN-gamma are seen in MS patients. TNF α is a pre-inflammatory cytokine and makes inflammation, but it also contributes to tissue repair in brain. Pre-inflammatory cytokines of IL-12 and IL-17 are also increased in CSF and brain lesions of MS patients [29]-[32]. Contributive 17 T cells contribute to the secretion of IL17 that is a pre-inflammatory cytokine, the increase of TH17 cells is induced by IL23 and IL17 itself causes absorption of neutrophils to the site of inflammation and create inflammation [18] [19]. Chemokine and its receptors also have a key role in calling leukocytes and the other cells to the site of inflammation. Entering inflammatory T cells inside CNS is the most important step in MS. Blood-cerebral blockage will be started by weak connection of these cells with endothelial cells. Then, they make a firm cellular connection and pass blood-cerebral blockage. Chemokine activate the connection of leukocyte cells with endothelial and this way they pass blood-cerebral blockage. Induction of proteolytic enzymes removes blood-cerebral blockage and chemokine keep entrance of the other cells to CNS. The expression of CCR5 in T cells is increased in the environmental blood of MS patients, besides, in recurrence which is representative of pathogenic role for +CTL CCR5 cells, the increase of expression of CXCR3 was observed in the T cells of some of the patients. T cells expressing CCR5 and CXCR3 produce high amount of TNF α and IFN-gamma and specific Th1 cells MBP express high level of CXCR3 and CXCR6. The expression of CXCR3 facilitates the entrance of T cells to CNS and CXCL10 keeps such cells in inflamed CNS. CCL4 is activated in parenchyma inflammatory cells (macrophage and microglia), CCL3 is activated in inflammatory cells of parenchyma and neuroglia and CCL5 is expressed in the inflammatory cells of pre-vessel and astrocytes. The other chemokine in active lesions of MS include: CCL2, CCL7, CCL8 and CXCL10. CXCR3 is expressed over most of the pre-vessel T cells of MS lesion and CCR5 is expressed over some of these cells.

CCR1 is expressed on the newly infiltrated monocytes, CCR2 and CCR3 are expressed on macrophage and CCR5 is expressed on the infiltrating monocytes and activated microglia cells. The role of chemokine and its receptors was verified in MS pathogenesis through a research on the animal model (EAE) of MS. The increase of CCL3, CCL2, CCL5 and CXCL10 in EAE is along with the progression of disease and their elimination results in recovery. Polymorphism in chemokine genes and its receptors cause sensitivity or immunity to MS, however, there is no certain evidence to confirm this hypothesis [29]. Ccl4 or chemokine ligand is a human protein being encoded by a gene under the same name and another name of that is MIP1. Its function is chemical absorption to call natural fatal cells, monocytes and the other immune system cells [23] [33]. Ccl5 is a human protein which is copied by a gene in the same name, this protein causes chemical absorption to absorb eosinophil and basophils and it has an active role in applying leucocytes in the site of inflammation [24]. The aim of this research is to investigate the impact of MS disease on production of cytokine and che-

mokine by the immune system of the patient. The results were the ones predicted according to the rule and the level of all 4 factors had an increase in expression, this represented that the patient had inflammatory reaction and the cytokines and chemokine are activated to call the other parts of immune system and they apply the other factors. In this study corticosteroid treatment was applied to test and prove its function. Using it for a period, the level of expression of these 4 genes were investigated. The result revealed that it decreased their expression influentially and it reduced inflammation.

4. Conclusion

According to the data it was specified that the 4 genes had an increase in expression and the increase of their expression leads to calling leucocytes and nervous harm; therefore, this increases and subsequently calling leucocytes to the site of nervous cells are harmful for cell and result in some harms that create disease, so however we can reduce the increase in expression, and the life expectancy and the analysis of high level of symptoms will be increased. According to the study, it can be stated that corticosteroid can be a reliable pharmaceutical, but the side effects must also be taken into account. It is recommended to investigate the lower factors of such genes as well and it is better to try herbal-based pharmaceuticals.

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Evaluating Hospital Admission/Discharge Rates at the Community Level

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Abstract

Hospital admission/discharge rates are generating increased attention from health care providers and payors. This study focused on evaluation of inpatient hospital admission/discharge rates for Syracuse and other New York State metropolitan areas during 2014 and 2015. It provided comparative information concerning this subject and suggested how this approach to analysis of hospital utilization could be carried out using publicly available data. The study data demonstrated that hospital admission/discharge rates per 1000 population increased with patient age in all of these areas. The study data suggested that differences in hospital admission/discharge rates among the New York State metropolitan areas were generally consistent between 2014 and 2015. Utica and New York City produced the highest rates. Rochester and Albany produced the lowest rates. Utilization rates for Syracuse were considerably lower than for Utica and New York City and slightly higher than for Rochester and Albany. This analysis demonstrated that most of the differences between aggregate rates for Syracuse and Rochester were produced by elderly patients, especially those aged 75 years and over. The analysis demonstrated that most of these differences in admission rates for the elderly were produced by adult medicine patients aged 75 years and over. Most of these differences were generated by patients with respiratory, digestive, and orthopedic disorders. Additional data suggested that the highest readmission rates for adult medicine and adult surgery were produced by patients aged 75 years and over.

Keywords

Hospitals, Hospitalization, Hospital Admissions

1. Introduction

In recent years, efforts by health care providers and payors in the United States to im-

prove services have focused on a number of issues related to utilization. Many of these concern improvements in the efficiency and outcomes of care [1].

Historically, health care providers have worked to deliver services more efficiently. Their efforts have included length of stay reduction programs stimulated by payments per discharge. Providers have been able to shorten stays by reducing expenses while maintaining revenue [2] [3].

Since the 1980s, health care researchers studied differences in hospital admission rates by region and approaches to reducing them. These efforts focused on local and regional variations in admission rates in the eastern and midwestern United States [4]-[7].

During the twenty first century, health care payors have developed new initiatives to reduce hospital admissions. These include programs that include primary care providers, statewide initiatives such as the Delivery System Reform Incentive Payment (DSRP) effort in New York, and initiatives developed by large insurers such as Blue Cross. Recently, efforts to increase the efficiency of care have also emerged from population based initiatives [7] [8].

Health care payors have also developed programs designed to improve hospital outcomes by reducing inpatient admissions. In 2009, Medicare began penalizing hospitals whose inpatient readmissions exceeded certain levels for specific diagnoses [9]-[13].

In recent years, a number of risk sharing arrangements involving health care providers and payors have developed in the United States. These have included Accountable Care Organizations designed to improve the efficiency and outcomes of care. They have also included payor driven initiatives to reduce hospital admissions, as well as efforts to reduce the use of long term care services for patients discharged from hospitals [14]-[17].

2. Population

The study evaluated inpatient hospital admissions and discharges, as well as related indicators in Syracuse, New York and other New York State metropolitan areas. The Syracuse area includes three acute care facilities, Crouse Hospital (19,776 discharges excluding well newborns, 2015), St. Joseph's Hospital Health Center (24,803 discharges, 2015), and Upstate University Hospital (28,237 discharges, 2015). The hospitals work with a combined medical staff of more than 1,800 physicians.

The Syracuse hospitals provide primary and secondary acute care to the metropolitan area with a population of approximately 600,000. They also provide tertiary care services to the Central New York Health Service Area with a population of approximately 1,400,000.

The Syracuse hospitals have worked cooperatively to improve the efficiency of acute care in the community through initiatives that involve the local health care system. A number of these initiatives have been developed through the Hospital Executive Council [18].

Historically, the Syracuse hospitals have maintained a conservative acute care admis-

sion rate compared with other metropolitan areas. An important cause of this admission rate was the decision not to construct another hospital in the northern suburbs of Syracuse during the 1970s. At the time this decision was made, the local hospitals sponsored the development of an organization to provide additional ambulatory care services in the area. In the 1980s, that entity became the sponsor of the community's first managed care plan.

During the 1990s, the managed care organization was acquired by another insurance plan. During the twenty first century, the Syracuse area has not had a locally based managed care plan. Since 2010, a number of health care payers, including Medicaid and Blue Cross, have begun discussions with hospitals in Syracuse and other New York State metropolitan areas concerning the potential for reducing inpatient admissions.

3. Method

This study evaluated recent hospital admissions/discharges in the metropolitan area of Syracuse, New York in comparison with other New York State metropolitan areas. The evaluation was based on hospital admissions/discharges per population in these areas during January-December 2014 and January-December 2015.

The study was based on resident data within which individual hospitals were not identified. It was developed as a community wide study by the Hospital Executive Council which serves as the research organization and the Ethics Committee for multi-hospital studies in the community. The study employed publicly available data which are not subject to hospital specific issues.

The study employed a simple descriptive analysis of hospital admission/discharge rates. These analyses were based on resident admissions/discharges compared with resident populations for each category of the analysis.

The article was based on summary data concerning hospital admission/discharge rates. The data used to calculate these rates are available from the authors on request.

The first component of the study compared hospital admission rates in Syracuse and other New York State metropolitan areas. This was a useful comparison because areas such as Utica and New York City have generated relatively high admission rates, while Rochester has produced relatively low rates. The comparison employed inpatient data from 2014 and 2015 in order to include the most current information.

For each of the two twelve month periods, the study data were analyzed by patient age including 0 - 17 years, 18 - 44 years, 45 - 64 years, 65 - 75 years, and 75 years and over, and total. This analysis provided information concerning the age levels of patients driving differences in admission/discharge rates between Syracuse and the other New York State metropolitan areas. Newborns were excluded from the analysis because they were not counted as admissions. Mental health patients were excluded because data for State mental health hospitals were not available.

Data for this portion of the study were obtained from the New York Statewide Planning and Research Cooperative System (SPARCS). This is a public data base that in-

cludes inpatient information from all hospitals in the State.

The second component of the study evaluated resident inpatient discharges by major hospital service for Syracuse and other New York State metropolitan areas in the population age groups where admissions and discharges per population in Syracuse appeared to be elevated. This analysis focused on adult medicine and adult surgery, which accounted for most inpatient admissions and discharges for these age groups.

The third component of the study evaluated resident inpatient discharges by clinical category for Syracuse and other New York State metropolitan areas in the major hospital service and population age groups where admissions and discharges per population in Syracuse appeared to be elevated. This analysis employed the Major Diagnostic Categories based on the All Patients Refined Diagnosis Groups developed by 3M™ Health Information Systems [19].

The fourth component of the study evaluated inpatient hospital readmissions within 30 days for patients by age level in the combined Syracuse hospitals. This analysis included numbers of readmissions and readmission rates for patients readmitted between January 2014 and December 2015. These data were based on the Potentially Preventable Readmissions software developed by 3M™ Health Information Systems.

These data were developed using the Potentially Preventable Readmissions algorithm developed by 3M Health Information Systems. The study data were obtained from the three Syracuse hospitals by the Hospital Executive Council through Business Associate Agreements.

4. Results

The first component of the study focused on resident hospital discharge rates for Syracuse and other New York State metropolitan areas including comparisons by age level. Related data are summarized in **Table 1**.

This information demonstrated that, consistent with experience in the hospital industry, admission/discharge rates for each metropolitan area increased with patient age. The rates for ages 75 years and over were 5 - 6 times the rates for ages 18 - 44 years and 3 - 4 times the rates for ages 45 - 64 years.

This information also demonstrated that, during 2015 and 2016, the highest resident hospital admission/discharge rates among the metropolitan areas were generated by Utica and New York City, while the lowest rates were produced by Rochester and Albany. Additional data demonstrated that the distributions of admission/discharge rates were similar between 2007 and 2015.

