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Health

An exploratory study on perceived relationship of alcohol, caffeine, and physical activity on hot flashes in menopausal women

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ABSTRACT

This study examined the effects of caffeine, alcohol, and physical activity (PA) on the perceived frequency and severity of hot flashes in menopausal women. Female employees at a Mid-Western university were invited to participate in an on-line survey. The 26-itemized Women's Health Survey (WHS) included questions regarding demographics, menopausal stage, experience of hot flashes, consumption of caffeinated beverages and alcohol, and participation in PA. One-hundred and ninety-six women completed the study. Ordinary Least Squares regressions revealed PA, caffeine, and alcohol intake were significant in predicting the severity of hot flashes (R^2 = 0.068, $F_{(6,180)}$ = 2.195, p = 0.046), though they did not predict frequency of hot flashes (R^2 = 0.043, $F_{(6,184)}$ = 1.39, p = 0.221). Participation in aerobic PA increased frequency of hot flashes (p = 0.031); while higher intensity of aerobic PA had an inverse relationship on both frequency and severity of hot flashes (p = 0.011, p = 0.003, respectively). Spearman correlations demonstrated a positive relationship between caffeinated soda intake and frequency (r = 0.17, p = 0.06) and severity (r = 0.19, p = 0.04) of hot flashes. Beverage consumption and PA may predict severity of hot flashes in women. Less frequent, higher intensity aerobic PA may lead to fewer, less severe hot flashes.

Keywords: Hot Flashes; Caffeine; Alcohol; Physical Activity

1. INTRODUCTION

Menopausal hot flashes, with varying degrees of severity, are a significant concern for women across the world. There are more than 40 million women in the United States over the age of 40, and it is estimated that approximately 46 million women in the U.S. will have reached menopause by the year 2020 [1]. Seventy-five percent of women over the age of 50 will experience hot flashes to some degree. For some, eight to ten flashes a day is not uncommon, interfering with their daily lives (North American Menopause Society) [2]. Episodes may last from 30 seconds to five minutes, generally averaging four minutes [1]. Women with hot flashes are more likely to experience disturbed sleep, depressive symptoms and significant reductions in quality of life as compared to asymptomatic women [3]. These symptoms may continue to occur for 5 years or more [4]. Geographic variation in the frequency of this phenomenon may be related to the diet and lifestyle of the area [1]. However, little research is available on the relationship of these factors to hot flashes.

A hot flash is described as a transient episode of flushing, sweating and a sensation of heat, often accompanied by palpitations and a feeling of anxiety, and sometimes followed by chills [5]. While the exact cause and mechanism is not well understood, there is a prevailing theory. As estrogen levels are decreased in women, due to surgery, chemicals, or age, the temperature regulation mechanism in the hypothalamus is affected. As a result, the core body temperature is lowered, and the threshold between acceptable and unacceptable body heat levels is more easily crossed. This causes signals to be sent to the rest of the body to release heat, causing perspiration from the sweat glands, leading to the dramatic rise in skin temperature associated with menopausal hot flashes [5].

Several factors have been studied for their contributions to the severity and frequency of hot flashes in menopausal women. Among those are dietary intake, biological factors, and modifiable behaviors. Studies

have also demonstrated a link between intensity and frequency of physical activity and characteristics of hot flashes. Some have found that increased activity leads to increase in menopausal symptoms; while others show that a more active lifestyle may lead to a decrease in occurrence [6-10]. According to current studies, women have reported alcohol consumption as precursors to hot flashes, with research both supporting and refuting this claim [1-3,5,9-11]. Others report a link between caffeine ingestion and menopausal symptoms; however, minimal research has been completed on this factor [7].

With new data regarding the association between these variables and characteristics of menopausal hot flashes, the need to more clearly define lifestyle recommendations for menopausal women has arisen. The purpose of this research study was to examine the effects of consumption of caffeine, alcoholic beverages, and physical activity on the perceived frequency and severity of hot flashes in menopausal women.

2. METHODS

2.1. Participants

Female employees at a Mid-Western University were invited to participate in an on-line survey. The inclusion criteria for participants were: 1) ≥ 40 years of age; 2) devoid of taking medications for treatment of menopausal symptoms; 3) absence of smoking; and 4) educational status of sixth grade or higher. The recruitment email informed participants of the following parameters related to the study: their random selection, criteria for participating, purpose of research procedures, and approximate time needed to participate in the study. Subjects were informed that by completing and submitting the survey, they were giving their consent. Duration for completion of the survey was one month. Ball State University's Institutional Review Board approved all aspects of this study.