The data demonstrated that, within these distributions, the admission/discharge rates for Syracuse were lower than those of Utica and New York City and higher than those of Rochester and Albany. The total discharge rates for Syracuse were 14.2 - 15.5 discharges per 1000 lower than for Utica and 4.2 - 8.5 discharges per 1000 lower than for New York City. The total rates for Syracuse were 1.0 - 3.6 discharges per 1000 higher than those of Rochester and 3.5 - 10.7 percentage discharges per 1000 higher than those of Albany.

Table 1. Resident inpatient hospitalization per 1000 population, medical/surgical, obstetrics, and pediatric/neonatal, New York state metropolitan areas, 2014-2015.

Resident County	2014					
	0 - 17 Years	18 - 44 Years	45 - 64 Years	65 - 74 Years	75 Years & Over	Total
Capital District (Albany, Schenectady, Rensselaer)	22.7	55.5	78.7	151.2	279.7	78.3
Erie County (Buffalo)	30.3	64.6	90.9	173.2	314.1	93.4
Monroe County (Rochester)	25.9	64.8	88.1	165.9	314.7	88.0
New York City (5 Burroughs)	37.6	69.8	103.2	194.9	388.0	97.5
Oneida County (Utica)	23.2	72.4	100.3	190.5	371.1	104.5
Onondaga County (Syracuse)	22.0	64.7	89.3	170.5	340.9	89.0
Resident County	2015					
	0 - 17 Years	18 - 44 Years	45 - 64 Years	65 - 74 Years	75 Years & Over	Total
Capital District (Albany, Schenectady, Rensselaer)	23.4	57.8	86.4	165.1	319.6	88.3
Erie County (Buffalo)	30.6	63.1	89.5	170.1	312.7	92.3
Monroe County (Rochester)	23.1	62.4	88.3	169.9	329.1	88.2
New York City (5 Burroughs)	36.6	68.5	101.2	190.7	386.5	96.0
Oneida County (Utica)	23.9	69.5	101.7	199.1	383.0	106.0
Onondaga County (Syracuse)	24.2	65.3	92.4	177.4	350.9	91.8

Data do not include well newborn (APR DRG 626, 640), mental health/substance abuse treatment (APR DRGs 740 - 776), and rehabilitation (APR DRG 860).
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The breakdowns of discharge rates by age level suggested that these differences were produced largely by elderly population subgroups. The Syracuse rates were less than 2 discharges per 1000 higher than those of Rochester and Albany for residents aged 0 - 17 years, less than 9 discharges per 1000 higher for residents aged 18 - 44 years, and less than 11 discharges per 1000 higher for residents aged 45 - 64 years. At the same time, the rates for Syracuse were 5 - 19 discharges per 1000 higher for residents aged 65 - 74 years and 26 - 60 discharges per 1000 higher for residents aged 75 years and over.

In this context, the comparisons between the rates for Syracuse and Rochester were

noteworthy because both markets are dominated by the same major insurer. The Syracuse rates were 3.9 discharges per 1000 lower—1.1 discharges per 1000 higher for ages 0 - 17 years, 0.1 discharges per 1000 lower—2.9 discharges per 1000 higher for ages 18 - 44 years, and 1.2 - 4.1 discharges per 1000 higher for ages 45 - 64 years. The Syracuse rates were 4.6 - 7.5 discharges per 1000 higher for ages 65 - 74 years and 21.8 - 26.2 discharges per 1000 higher for ages 75 years and over.

These comparisons suggested that hospital admission/discharge rates in the Syracuse metropolitan area were conservative compared with those of other areas in New York State. They also indicated that most of the differences between Syracuse and those of Rochester and Albany were generated by elderly populations.

The second component of the analysis focused on hospital admission/discharge rates for major inpatient services in population age groups where rates in Syracuse appeared to be elevated, ages 65 - 74 years and 75 years and over. Relevant data are summarized in **Table 2**.

This information included admission/discharge rates for adult medicine and adult surgery. These were the two largest inpatient services in the Syracuse hospitals and the only services with substantial numbers of discharges aged 65 - 74 years and 75 years and over.

This information demonstrated that, consistent with the experience of the hospital industry, admission/discharge rates for adult medicine were substantially higher than for adult surgery. These differences increased with patient age.

These data demonstrated that adult medicine admission/discharge rates for Syracuse were lower than those of New York City and Utica for both 2014 and 2015. For ages 65 - 74 years, discharge rates for Syracuse were 17.9 - 22.9 discharges per 1000 lower than those of Utica and 25.5 - 33.5 discharges per 1000 lower than those New York City. For ages 75 years and over, discharge rates for Syracuse were 28.3 - 29.7 discharges per 1000 lower than those of Utica and 40.0 - 51.3 discharges per 1000 lower than those of New York City. These differences were consistent with the differences in the aggregate discharge rates among these communities.

The data in **Table 2** also demonstrated the adult medicine admission rates for Syracuse were generally higher than those of Rochester and Albany for both 2014 and 2015, especially for ages 75 years and over. For ages 65 - 74 years, discharge rates for Syracuse were 2.3 discharges per 1000 lower—4.7 discharges per 1000 higher than those of Rochester and 5.8 - 10.1 discharges per 1000 higher than those of Albany. For ages 75 years and over, discharge rates for Syracuse were 18.4 - 23.0 discharges per 1000 higher than those of Rochester and 30.3 - 47.1 discharges per 1000 higher than those of Albany for ages 75 years and over.

The data in **Table 2** suggested that differences in hospital admission/discharge rates for adult surgery among Syracuse, Utica, and New York City in these age groups were less clear than for all ages combined. For ages 65 - 74 years, rates for Syracuse were 2.1 discharges per 1000 lower-1.1 discharges per 1000 higher than those of Utica, while rates were 9.1 - 12.1 discharges per 1000 higher than those of New York City. For ages

Table 2. Resident inpatient medical/surgical hospitalization per 1000 population, ages 65 years and over, New York state metropolitan areas, 2014-2015.

Resident County	2014			
	65 - 74 Years		75 Years & Over	
	Adult Medicine	Adult Surgery	Adult Medicine	Adult Surgery
Capital District (Albany, Schenectady, Rensselaer)	96.7	54.4	218.3	61.3
Erie County (Buffalo)	102.4	66.2	241.7	77.6
Monroe County (Rochester)	109.1	56.8	247.0	67.8
New York City (5 Burroughs)	140.3	54.6	316.7	71.3
Oneida County (Utica)	124.7	65.8	295.1	76.0
Onondaga County (Syracuse)	106.8	63.7	265.4	75.5
Resident County	2015			
	65 - 74 Years		75 Years & Over	
	Adult Medicine	Adult Surgery	Adult Medicine	Adult Surgery
Capital District (Albany, Schenectady, Rensselaer)	104.6	60.5	244.2	75.4
Erie County (Buffalo)	101.8	68.4	235.7	77.0
Monroe County (Rochester)	105.7	64.2	251.5	77.6
New York City (5 Burroughs)	135.9	54.8	314.5	72.0
Oneida County (Utica)	133.3	65.8	302.8	80.2
Onondaga County (Syracuse)	110.4	66.9	274.5	76.0

Adult medicine data exclude Diagnosis Related Groups concerning surgery, obstetrics, psychiatry, alcohol/substance abuse treatment, and rehabilitation.
 Adult surgery data exclude Diagnosis Related Groups concerning medicine, obstetrics, psychiatry, and alcohol/substance abuse treatment.
 Prepared by Hospital Executive Council.

75 years and over, rates for Syracuse were 0.5 - 4.2 discharges per 1000 lower than those of Utica and 4.0 - 4.2 discharges per 1000 higher than those of New York City. These differences contrasted with the data for all ages where rates for Syracuse were lower than those in these communities.

The study data also demonstrated that differences in adult surgery hospital admission/discharge rates among Syracuse, Rochester, and Albany were also less clear than

for all ages combined. For ages 65 - 74 years, rates for Syracuse were 2.7 - 6.9 discharges per 1000 higher than those of Rochester and 6.4 - 9.3 discharges per 1000 higher than those of Albany. For ages 75 years and over, discharge rates were 1.6 - 7.7 discharges per 1000 higher than those of Rochester and 0.6 - 14.2 discharges per 1000 higher than those of Albany.

Among these data, the clearest differences between Syracuse, Rochester, and Albany were for adult medicine patients aged 75 years and over. For this population, discharge rates in Syracuse exceeded those of the other communities by relatively large margins. This information suggested that this population was probably the source of most of the differences in rates that were identified in the aggregate data.

The third component of the study focused on hospital admission/discharge rates for Syracuse and other New York State metropolitan areas for adult medicine patients aged 75 years and over by Major Diagnostic Category. Relevant data are summarized in **Table 3**.

This information focused on resident hospital admission/discharges among Major Diagnostic Categories with the highest rates among New York State metropolitan areas. These categories included cardiology, respiratory medicine, urology, neurology, the digestive system, and infectious/parasitic diseases.

The data demonstrated that, consistent with previous analyses, admission rates for these adult medicine diagnoses for patients aged 75 years and over in Syracuse were generally lower than those of Oneida County and New York City for diagnoses involving the circulatory Major Diagnostic Category (11.9 - 14.7 discharges per 1000 lower). This category had the highest overall rates among the metropolitan areas. Differences between rates for Syracuse and the other areas for other Major Diagnostic Categories varied.

The data in **Table 3** also demonstrated that resident admission/discharge rates in Syracuse were higher than those in Rochester for diagnoses involving a number of adult medicine diagnoses. These included the respiratory system, kidney and urinary tract, and orthopedics for both 2014 and 2015. These differences were greatest for the respiratory Major Diagnostic Category where the rate for Syracuse was 12.7 discharges per 1000 higher in 2014 and 13.7 discharges per 1000 in 2015. Additional information concerning inpatient hospital admissions/discharges in 2014 and 2015 was generated by an analysis of inpatient readmissions by age level for these time periods. Relevant data are summarized in **Table 4**.

This information includes numbers of readmissions and readmission rates for the Syracuse hospitals for the same age levels evaluated in the data for resident hospital readmission rates. These data are included in the resident admission data for Syracuse included in previous components of the study.

The data demonstrated that in both 2014 and 2015, readmission rates increased by age level from 1 - 2 percent for patients aged 0 - 17 years to 8 - 9 percent for patients aged 75 years and over. The data also indicated that the largest numbers of readmissions in the Syracuse hospitals were produced by patients aged 45 - 64 years, 1510 -

Table 3. Resident adult medicine inpatient hospitalization per 1000 population, ages 75 years and over by major diagnostic category, New York state metropolitan areas, 2014-2015.

MDC/Description		2014					
		Onondaga County	Capital District	Erie County	Monroe County	New York City	Oneida County
01	NERVOUS SYSTEM	27.1	22.2	27.4	27.9	29.5	29.1
04	RESPIRATORY SYSTEM	44.7	34.8	34.5	32.0	42.8	52.3
05	CIRCULATORY SYSTEM	53.2	54.9	57.7	57.6	74.1	65.1
06	DIGESTIVE SYSTEM	30.1	24.0	28.7	26.5	35.1	33.4
07	HEPATOBIILIARY SYS & PANCREAS	5.5	3.9	4.6	4.6	6.8	6.8
08	MUSCULOSKEL SYS & CONN TISSUE	15.4	12.5	12.8	12.7	16.1	15.6
09	SKIN, SUBCUT TISSUE & BREAST	8.4	6.5	5.9	6.5	9.8	9.3
10	ENDOCRINE, NUTRIT & METABOLIC	10.7	6.5	8.9	9.5	14.8	14.6
11	KIDNEY & URINARY TRACT	26.7	20.1	20.7	21.6	28.6	27.4
18	INFECTIOUS & PARASITIC DISEASES	28.4	20.1	25.1	34.5	35.8	21.2
All Others		15.2	12.8	15.3	13.4	23.3	20.3
Total		265.4	218.3	241.7	247.0	316.7	295.1
MDC/Description		2015					
		Onondaga County	Capital District	Erie County	Monroe County	New York City	Oneida County
01	NERVOUS SYSTEM	29.0	25.9	28.7	26.5	29.9	28.6
04	RESPIRATORY SYSTEM	49.4	43.7	35.2	35.7	43.3	60.9
05	CIRCULATORY SYSTEM	51.7	49.6	54.8	53.9	72.5	66.4
06	DIGESTIVE SYSTEM	30.4	26.4	26.8	57.5	33.4	32.8
07	HEPATOBIILIARY SYS & PANCREAS	4.8	4.3	4.0	4.7	6.6	5.1
08	MUSCULOSKEL SYS & CONN TISSUE	16.3	13.7	11.5	12.7	16.0	14.6
09	SKIN, SUBCUT TISSUE & BREAST	7.8	7.1	5.3	5.8	9.4	9.1
10	ENDOCRINE, NUTRIT & METABOLIC	10.4	7.3	9.1	10.7	14.2	13.5
11	KIDNEY & URINARY TRACT	25.1	25.1	21.1	20.8	28.3	26.9
18	INFECTIOUS & PARASITIC DISEASES	33.8	26.1	24.4	38.0	38.1	23.6
All Others		15.7	14.9	14.9	15.3	23.0	21.4
Total		274.5	244.2	235.7	251.5	314.5	302.8

Data exclude Diagnosis Related Groups concerning surgery, obstetrics, mental health, and alcohol/substance abuse treatment, and rehabilitation.

Prepared by Hospital Executive Council.

Table 4. Potentially preventable readmissions, total readmissions by age level, Syracuse hospitals, 2014-2015.

	2014					Total
	0 - 17 Years	18 - 44 Years	45 - 64 Years	65 - 74 Years	75 Years & Over	
Number of Readmissions	200	981	1,537	789	1,298	4,805
At Risk Population	11,612	18,886	20,610	10,668	14,577	76,353
Readmission Rate (Percent)	1.72	5.19	7.46	7.40	8.90	6.29
	2015					Total
	0 - 17 Years	18 - 44 Years	45 - 64 Years	65 - 74 Years	75 Years & Over	
Number of Readmissions	241	836	1,510	781	1,298	4,666
At Risk Population	11,933	18,491	20,652	11,035	14,858	76,969
Readmission Rate (Percent)	2.02	4.52	7.31	7.08	8.74	6.06

Prepared by Hospital Executive Council

1537 patients, and ages 75 years and over, 1298 patients for both years. The total numbers of patients at risk of readmission for all ages combined in the Syracuse hospitals varied only slightly between the two years, 76,353 - 76,969.

This information demonstrated that, like resident hospital admission/discharge rates by age level in Syracuse, readmission rates were consistent between 2014 and 2015. The data also suggested that, like resident admission rates, readmission rates were highest for patients aged 75 years and over.

5. Limitations

The study analysis and resulting conclusions were based on the data concerning resident hospital admission/discharge rates for the New York State metropolitan areas identified. These data were developed for January-December 2014 and 2015. The data do not relate to other areas or time periods.

6. Discussion

Hospital admission/discharges rates are generating increased attention from health care payors. Medicare risk programs, as well as initiatives by Medicaid and commercial insurance companies are supporting increased use of primary care, long term care, and other programs as means of reducing hospital utilization and related costs.

This study focused on evaluation of inpatient hospital admission/discharge rates for Syracuse and other New York State metropolitan areas during 2014 and 2015. It provided comparative information concerning this subject and suggested how this approach to analysis of hospital utilization could be carried out using publicly available data.

The study data demonstrated that hospital admission/discharge rates per 1000 population increased with patient age in all of these areas. They also showed that, among the elderly, the population with the highest rates, adult medicine patients' conditions such

as respiratory and circulatory conditions produced the largest utilization rates.

The study data suggested that differences in hospital admission/discharge rates among the New York State metropolitan areas were generally consistent between 2014 and 2015. Utica and New York City produced the highest rates. Rochester and Albany produced the lowest rates. Utilization rates for Syracuse were considerably lower than for Utica and New York City and slightly higher than for Rochester and Albany.

Admission rates for the metropolitan areas during 2014 and 2015 were also evaluated by age level, a major cause of variations in this indicator. This analysis demonstrated that most of the differences between aggregate rates for Syracuse and Rochester were produced by elderly patients, especially those aged 75 years and over.

The analysis demonstrated that most of these differences in admission rates for the elderly were produced by adult medicine patients aged 75 years and over. Most of these differences were generated by patients with respiratory, digestive, and orthopedic disorders. Additional study data suggested that the highest readmission rates for adult medicine and adult surgery patients were produced by those aged 75 years and over.

The results of this study concerning resident hospital utilization rates were not earthshaking, in the context of increasing interest in this subject by health care providers and payors. At the same time, they suggested that resident data could be used to focus planning on populations with higher utilization rates and the development of services to address their needs. In the case of Syracuse, this meant the frail elderly aged 75 years and over and not those with commercial insurance aged 18 - 64. In other communities, the focus could be different.

This type of planning used to be more common when managed care was widespread. The increased attention being devoted to it by Accountable Care Organizations, Medicaid, and Blue Cross may suggest a wave of renewed interest in this type of care delivery and related planning. Such efforts could take advantage of additional indicators such as severity of illness and the specificity of ICD 10 diagnosis codes.

7. Recommendations

The study suggests that health care providers and payors should consider reviewing hospital admission rate data as part of their planning efforts. Numerous changes in the delivery of health care services have occurred during the last twenty years. In the context of these developments, such as telemedicine, long term acute care, and intensive rehabilitation, this could be a valuable exercise. It could lead to reductions in hospital admissions, improved outcomes, and lower costs for all of those involved with health care.

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A New Type of Double-Lumen Catheter to Replace Current One in RCA

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Abstract

Objective: To replace the peripheral venous puncture for blood sampling with taking blood samples extracorporeally from arterial line before predilution during RCA-CRRT performed. **Methods:** A new type of double-lumen femoral catheter was used instead of the current tubes. The new type of double-lumen tube had a greater distance from the inner venous ports to the inner arterial ports than current tubes. The minimum distance from the venous port to the arterial port was greatly lengthened. Replacement solution contained citrate, zero Ca^{2+} , zero bicarbonate, low Na^+ . Blood samples were synchronously collected from the arterial line before the infusion of citrate replacement fluid and from the peripheral vein. The iCa concentration data of two groups were analyzed to observe the difference between iCa concentration levels in the arterial line and in peripheral vein; the anticoagulant effect of RCA and possible complications were observed, such as bleeding, clottings and hypocalcaemia. **Results:** 28 times of RCA-CRRT were performed on 17 AKI and CRF patients with active bleeding or at the high risk of bleeding; 336 blood samples were collected. Statistics showed that the difference of iCa concentration between arterial line group and the peripheral vein group was not significant ($P = 0.9$), there is a high degree of similarity between the iCa concentration of arterial line blood and the peripheral venous blood. None of the patients developed citrate toxicity or metabolic alkalosis. None induced bleeding, or bleeding aggravated. No obvious clotting occurred. Systemic calcium concentration was achieved in the ideal range. **Conclusion:** In clinical practice, the data of iCa concentration from arterial line can be used to replace that from peripheral vein when the new type of double-lumen femoral catheter is placed in femoral vein. RCA-CRRT therapy is safe and effective.

Keywords

RCA-CRRT, Ionized Calcium, Arterial Line, Peripheral Vein, New Type of

Double-Lumen Femoral Catheter

1. Introduction

With rapid popularization of blood purification, the morbidity of bleeding or high risk of bleeding is gradually increasing in patients who required continuous renal replacement therapy (CRRT) in ICU. Regional citrate anticoagulation (RCA) for CRRT is associated with a longer filter life span and fewer bleeding events. However, the complexity of the regimen is the major hurdle preventing widespread application [1] [2] for example, frequent peripheral vein puncture for blood sampling. In order to simplify the cumbersome operating procedures of blood sampling, a new type of double lumen catheter was located in the patient's femoral vein, in which the minimum distance from the inner venous port to the inner arterial port was lengthened to 6 cm long, then, extracorporeal circulation connected via arterial and return lines. Blood samples were taken both from the arterial line before predilution and the peripheral vein synchronously, and in turn, were tested immediately. The difference of iCa levels was assessed between arterial line and peripheral vein, which if, was caused by recirculation. If there is a high degree of consistency to two groups, the iCa data tested from arterial line blood sample can be used to replace that from peripheral vein, finally the cumbersome sampling by venous puncture from peripheral vein can be left out and be substituted by taking from arterial line. As a result, a new simplified sampling method will be provided for a completely automatic RCA machine with the function of iCa automatic monitoring online. The currently used double lumen femoral catheters seem not competent to this machine due to blood recirculation [3].

2. Methods

Seventeen critically ill patients with bleeding or at the high risk of bleeding were evaluated in ICU during the period of 2014.10-2016.7. The study was approved by the Ethics Committee of Xili Hospital. 28 times of RCA-CRRT were performed after obtaining informed consent. The median age of patients was 49.1 years (range, 13 to 80); 9 men, 8 women; Of 17 patients with bleeding or at the high risk of bleeding, 2 AKI caused by acute heart failure, 1 Acute exacerbation on CRF, 2 Cerebral hemorrhage, 1 ARDS, 1 HELLP syndrome, 1 Thermoplegia, 1 Sepsis, 1 acute nephritis, 3 occurred after bone marrow transplantation, 1 after renal transplantation, 3 CRF respectively complicated with hyperkalemia, fundus hemorrhage and gastrointestinal hemorrhage. CVVH was performed using the Fresenius Medical Care with AV-400/600 filter. The circuit was run for 6 - 20 hr, 11 hr on average; a new type of double lumen YXD-12F.24cm catheter (YXD. Co., Ltd., Shenzhen, Guangdong, China) was placed into femoral vein for vascular access by the physicians. The minimum distance from the inner venous port to the inner arterial port was lengthened to 6 cm long; the length inside the vein of this new type of double lumen catheter stands for 24 cm long: See **Figure 1**, **Photo 1** and **Photo 2**.

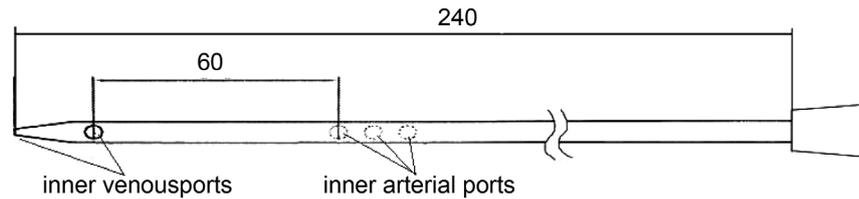


Figure 1. Distance from the inner venous port to the inner arterial port.



Photo 1. The object of distance from the inner venous port to the inner arterial port.



Photo 2. The object of distance from the inner venous port to the inner arterial port.

The blood flow rate was maintained at 150 mL/min, and replacement solution contained 13.3 mmol/L citrate, zero Ca^{2+} , zero bicarbonate [4] running at three different rates of 2000 - 2500 - 3000 ml/h was the only predilution replacement fluid. The rate of predilution replacement fluid varied with patients' coagulation test result before the treatment. 336 blood samples were synchronously collected from the arterial line in the circuit before the infusion of citrate replacement fluid, from the peripheral vein (iCa waved preferably from 0.99 - 1.25 mmol/L) and from the return line. The one from arterial line was put into group 1; that from peripheral vein group 2. During the treatment, systemic iCa, serum electrolytes, and arterial blood gases were measured at the end of first hr, then every 3 h for 20 h. Additional blood tests were performed as needed based on clinical indications. 10% calcium gluconate was infused in the way of echelon [5] into return line at the point just before the entrance into the body via a 3-way stopcock and was titrated to achieve a systemic iCa level ranged at 0.99 - 1.25 mmol/L. Potassium was added to the pre-replacement fluid for those patients who required supplementation. Rates of hemofilter clotting, significant bleeding events, metabolic alkalosis (defined as $\text{pH} > 7.50$ [1]) and evidence of citrate toxicity were observed (Table 2).

3. Statistical Analysis

Data are reported as the median and standard deviation. The analysis was performed by the Statistical Center of Shenzhen University Town. SPSS version 18.0.

4. Results

The data of our study are shown in Table 1, Table 2 and Figures 2-6. The relation

Table 1. Relationship between arterial line iCa and the peripheral vein.

Paired T test	Median (mmol/L)	SD (mmol/L)	Sampling times	T Value	P value
Group1	1.10858	0.090904	112	0.000	1.000
Group2	1.10858	0.090337	112	0.000	1.000

Table 2. Clinical characteristics and laboratory parameters.

	RR	BP	HR	Scr	APTT	PH	Lip Numbness	Clotting
1 h (n – 28)	21 ± 5	134 ± 17/81 ± 14	91 ± 25	602 ± 467	39 ± 10	7.33 ± 0.08	0	0
2 h (n – 28)	19 ± 2	130 ± 21/78 ± 14	89 ± 21		40 ± 9		0	1
8 h (n – 26)	16 ± 2	126 ± 25/78 ± 13	89 ± 20		37 ± 8		0	0
11 h (n – 22)		130 ± 14/79 ± 10		339.9 ± 268.5		7.43 ± 0.025	0	0
14 h (n – 6)		123 ± 4.6/76 ± 3.7			44 ± 7.9	7.46 ± 0.016	0	0
17 h (n – 1)	17	122/74	80		27.3		0	0
20 h (n – 1)	18	120/75	82		37	7.47	0	0

n: Number of patient enrolled; RR: Respiratory rate; BP: Blood pressure; HR: Heart rate; SCR: Serum creatinine; Lip numbness: a symptom of citrate toxicity.

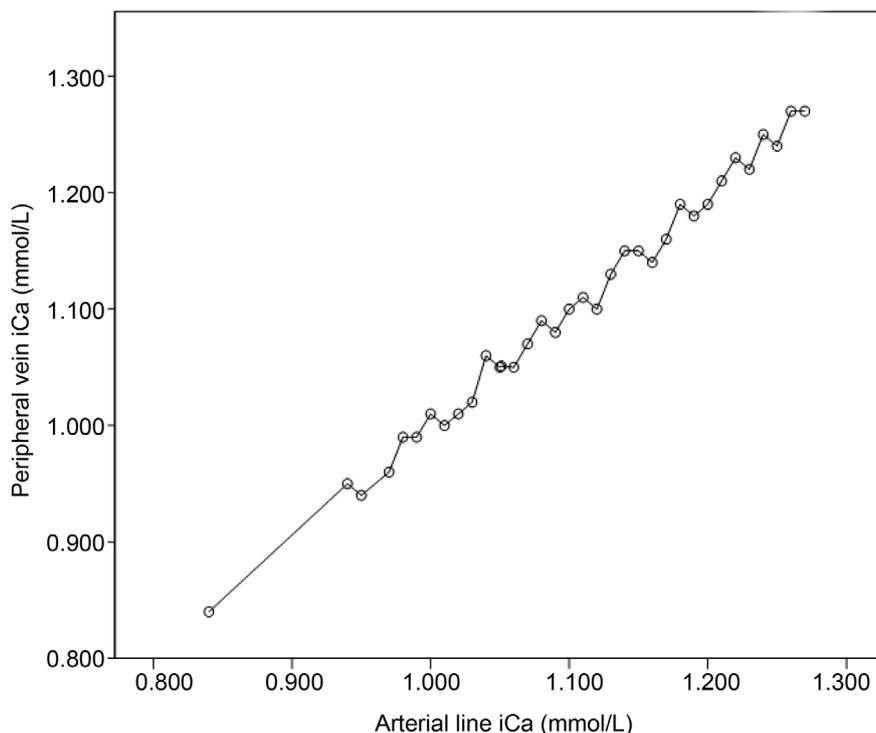


Figure 2. One element linear regression scatter plot for iCa of arterial line and the peripheral vein.

between group 1. (1.10858 ± 0.0909 mmol/L) and group 2. (1.10858 ± 0.0903 mmol/L) were analyzed by means of paired t-test, two element correlation and one-element linear regression. T-test result shows: $P > 0.9$, there is not significant difference between

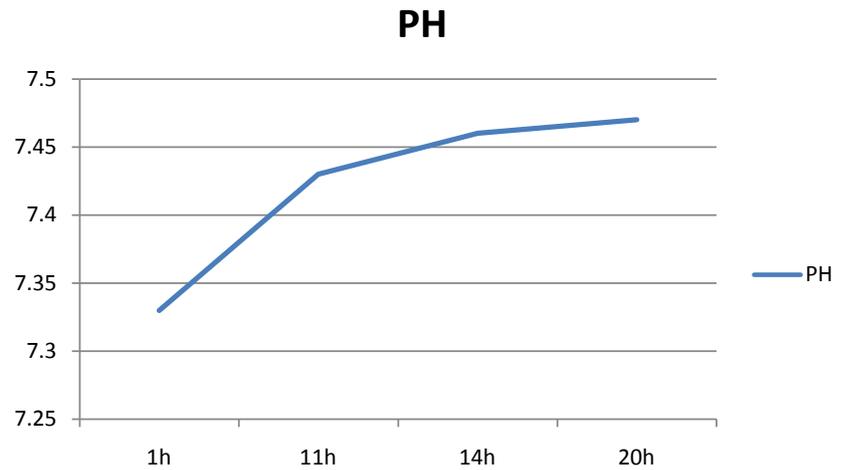


Figure 3. pH variation during treatment.

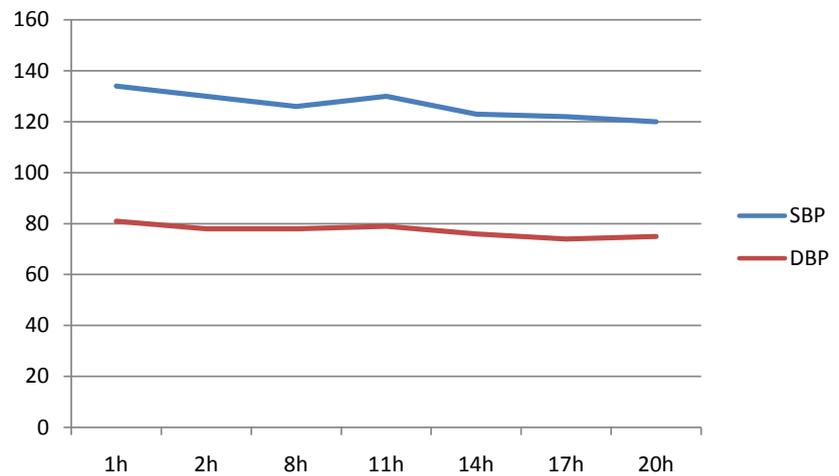


Figure 4. Blood pressure variation during treatment. SBP: systolic blood pressure; DBP: diastolic blood pressure.

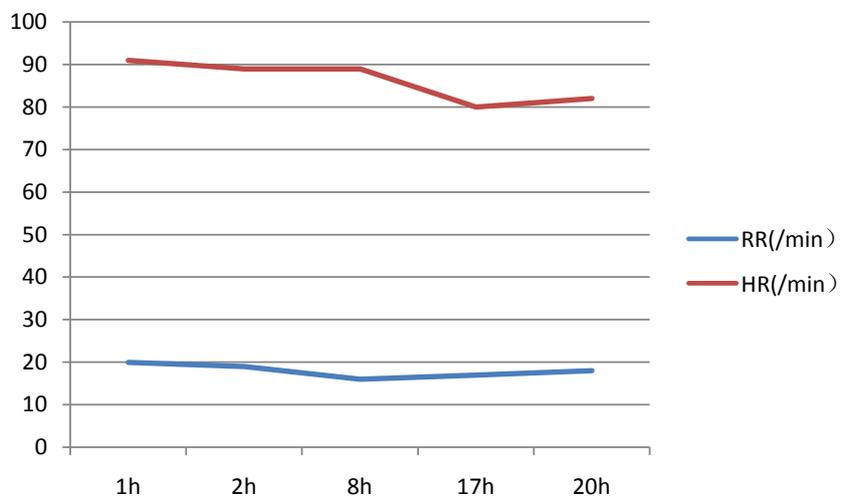


Figure 5. Respiratory rate and heart rate variation during treatment.

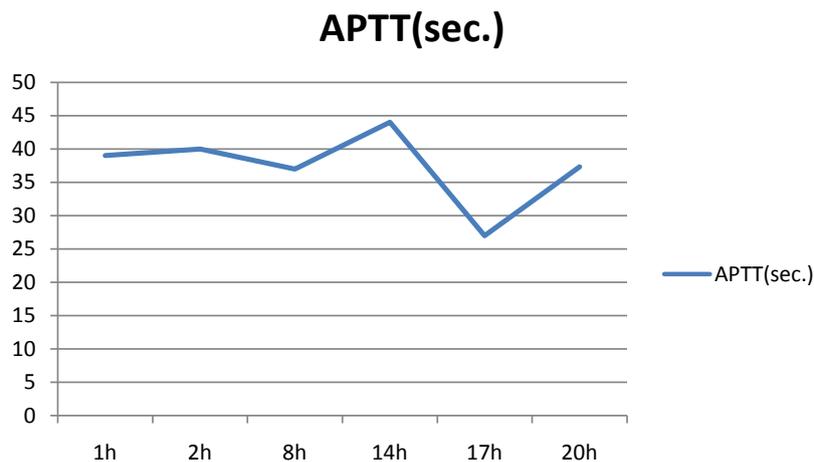


Figure 6. APTT variation during treatment.

the values of iCa concentration of two groups; correlation: $R = 0.992$; linear regression equation is fitted: arterial line $iCa = 0.001 + 0.999 * \text{Peripheral } iCa$, $R^2 = 0.985$, $P < 0.001$. The results analyzed above suggested that the iCa value from arterial line has a high degree of consistency to that from the peripheral vein; The iCa value of arterial line can be used to replace the one punctured from peripheral vein when this new type of double lumen femoral catheter is applied during RCA-CRRT. See **Table 1** and **Figure 2**.

The values of PH, blood pressure, respiratory rate, heart rate and APTT waved as shown in **Figures 3-6**. PH value improved and elevated (from 7.33 ± 0.08 to 7.46 ± 0.016 , even to 7.47, $n = 1$) gradually during the treatment period (**Figure 3**). But no other clinical metabolic alkalosis manifestations. No citrate toxicity such as lip numbness was observed (**Table 2**). Blood pressure gradually reduced from $134 \pm 17/81 \pm 14$ mmHg to $123 \pm 4.6/76 \pm 3.7$ mmHg during treatment without clinical significance (**Figure 4**). Respiratory rate (range from $21 \pm 5/\text{min}$ to $16 \pm 2/\text{min}$) and heart rate ((range from $91 \pm 25/\text{min}$ to $89 \pm 20/\text{min}$) fluctuated within a narrow range (**Figure 5**). APTT varied in normal range (**Figure 6**).

Other characteristics were shown in **Table 2** and **Figures 3-6**.

In one patient a filter was changed because of clotting caused by malposition of catheter tip and the failure to deal with the machine alarm; other circuits were run for 6 - 20 hr, 11 hr on average and stopped electively. Systemic iCa level was maintained within the target level (0.95 - 1.27 mmol/L); iCa level from return line maintained at 0.17 - 0.49 mmol/L. Blood flow was maintained at 150 mL/min for all patients. No additional sodium bicarbonate was given to any of the patients. The pH and base excess improved gradually during the treatment period. No citrate toxicity and metabolic acidosis occurred.

5. Discussion

The cumbersome blood sampling by venous puncture from peripheral vein is the main

part of the complexity of RCA regimen. In order to solve this problem, we tried to take the blood samples extracorporeally from arterial line before predilution instead of venous puncture for blood sampling from peripheral vein.

The currently used double lumen femoral catheters seem not competent to offer such a sample due to blood recirculation [3]. The tested value of iCa from arterial line is not equal to that from peripheral vein because of recirculation. To attempt greatly reduce the this regional recirculation, we used a new type of double lumen catheter in the patient's femoral vein, in which the minimum distance from the inner venous port to the inner arterial port was lengthened to 6 cm long, and the length inside the vein of this new type of double lumen catheter stands for 24 cm long. The outcome of our study shows that there is a high degree of consistency to group 1 and group 2, the iCa data tested from arterial line blood sample can be used to replace that from peripheral vein. The utilization of this new type of femoral double lumen catheter made it possible to reduce the regional blood recirculation close to zero, furthermore, realized detection of systemic iCa level without cumbersome venous puncture when RCA-CRRT is performed. In the future, this new simplified sampling method will be used for a completely automatic RCA machine with the function of iCa automatic monitoring online.

Our study enrolled 17 cases, it is not a large sample statistically. With the sample extending , more concrete data will be reached. The shortage of this catheter is that the femoral vein is the only location to be placed, it can not be used in jugular vein. Finally, if there is a new totally coagulating-proof hemofilter and extracorporeal circuit set developed successfully, this new catheter will work only as an ordinary double-lumen catheter with the superiority described above lost.

From a clinical point of view, the variations of other clinical parameters such as Bp, HR, RR, APTT were within normal range. SCr reduced reasonably.

6. Conclusion

RCA-CRRT is safe, effective, easy to handle. This type of double-lumen catheter will replace the current one in RCA. It will contribute to completely automatic RCA machine with the function of iCa automatic monitoring online in the near future.

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Safety and Efficacy of Oral Mirodenafil in Mexican with Erectile Dysfunction

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Abstract

Erectile dysfunction is treated with 5-phosphodiesterase inhibitors as Mirodenafil, which has shown its efficacy and safety in Koreans, however; no information in other populations is available. An open clinical trial study was designed to evaluate the efficacy and safety in real life of a fixed-dose of Mirodenafil in Mexican patients with erectile dysfunction. Forty-seven male patients received a 100 mg tablet of Mirodenafil, during 12 weeks. Primary outcome efficacy measure was the percentage of male patients with successful intercourse. Secondary outcomes measures included patient satisfaction, mood and self-esteem level. Safety assessments included laboratory tests, vital signs, physical examination, 12-lead electrocardiogram recordings, and incidence of adverse events by patients. Oral administration of Mirodenafil improved in an 80% - 90% the number of successful intercourses from 7 to 84 days of treatment. Moreover, patients reported a significant increment in their sexual satisfaction, mood and self-esteem. Mirodenafil treatment did not modify vital signs nor anthropometric parameters during 84 days. Mild headache was the most frequent adverse event (17.0%) and there were no severe adverse events during pharmacological treatment. Data suggest that oral Mirodenafil is safety, well tolerated and effective in the Mexican population with erectile dysfunction.

Keywords

Efficacy, Erectile Dysfunction, Mexicans, Mirodenafil, Safety

1. Introduction

Erectile dysfunction is defined as a consistent inability to achieve or maintain an erec-

tion that is sufficient for satisfactory sexual intercourse [1]. Erectile dysfunction is an important worldwide health issue that has a significant negative impact on sexual satisfaction, mood and self-esteem of the affected men [1] [2]. In United States, the Massachusetts Male Aging Study reported a combined prevalence of minimal, moderate and complete impotence of 52% in subjects between 40 and 70 years [2]. In addition, a recent revision points out that more than 75% of men over 70 years old have erectile dysfunction [3]. In South Korea, the overall reported prevalence rate of dysfunction erectile is ranged between 18% - 36.6% [1], whereas in Mexico, it is around 55% in men over 40 years old [4]. In addition, it has been reported that incidence of erectile dysfunction is higher in men with diabetes mellitus, hypertension, high body weight, dyslipidemia, atherosclerosis or other diseases associated with endothelial dysfunction [1] [5]-[7].

The first-line of pharmacological treatment of erectile dysfunction is phosphodiesterase type 5 inhibitors. Currently, Mirodenafil, Sildenafil, Tadalafil Vardenafil, Udenafil and Avanafil belong to this pharmacological group [1] [8]. Mirodenafil is an analogue of the pyrrolopyrimidinone launched in Korea in 2007. Preclinical studies suggest that Mirodenafil enhances nitric oxide-cGMP-K⁺ channels pathway resulting in a significant relaxation of the corpus cavernosum with the consequent penile erection [9] [10]. The pharmacokinetics of oral Mirodenafil is dose-dependent due to the saturable hepatic metabolism of this drug [11], it is primarily metabolized via hepatic cytochrome P450-1A1/2, -2B1/2, -2D subfamily and -3A1/2 [12] and it reaches significantly higher C_{max} and AUC values than those of sildenafil in the plasma and corpus cavernosum tissue [13].

Clinically, the first trial of Mirodenafil reported a significant improvement in the erectile function with respect to placebo group after 12 weeks of treatment. In addition, this study showed that the dose of 50 or 100 mg of Mirodenafil was well tolerated, since the most frequent adverse events were mild and resolved spontaneously [14]. In line with these results, a recent meta-analysis performed from three randomized, double blind and placebo-controlled clinical trials with a total of 374 participants showed that Mirodenafil was more effective than placebo, and well tolerated, after 12 weeks of treatment [15]. In fact, Mirodenafil has resulted useful in the treatment of erectile dysfunction in diabetic, hypertensive and benign prostatic hyperplasia-induced lower urinary tract symptoms patients [16]-[18]. Notwithstanding, all studies have been made in Korean men and the information in other populations is lacking. Considering that ethnicity is a key factor that may modify both pharmacokinetics and pharmacodynamics of drugs, resulting in variability in response to drug therapy [19], as well as, the limited information available between Mexicans and Koreans. The current hypothesis was raised since previous studies have reported ethnic differences in allele frequencies for selected enzymes, transporters, and pharmacologic targets between White, Black and Asian populations [20]. In addition, our group has reported that some drugs metabolized by CYP3A as sildenafil, an analogue of mirodenafil, reach higher plasmatic levels in Mexicans in comparison with Whites [21]. For these reasons, we consider that it is

necessary to perform clinical studies in different ethnic populations to rationally establish drug dosing and, if necessary, optimize its efficacy and safety for each population, especially, when the information is unknown. The current study investigated the efficacy and safety in real life of a tablet of 100 mg of Mirodenafil per week, on an “as needed” basis in Mexican men with erectile dysfunction.

2. Patients and Methods

2.1. Subjects

A prospective, open clinical trial was performed between January and June 2011 to evaluate the efficacy and safety in real life of a 100 mg tablet of Mirodenafil per week during 84 days, in Mexican men with erectile dysfunction. Forty-seven subjects were included in the study. All of them were between 18 and 65 years old, with erectile dysfunction for at least three months and active sexual life. In addition, no one was taking any other dysfunction erectile treatment. Exclusion criteria were subjects who had a penile anatomical problem, had undergone penile surgery or spinal cord injury, were on medication containing nitrate or nitric oxide preparations or antiandrogen therapy, had a primary diagnosis of sexual disorder different to erectile dysfunction, had alcoholism or drug addiction and had previously hypersensitive reactions to phosphodiesterase type 5 inhibitors. In addition, the health status of patients was determined by medical history, clinical examination and suitable laboratory tests as blood chemistry, hematic biometry, urinalysis and electrocardiogram. If patients had peptic ulcer, ischemic heart disease, urinary tract infection, diabetic retinopathy, diabetic neuropathy, ketoacidosis or other severe concomitant medical conditions were also excluded from the study. This study was carried out following the recommendations of the latest version of the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects [22]. The Institutional Research and Ethics Committees of our institution approved the current protocol.

2.2. Study Design

All participants read the protocol and provided written informed consent for their participation in the study. After obtaining written informed consent, patients received a blister with 12 tablets of 100 mg of Mirodenafil. Landsteiner Scientific, S. A. de C. V. (Mexico City, Mexico) provided all Mirodenafil tablets. Patients were informed about the time to onset and duration of the effects of 100 mg Mirodenafil. Furthermore, all patients were instructed to take only a capsule of 100 mg of Mirodenafil per week, on an “as needed” basis, one hour before sexual intercourse. All patients attended four visits. The efficacy and safety of this dose of Mirodenafil were assessed at 7 (visit 2), 42 (visit 3) and 84 (visit 4) days after drug treatment. General outline is shown in the **Figure 1**. The primary efficacy outcome was the percentage of male patients with successful intercourse, which was assessed during each visit as the percentage of patients achieving ejaculation during all attempted sexual intercourse. The secondary efficacy outcomes were patient satisfaction, mood and self-esteem level. Patient satisfaction was

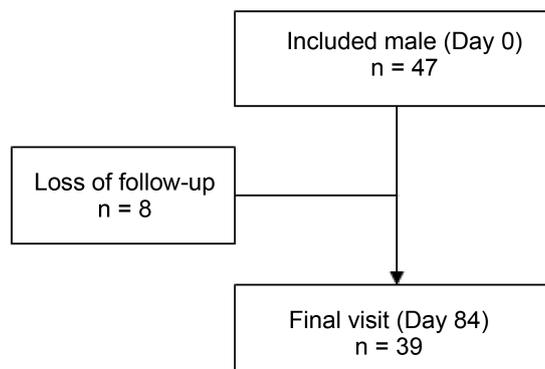


Figure 1. General outline of the study (Reyes-García *et al.*).

evaluated using a 10 cm scale without points or numbers within the scale, with exception of 0 and 10, where 0 means no satisfaction and 10 the best intercourse imaginable. The mood was assessed by the question How do you feel in your intercourse with the pharmacological treatment? and a four-point Likert scale that had the words: frustrated 1), dissatisfied 2), satisfied 3) and pleased 4); whereas the self-esteem level was evaluated using the question: How has affected your self-esteem level the pharmacological treatment?, and a second four-point Likert scale that had the words: nothing (0), very little (1), moderately (2) and definitely (3). For safety monitoring, all vital signs were assessed at baseline, and subsequently, blood pressure and heart rate were checked at every visit. Blood chemistry, hematic biometry, urinalysis and electrocardiogram were done at baseline and visit 4 (84 days). Notwithstanding, it is fair to say that in case of alarm signals to the clinical examination, the protocol contemplated earlier laboratory studies. Patients were also asked about concomitant medications and for adverse events at every visit. Causality of adverse events to the pharmacological treatment was determined by Naranjo's algorithm where more than 9 points indicates a definite causality, 5 to 8 a probable causality, 1 to 4 a possible causality and 0 is equal a doubtful causality.

2.3. Data Analysis

The efficacy of Mirodenafil was evaluated using a per protocol analysis based in the number of patients who completed the study. Within Mirodenafil group, statistical differences between the percentage of male patients with successful intercourse and unsuccessful intercourse in each visit was evaluated by χ^2 test, whereas statistical analysis of the time-courses obtained from patient satisfaction, patient mood and patient self-esteem were assessed by Kruskal-Wallis' test followed by Dunn's test. Statistical significance was accepted at the $P < 0.05$ level. The number of patients evaluated in the current study was based on data obtained previously [16], and considering a 90% of confidence interval ($z = 1.64$), 80% of prevalence of success (p), 20% of prevalence of failure (q) and 10% of precision (d). According to the equation for determine a success proportion ($n = (z^2)(p)(q)/d^2$), and estimating a 10% drop out, a total of 47 patients were required for efficacy analysis. Adverse events were summarized using Medical Dictio-

nary for Regulatory Activities Preferred Terms. Change from baseline in continuous safety variables as blood pressure, heart rate and other vital signs were evaluated by Repeated Measures Analysis of Variance followed by Dunnett's test, whereas laboratory analysis and electrocardiogram parameters were assessed by a paired Student t-test.

3. Results

3.1. Demographic Data

Demographic data obtained from included male with erectile dysfunction are shown in **Table 1**. Volunteers were a mean \pm standard deviation (S.D.) age of 53.9 ± 8.7 years with a range of 31 to 65 years. In the study were included 5 patients < 40 years and 42 patients ≥ 40 years old. They had a sexual satisfaction of 3.32 ± 1.5 in the analogue visual scale (from 0 to 10) at the first visit. From 47 patients, 27 (57.4%) were diabetic, 10 (21.2%) hypertensive, 10 (21.3%) dyslipidemic, 10 (21.3%) smokers, and 1 (2.1%) patient had benign prostatic hyperplasia. Respect to concomitant medications, 38 (80.9%) were using other concomitant medications. Twenty seven (57.4%) patients were taking hypoglycemic drugs as metformin, glibenclamide and insulin; 13 (27.6%) were prescribed with multivitaminics as B vitamins; 10 (21.2%) patients were taking antihypertensive drugs as enalapril, captopril and amlodipine among others; 9 (19.1%) patients were using antidyslipidemic drugs as pravastatine and benzafibrate; and 10 (21.2%) patients had other concomitant medications.

3.2. Efficacy of Mirodenafil

Analysis of the primary outcome efficacy measure revealed that Mirodenafil improves the percentage of male patients achieving ejaculation during intercourse, in a significant statistically manner ($P < 0.05$), from the second to the fourth visit (**Figure 2**). In the second visit (7 days), 78.7% of male patients reported successful intercourse, whereas 88.9% (42 days) and 89.7% (84 days) of volunteers stated successful intercourse in the third and fourth visit, respectively.

Consequently, patients reported a significant increment ($P < 0.05$) in their satisfaction regarding sexual activity using a 100 mg of Mirodenafil tablet weekly, on an "as

Table 1. Demographic data.

Characteristic	n = 47
Age (years)	53.9 ± 8.7
Weight (kg)	78.3 ± 11.1
Height (m)	1.7 ± 0.1
Systolic blood pressure (mmHg)	124.8 ± 14.3
Diastolic blood pressure (mmHg)	76.9 ± 11.2
Temperature ($^{\circ}$ C)	36.4 ± 0.3
Heart rate (beats per minute)	70.9 ± 7.3
Respiratory frequency (breaths per minute)	18.6 ± 1.5

Data expresses the mean \pm S.D.

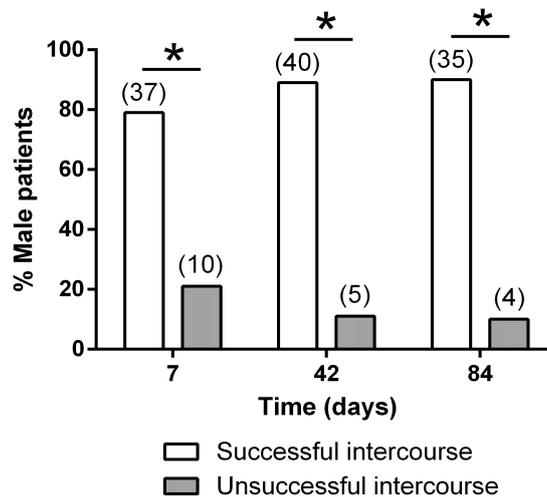


Figure 2. Percentage of male patients that reach the ejaculation during the intercourse after oral administration of a tablet of 100 mg of Mirodenafil (–1 h). *Statistically different regarding the number of patients who did not achieve ejaculation in the same visit by χ^2 . A $P < 0.05$ was significant (Reyes-García *et al.*).

needed basis”, one hour before sexual intercourse. Baseline mean \pm S.D. of sexual satisfaction was increased from 3.3 ± 1.9 to 6.1 ± 2.4 , 7.1 ± 2.2 and 7.4 ± 2.1 at 7, 42 and 84 days, respectively (**Figure 3(a)**). In addition, volunteers indicated that their mood after an intercourse improved from dissatisfied to satisfied during pharmacological treatment. In this regard, patients reported an average \pm S.D. basal mood of 1.8 ± 0.6 , which was improved significantly ($P < 0.05$) to 2.8 ± 0.7 , 3.0 ± 0.6 and 3.0 ± 0.7 at 7, 42 and 84 days (**Figure 3(b)**). Regarding patient’s self-esteem, they reported a positive change in their self-esteem between very little and moderate at the 7 days (1.6 ± 0.9), and a positive change clearly moderate at 42 and 84 days (2.0 ± 1.0 and 2.1 ± 0.9 , respectively). The increment in the patient self-esteem was statistically different ($P < 0.05$) of basal since day 7 (**Figure 3(c)**).

3.3. Safety of Mirodenafil

A tablet of 100 mg of Mirodenafil weekly was well tolerated and safe, since only 22 patients (46.8%) stated 34 adverse events during 84 days, and only 5 patients (10.6%) presented 5 mild and transient adverse events definitively related to the pharmacological treatment according Naranjo’s algorithm (**Table 2**). Adverse events related to the pharmacological treatment were tachycardia, conjunctivitis, eye irritation, facial flushing and nasal constipation. Thirty-one of total adverse events were mild (91.2%) and 3 moderate (8.8%). There were not severe adverse events and no patient was withdrawn from the study due adverse events. However, eight patients were lost to follow-up (**Figure 1**). In addition, no clinically significant changes in anthropometric parameters or vital signs were detected at every visit; consequently, laboratory tests and electrocardiogram recordings were not altered at the end of the study respect to the basal values.

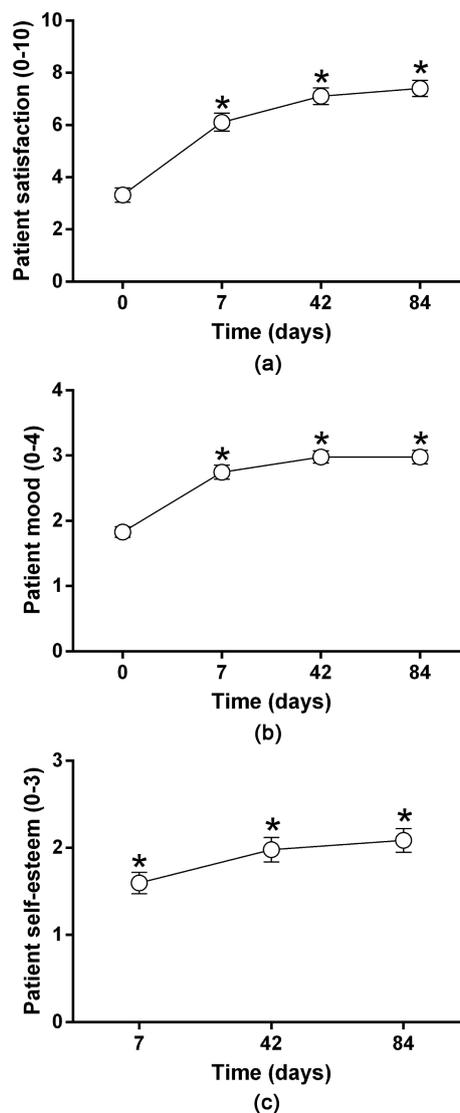


Figure 3. Improve of patient satisfaction, patient mood and patient self-esteem after oral administration of a tablet of 100 mg of Mirodenafil 1 hour before intercourse. *Statistically different from basal data (day 0) by Kruskal-Wallis followed by Dunn’s test. A $P < 0.05$ was significant (Reyes-García *et al.*).

Table 2. Adverse events reported by male patients, who took a tablet of 100 mg of Mirodenafil per week during 84 days, with an incidence greater than 3%.

Adverse event	Number (%)
Headache	8 (17.0)
Conjunctivitis	2 (4.3)
Eye irritation	2 (4.3)
Urinary tract infection	2 (4.3)
Total	34 (100)
Related to drug treatment	5 (14.7)
Mild	31 (91.2)
Moderate	3 (8.8)
Severe	0 (0)

4. Discussion

Mirodenafil is a phosphodiesterase type 5 inhibitor developed to treat erectile dysfunction due it seems to induce a significant relaxation of the corpus cavernosum by activation of nitric oxide-cGMP-K⁺ channels pathway [9] [10]. However, the evidence of its efficacy and safety in the treatment of erectile dysfunction is limited to Korean population [14] [15]. In this study, it was evaluated the efficacy and safety of an oral tablet of 100 mg of Mirodenafil once a week during 84 days in a Mexican population.

Results showed that Mirodenafil increased in a significant manner the percentage of male patients reaching ejaculation during intercourse. In a similar way, the patients reported an improvement in their sexual satisfaction, as well as a moderate increment in their self-esteem and mood with the oral administration of Mirodenafil. Our data agree with a multicenter, randomized, double-blinded, placebo-controlled study performed in 223 subjects, who took placebo or fixed doses of 50 mg or 100 mg of Mirodenafil for 12 weeks on an “as needed” basis. In this study, authors demonstrated that Mirodenafil in both doses was superior to placebo since it showed a greater improvement in the whole sexual life of patients including erectile function, orgasmic function, sexual desire and intercourse satisfaction [14]. Similarly, a second placebo-controlled study performed in 180 subjects showed that the daily administration of 50 mg of Mirodenafil during 12 weeks increases the rate of successful intercourse [23]. In fact, a recent meta-analysis study from three randomized controlled trials concluded that mirodenafil was well tolerated and more effective than placebo after 12 weeks [15]. In this regard, the current study extends the observations made about effectiveness of Mirodenafil in the treatment of dysfunction erectile in Korean male to Mexican male.

The risk of erectile dysfunction increases in patients with diabetes, hypertension, hyperlipidemia, tobacco use and urinary tract disorders, among other factors [5] [24]. In our study, Mirodenafil demonstrated to be effective and well tolerated in a heterogeneous Mexican population, where more than 50% of volunteers were diabetic; furthermore, around one-fifth were hypertensive, one-fifth dyslipidemic, one-fifth smokers and one patient had benign prostatic hyperplasia. According with our results, a double-blind study performed in 112 Korean diabetic male showed that 100 mg of Mirodenafil “as needed” basis had greater improvements in sexual life and partner relationship than the placebo group [16]. In a similar way, another double blind study conducted in 109 patients taking antihypertensive drugs exhibited a greater efficacy in the 100 mg of Mirodenafil group, compared with the placebo group, in sexual function [17]. Furthermore, Mirodenafil, not only improve sexual function in patients with benign prostatic hyperplasia, but also, the lower urinary tract symptoms associated to benign prostatic hyperplasia when it was administered concomitantly with a α_1 -blocker as tamsulosin and alfuzosin [18]. Moreover, some studies have demonstrated the efficacy of Mirodenafil in heterogeneous Korean populations with erectile dysfunction and associate risk factors as hyperlipidemia, tobacco use, alcohol consumption, benign prostatic hyperplasia, diabetes mellitus and hypertension [14] [16] [17] [23]. In summary, Mirodenafil seems to be effective in patients with erectile dysfunction and associated

risk factors. Likewise, our study extends this conclusion to Mexican population.

The effectiveness of Mirodenafil in the treatment of erectile dysfunction seems obey to the activation of the nitric oxide-cGMP-K⁺ channels pathway since its chemical structure is very similar to other phosphodiesterase 5 inhibitors [9]. In this regard, a preclinical study performed in the rat model of cavernosal nerve injury showed that Mirodenafil chronic administration was able to increase the levels of nitric oxide synthase and cGMP, as well as, to improve intracavernosal pressure of rats with cavernosal nerve injury [10].

Regarding Mirodenafil safety and tolerability, in the current study, 34 adverse events were reported during 84 days, and only five mild and transient adverse events were related to the pharmacological treatment. From total adverse events, headache was the most frequent adverse event. In line with our results, other studies have reported that Mirodenafil between 50 mg and 150 mg is well tolerated with a few mild or moderate total adverse events, being the headache and flushing the most common adverse events [9] [14]-[17] [23].

Furthermore, in our study, Mirodenafil seems to have good tolerability in patients who receive concomitant medications, since there were no clinically significant changes in their laboratory tests, anthropometric parameters, vital signs and electrocardiogram recordings at the end of the study. According with our results, Mirodenafil has been well tolerated in clinical trials where patients was taking concomitantly antidiabetic, anti-hypertensives or α_1 -blockers [16]-[18]. The current study confirms the observations above mentioned and, extends these observations to concomitant administration of Mirodenafil with multivitamins, antidyslipidemic and some analgesic drugs as acetylsalicylic acid, acetaminophen, naproxen and indomethacin.

5. Conclusion

In conclusion, the data suggest that Mirodenafil is effective, safety and well tolerated in the treatment of erectile dysfunction in Mexican male with erectile dysfunction. However, clinical trials in other populations with larger cohorts and long-term follow up are necessary to generalize the Mirodenafil efficacy and safety worldwide.

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Conflict of Interest

NS-C works in Landsteiner Scientific, S.A. de C.V., as medical director. Authors declare that there is no any other conflict of interest.

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Risk Factors for Stroke in Sulaimaniyah Iraqi Kurdistan Region-Iraq

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Abstract

Background: Stroke is a frequent medical problem and a leading cause of death and disability worldwide. Several conditions and lifestyle factors have been associated with stroke. **Aim:** To evaluate risk factors in stroke patients in Sulaimani city. **Results:** 110 patients with stroke were included in this study, hypertension was found to be the most common risk factor in current study. Out of 110 cases, 83 (75.5%) were hypertensive. Peak stroke-prone age was (60 - 69) year for male, (70 - 79) year for female. We found a statistically significant relation between level of TSC, LDL with ischemic stroke ($r = 0.4047$, $P < 0.0001$) and ($r = 0.4052$, $P < 0.0001$) respectively. While there was a significant inverse relation between HDL and ischemic stroke (Correlation coefficient = -0.4862 , $P < 0.0001$). On the other hand, there was no significant relation between level of TG and ischemic stroke ($r = 0.2403$, $P < 0.0114$). Also correlation statistic between TSC/HDL, LDL/HDL and result of CT scan, showed that there is statistical significance correlation between infarction and value of atherogenic index, ($r = 0.5301$, $P < 0.0001$, $r = 0.4990$, $P < 0.0001$) respectively, but there is no correlation between haemorrhage & the index. **Conclusion:** Hypertension is the leading risk factor of stroke. It is therefore essential to detect and treat hypertension at its outset. High value of atherogenic index mostly associated with ischemic stroke .while no relation found with haemorrhagic stroke.

Keywords

Stroke, Risk Factors, CT Scan, Brain, Lipid Profile, Echocardiography

1. Introduction

Cerebrovascular diseases include disorders in which there is a disturbance of blood supply to the brain. Stroke occurs when an artery supplying blood to a part of the brain

suddenly becomes blocked (ischaemic stroke) or bleeds (haemorrhagic stroke), accounting for about 85% & 15% of cases respectively [1]. This causes loss of function of part of the brain and may affect functions [1] [2]. Transient ischaemic attack (TIA), sometimes called “mini-stroke”, is a temporary manifestation of cerebrovascular disease [3]. Stroke is a major public health problem, being among the top three causes of death in most countries. It affects the brains of almost a half million people every year. Ischemic stroke accounts for more than 80 percent of all strokes. Intracranial Haemorrhage (ICH) usually accounts for 10 to 30 percent of cases depending on the origin of the patient, with greater relative frequencies reported in Asians and blacks [4].

Stroke is classified into two major types: **Brain ischemia** due to thrombosis, embolism, or systemic hypoperfusion. **Brain hemorrhage** due to intracerebral hemorrhage or subarachnoid hemorrhage [5]. Risk factors for stroke comprise both modifiable and nonmodifiable characteristics; **no modifiable** include **Age**: Doubles for each decade of life after age 55(6). **Heredity (family history) and race**: Greater if a parent, grandparent, sister or brother has had stroke [6] [7]. **Gender**: Stroke is more common in men than in women. In most age groups, more men than women will have a stroke in a given year [8]. **Prior stroke, TIA or heart attack**—The risk of stroke for someone who has already had one is many times that of a person who has not. TIAs are strong predictors of stroke. A person who’s had one or more TIAs is almost 10 times more likely to have a stroke than someone of the same age and sex who hasn’t [9]. While **modifiable risk factors include**: **High blood pressure**—High blood pressure is the most important controllable risk factor for stroke [10] [11]. **Cigarette smoking**—In recent years, studies have shown cigarette smoking to be an important risk factor for stroke [11]. **Diabetes mellitus**—Diabetes is an independent risk factor for stroke. Many people with diabetes also have high blood pressure, high blood cholesterol and are overweight [11]. **Carotid or other artery disease**—The carotid arteries supply blood to brain. A carotid artery narrowed by fatty deposits from atherosclerosis may become blocked by a blood clot. **Atrial fibrillation**—This heart rhythm disorder raises the risk for stroke. **Other heart disease**—People with coronary heart disease or heart failure have a higher risk of stroke than those with hearts that work normally. Dilated cardiomyopathy, heart valve disease and some types of congenital heart defects also raise the risk of stroke [11]. **High blood cholesterol**—People with high blood cholesterol have an increased risk for stroke. Also, it appears that low HDL (“good”) cholesterol is a risk factor for stroke [10] [11]. **Poor diet**—Diets high in saturated fat and cholesterol can raise blood cholesterol. Diets high in sodium (salt) can contribute to increased blood pressure. Diets with excess calories can contribute to obesity [9]-[11]. **Physical inactivity and obesity**—Being inactive, obese or both can increase your risk of high blood pressure, high blood cholesterol, diabetes, heart disease and stroke [11]-[17].

2. Patients and Methods

This cross sectional study was approved by the scientific committee of the directory of health in sulaimani city and was conducted to evaluate risk factors for stroke in those

patients admitted to the General Teaching Hospital in Sulaimani city-Iraq, from November 2009 to November 2010. The total sample size was 110 patients.

2.1. Inclusion & Exclusion Criteria

All patient with proven stroke by CT-scan included in this study, those who have no CT-scan & having space occupying lesion on CT where excluded from study. **Trans-thoracic echocardiography** performed using PHILIPS EnVisor C machine combines real-time two-dimensional imaging of the heart and cardiac valves. All patients in the study had a C.T scan (SOMATOM AR.SP, version B41A) of the brain (without contrast) to confirm the clinical diagnosis of stroke and the results was read by expert radiologists within 24 hours of presentation [17]. Estimation of lipid profile (TSC, TG, HDL, and LDL) was done using TECO DIAGNOSTICS kits, Interpretation of result was done according to the National Cholesterol Education Program (**ATP III Guidelines**). Atherogenic index measured by dividing LDL over HDL, also ratio of TSC/HDL was measured [18] [19].

2.2. Statistical Analyses

Data were translated into codes using a specially designed coding sheet, and then converted to computerized database. An expert statistical advice was sought and statistical analyses were done using (**SPSS**) (Statistical Package for Social Science) version 17 computer software. The degree of association between the variables (lipid profile and CT scan results) calculated using Rank correlation *i.e.* Spearman's rho and/or Kendall's tau rank correlation coefficients. P-value < 0.05 regarded as statistically significant.

3. Results

A total of 110 patients with stroke were enrolled in the study; 73 ischemic strokes and 27 hemorrhagic ones (**Table 1**). Altogether, males (59%) outnumbered females (41%). Peak stroke-prone age was (60 - 69) year for male, (70 - 79) year for female. **Table 2** shows the patients' age and gender distribution, males out number females in most age groups. Hypertension was found to be the most common risk factor in current study (75.5%),. Out of 110 cases, 83 (75.5%) were hypertensive, followed by cigarette smoking (52.7%) and ischemic heart disease (37.3%) (**Table 3**). The fasting serum total cholesterol was within its normal reference range in 84.5% of the patients. However, serum LDL levels were elevated in 25.4% and serum HDL was low in 78.2.% of the patients, We found a statistically significant relation between level of TSC, LDL with ischemic stroke ($r = 0.4047$, $P < 0.0001$) and ($r = 0.4052$ $P < 0.0001$) respectively (**Table 4**). While there was a significant inverse relation between HDL and ischemic stroke (Correlation coefficient = -0.4862 , $P < 0.0001$).On the other hand, there was no significant relation between level of TG and ischemic stroke ($r = 0.2403$, $P < 0.0114$) (**Table 4**). Also correlation statistic between TSC/HDL, LDL/HDL and result of CT scan, showed that there is statistical significance correlation between infarction and value of atherogenic index, ($r = 0.5301$, $P < 0.0001$, $r = 0.4990$, $P < 0.0001$) respectively, but there is no correlation

Table 1. Stroke subtype, note that ischemic stroke is more frequent than hemorrhagic stroke, (73% versus 27%).

Ischemic stroke	73%
Hemorrhagic stroke	27%

Table 2. Age and sex distribution of stroke.

Age	No. (n = 110)	Male (%)	Female (%)	P value
30 - 39	3	1 (33.3)	2 (66.7)	0.9935
40 - 49	12	6 (50)	6 (50)	0.6831
50 - 59	17	13 (76.5)	4 (23.5)	0.0085
60 - 69	40	27 (67.5)	13 (32.5)	0.0002
70 - 79	32	18 (56.2)	14 (43.7)	0.4777
80 - 89	5	1 (20)	4 (80)	0.2059
90 - 99	1	0 (0)	1 (100)	0.0001

Table 3. Risk factors in stroke patients in decreasing order of frequency; note that hypertension was the commonest one.

Risk factors	No. {n = 110} (%)
Hypertension	83 (75.5)
Smoking	58 (52.7)
Ischemic heart disease	41 (37.3)
History of prior stroke	40 (36.4)
Family history of stroke	33 (30.8)
Diabetes mellitus	24 (21.8)
Atrial fibrillation	21 (19.1)
Valvular heart disease	7 (6.2)
Oral contraceptive pills	9 (8.2)
Increased hematocrit	8 (7.3)
Alcohol	7 (6.4)
On Anticoagulants	2 (1.8)

between haemorrhage and the index. Trans-thoracic echocardiography showed that 56.4% of the patients have hypertensive heart disease and that 27.3% of the patients demonstrated evidence of ischemic heart disease; the study was unremarkable in 15.5% of the patients (**Table 5**).

4. Discussion

The patients included in the present study represent a random sample of patients hospitalized in medical department with acute stroke with variable duration of in-patient care. The frequency of these risk factors in our study distributed as follows (in decreasing order):

Table 4. Fasting serum lipid profile results in strokes patients (n = 110).

Lipid profile	No.	%
Normal TSC	93	84.5
High TSC	17	15.4
Low HDL	86	78.2
High HDL	24	21.8
Normal LDL	82	74.5
High LDL	28	25.4
Normal TG	96	87.3
High TG	14	12.7

Table 5. Transthoracic echocardiographic findings of stroke patients. This table shows that “hypertensive heart disease” is the main finding (56.3%).

TTE finding	No. of patients (n = 110)	Percentage of patients
Hypertensive heart disease	62	56.4
Ischemic heart disease	17	27.3
Normal	13	15.5
Aortic sclerosis	9	8.2
Valvular heart disease	7	6.3
Atrial septal defect (secondum type)	1	0.9
Atrial myxoma (left-sided)	1	0.9

Hypertension is the commonest risk for stroke (75%), smoking (52%), ischemic heart disease (37%), history of prior stroke (36%), family history of stroke (30%), diabetes mellitus (21%), atrial fibrillation (19%), valvular heart disease (8%), oral contraceptive pills (8%), increased hematocrit (7%), alcohol (6%), and anticoagulant use (1%).

Stroke rates increase dramatically with age. About two thirds of all strokes occur after the age of 65. in our study the most affected age group was between (60 - 70) years of age [20].

Stroke is more common in male sex than female’s one according to many series [20] [21]; in our study it was also more common in male sex (59%) as compared to that of female (41%).

Stroke subtype: Ischemic stroke had been reported to be more frequent than hemorrhagic stroke and accounted for 73% versus 27% of that of haemorrhagic stroke in our study. This percentage of hemorrhagic stroke (27%) is a slightly higher than the western figures (especially in USA, which is around (10% - 15%), but coincides with the results of studies done on Asian populations [22]-[24].

Hypertension: The above findings indicate that hypertension is the commonest risk factor identified and is the most important risk factor for stroke, and this observation is consistent with other studies. For people of all ages and both sexes, higher levels of both systolic and diastolic blood pressure have been associated with an increased incidence

of ischemic and hemorrhagic stroke [25]-[34].

Smoking: Smoking has been seen as a risk factor for stroke incidence in some studies [35] [36]. In the Oslo study of men, smoking was found to be a stronger predictor of stroke mortality than incidence [37]. A dose response was seen with cigarette smoking, and smoking cessation reduced the stroke incidence risk [38]. Other studies have shown this effect, which suggests that a real way to reduce both stroke occurrence and mortality is to encourage smoking cessation. In line with these observations, in our study 58 cases (among 110 cases) were smokers.

Diabetes mellitus: In our study most cases were of type 2 diabetes, and its contribution to stroke (21%) was a little bit higher than many other studies (5% - 10%). This difference might be explained by the fact that some cases were previously undiagnosed and many others were poorly controlled. Many studies have observed an independent association—in both men and women—of diabetes with an elevated risk of stroke [39], with relative risks of ischemic stroke and stroke of all types of 1.8 to 3.0 for both diabetic men and diabetic women [40] [41].

Dyslipidemia: The relation between serum cholesterol levels and the risk of stroke is not clear. A U-shaped relation between the serum level of total cholesterol and the risk of stroke of all types has been proposed, derived from an inverse association with hemorrhagic stroke and a direct association with ischemic stroke. The inverse relation with hemorrhagic stroke has been observed in numerous studies of populations of Japanese origin [41]-[44].

In our study, we found that patients with high total serum cholesterol (Correlation coefficient $r = 0.4047$; $P < 0.0001$; 95% Confidence interval for $r = 0.2353$ to 0.5503) and low HDL cholesterol (Correlation coefficient $r = -0.4862$; $P < 0.0001$; 95% Confidence interval for $r = -0.6173$ to -0.3289) were mostly associated with ischemic stroke; we measured the atherogenic index (LDL/HDL cholesterol) and the atherogenic ratio (total serum cholesterol/HDL cholesterol) and found that a statistically significant association was established between high atherogenic index (Correlation coefficient $r = 0.4990$; $P < 0.0001$; 95% Confidence interval for $r = 0.3439$ to 0.6276) and high atherogenic ratio (Correlation coefficient $r = 0.5301$; $P < 0.0001$; 95% Confidence interval for $r = 0.3807$ to 0.6526) with ischemic stroke. Also we found that a normal total serum cholesterol does not confirm a protection against stroke, because 28 patients had a high LDL (more than 160 mg/dl; $n = 110$) and 86 patients had a low HDL cholesterol (less than 40 mg/dl; $n = 110$) within the normal total cholesterol range.

Alcohol: The relation of moderate alcohol consumption to the risk of stroke has not been conclusively determined. Several methodologic problems have hampered research, including the contamination of the reference group of lifelong abstainers with former drinkers, which may contribute to the J-shaped relation observed in many studies. Only 6% patients in our study were drinkers; this small number is consistent with other studies about alcohol and stroke [45] [46].

Oral contraceptive pill: Higher-dose formulations of oral contraceptives were found to increase the risk of stroke in some subgroups of women, including women over 35

years of age, cigarette smokers, women with hypertension, and women with a history of migraine headaches [47] [48]. A recent meta-analysis combined the results of 47 case-control and cohort studies and established a relative risk among users, an increased risk of stroke have been observed [49].

In our study 9 cases (8%) had history of taking oral contraceptive pills, six of them were ischemic stroke, which can be explained by its adverse effects, such as increased thrombosis and three cases were haemorrhagic stroke which might be related to its effect on raising blood pressure [50].

Atrial fibrillation: Abnormal contraction of the atria may result in thrombus formation. The risk of stroke secondary to thromboembolism related to atrial fibrillation is approximately 3% to 5% per year [51]. In a study of primary prevention in patients with atrial fibrillation, the annual risk of stroke was 6.3% with no treatment, 3.6% with aspirin therapy, and 2.3% with warfarin therapy [52]. In our study out of 110 stroke patients 21 cases (19%) had atrial fibrillation, 20 cases were ischemic stroke, and only 1 case was hemorrhagic stroke which had history of using anticoagulant drug (warfarin), possibly been over anticoagulated. Randomized treatment trial data have shown that anticoagulation with warfarin can reduce the relative risk of stroke by 70% to 80% in the highest-risk groups (age older than 75 years, prior thromboembolic event, history of hypertension, impaired left ventricular function and diabetes), with low risk of hemorrhagic complications and acceptable adverse-effect profiles [53]. In the current study only 2 cases (1.8%) were they had history of using anticoagulant (both of them had AF), one ischemic and the other was hemorrhagic stroke, which might be one of them under treated or the other been over treated respectively.

Ischemic heart disease: People with coronary heart disease or heart failure have a higher risk of stroke than those with hearts that work normally [54] [55].

In the present study 41 cases (37%) had previous history of coronary heart disease and also 17 cases (27%) by transthoracic echocardiography had this disease.

12-Increased hematocrit: A high hematocrit is expected to be associated with an increased risk of thrombosis or embolism. Numerous reports from patients with polycythemia vera and pseudopolycythemia confirm a correlation of elevated hematocrit levels and the incidence of thrombosis [56] [57]. The Framingham study established a positive correlation between the hematocrit value and the risk of cerebral infarction [58] and in a prospective study a hematocrit level higher than 0.51 was found to be an independent risk factor for stroke [59]. Incyanotic congenital heart disease, exceedingly high hematocrit values of up to 0.80 have been recorded, and cerebral and pulmonary infarcts as well as cerebral venous thrombosis correlate with hematocrit levels [60]. In line with these observation, in our study 8 cases (among 110 cases) were detected to have a high hematocrit level all of them were ischemic stroke except one.

Transthoracic echocardiography: Echocardiography is the investigation of choice when a cardiac source of embolism is suspected. However, debate persists about which patients with a stroke or thromboembolism requires imaging. This is in part a result of the increasing pressure on already overloaded echo services and a need for prioritiza-

tion, but it also reflects considerable variation in physicians [61]-[64].

Echocardiographic finding in our patients was like that: Hypertensive heart disease (56%), Ischemic heart disease (27%), Normal (15%), Aortic sclerosis (8%), Valvular heart disease (6%), Atrial septal defect (0.9%), atrial myxoma (0.9%). According to the above findings, hypertensive heart disease (left ventricular hypertrophy (LVH) + diastolic dysfunction) was the main echocardiographic finding among our patients. This observation is consistent with other studies: LVH and abnormal LV geometry are independently associated with increased stroke risk [65]. In hypertensive patients, concentric and eccentric hypertrophy was associated with an ≈ 2 -fold increase in stroke incidence, whereas concentric remodeling did not carry increased risk [66] [67].

Aortic sclerosis: The early build-up of calcium deposits that causes the valve to be thicker and more rigid than normal. Aortic sclerosis is diagnosed on echocardiography as focal areas of increased echogenicity on the valve leaflets with normal valve motion and a normal, or only mildly increased, antegrade velocity across the valve [68]-[70]. In our study (8%) of the cases were they have this finding on echocardiography.

Atrial septal defect: The association of ASD with cerebral embolic events has been less well studied. In one series of 103 patients (mean age 52 years) with a presumed paradoxical embolism and an atrial septal abnormality undergoing percutaneous closure, a PFO alone was present in 81, an ASD alone in 12, and both a PFO and ASD in 10 [71] [72]. In our study only one case (0.9%), was had this finding (Atrial septal defect).

Atrial myxoma: The most common benign cardiac tumour, is found more commonly in young adults with stroke or transient ischemic attack (1 in 250) than in older patients with these problems (1 in 750). Strokes are often recurrent, and may be embolic or hemorrhagic, the presentation ranging from progressive multi-infarct dementia, to massive embolic stroke causing death. Because tumour fragments or adherent thrombus may embolize [73] [74]. In our study only one case (0.9%) was detected by trans-thoracic echocardiography.

5. Conclusions

- Hypertension is the leading risk factor of stroke. It is therefore essential to detect and treat hypertension at its outset.
- Stroke incidence increases with age and it is more common in male gender.
- Echocardiography is a useful test as it is cheap, non-invasive and available, to find risk factors for stroke or a complication of these risk factors on the heart.
- Normal total serum cholesterol (TSC) does not exclude the absence of dyslipidemia, as most of our patients have low serum HDL, and or high LDL.
- High TSC and low serum HDL are mostly associated with ischemic stroke.

6. Recommendations

- 1) Hypertension is the main risk factor for stroke, so early diagnosis and prompt management of it is the main stay in preventing stroke.
- 2) Inpatient stroke unit operational under the direction of stroke director.

- 3) Stroke clinic to provide outpatient consultations for stroke care.
- 4) Ongoing program for primary and secondary stroke prevention.
- 5) Stroke nurse-coordinator.

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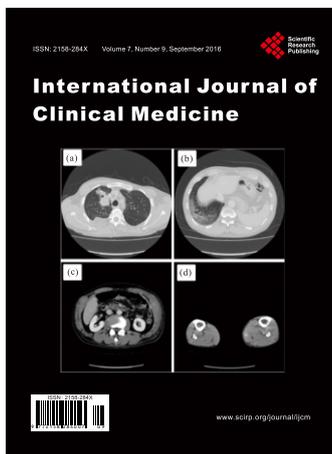
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