2.2. Instrumentation

The 26-itemized Women's Health Survey (WHS), developed by the researchers, was accepted for face validity by three experts (two dietitians, one physician). Reliability was established by utilizing a small (n = 20) sample of subjects other than the study population who were of similar characteristics. Subjects took the survey twice, with two weeks between each administration. Test-retest results were observed from the same participants to assess similarity of answers for each test. The Kappa coefficients from the test-retest ranged from a low of 0.44 to 1.00, with a median coefficient of 0.77. Except for two questions where the Kappa coefficient could not be calculated due to zero variance in responses, all coefficients were statistically significant (see Appendix).

The WHS included questions regarding demographics, stage of menopause, hot flashes, average daily consumption of caffeinated beverages, alcohol, and participation in physical activity. Caffeinated beverages were divided into subcategories based on usual number of servings. Weekly consumption of coffee, tea, and cocoa were listed as eight fluid ounces (236.56 mL), while energy drinks and soda were 12 fluid ounces (354.84 mL). Intake of caffeinated pills, diet pills containing caffeine, and dark chocolate were also recorded. Usual weekly intake of alcoholic beverages was divided into subcategories, namely, beer (12 fluid ounces, 354.84 mL), white wine or champagne (5 fluid ounces, 147.85 mL), red wine (5 fluid ounces, 147.85 mL), and mixed drinks (1.5-2 fluid ounces, 44.36-59.14 mL). Usual physical activity per week assessed separately 30 minute intervals of aerobic (e.g. running) and strength activity (e.g. weight lifting). Usual intensity of physical activity was measured using descriptors mild (don't break a sweat during activity), moderate (break a light sweat), or heavy intensity (break a sweat, heart rate very increased).

Usual daily frequency and severity of hot flashes were evaluated with rating scales. The subjective 10-point rating scale ranged from 1 being very mild (a warm sensation without sweating or disruption of normal activity) to 10 being very severe (heat sensation with sweating that may have interrupted daily activities) [5].

2.3. Statistical Analysis

Separate ordinary least squares (OLS) regressions were used to evaluate frequency and severity of hot flashes. Level of self-reported physical activity, average daily caffeine, and alcohol intake were the predictors. Spearman rank correlations were used to examine the relationships of categories of beverage intake levels with hot flash frequency and severity. This analysis was performed in order to look at beverages individually after excluding those who never consumed the beverage in the last week. Significance was established at $p < 0.05$.

3. RESULTS

3.1. Demographics and Menopausal Characteristics

One-hundred and ninety-six women successfully completed the study. As observed in **Table 1**, more than half were 50-59 years, Caucasian, and in the naturally postmenopausal reproductive stage. Most participants had experienced hot flashes (81.1%), and were not taking medications (92.9%) or using alternative therapies

(91.3%). Mean number of hot flashes ± standard deviation (SD) were 2.2 ± 1.5 per week, while the mean usual severity ± SD was 3.26 ± 2.58 on a scale of one to ten.

3.2. Physical Activity

More than half the subjects reported participating in aerobic physical activity 0-2 times per week (n = 110, 56.2%) and at moderate intensity (n = 89, 45.4%). In reference to strength activity, 60.2% participated 0-2 times per week with 38.3% performing at light to moderate intensity. Mean ± SD weekly participation in 30 minutes of aerobic physical activity and strength exercises were 2.48 ± 1.25 times and 1.51 ± 0.724, respectively (**Table 2**).

3.3. Caffeine and Alcohol

Based on reported intake of caffeinated beverages (majority consumed 0-3 servings of each beverage), total mean ± SD caffeine intake was 1144 mg ± 1008 mg, while total mean ± SD servings of alcohol was 2.52 ± 3.46 servings per week. The median reported weekly intake of caffeine and alcohol among participants were 1080 mg and 1.20 servings, respectively.

Although 196 women participated in this research, due to insufficient information, only data for 188 were analyzed using Ordinary Least Squares (OLS) regression. Overall, the regression results revealed that the effects of self-reported physical activity, average daily caffeine, and alcohol intake were not significant in predicting the frequency of hot flashes (R^2 =0.043, $F_{(6,184)}$ = 1.39, p = 0.221). However, after controlling for the other independent variables, the regression indicated that, relatively, more participation in aerobic physical activity increased frequency of hot flashes (B= 0.241, β = 0.20, p = 0.031); while higher intensity of aerobic physical activity had an inverse relationship (B = –0.423, β = –0.261, p = 0.011). All other variables remained statistically insignificant (**Table 3**).

Overall, regression analysis also revealed a small, but statistically significant effect of physical activity, caffeine, and alcohol on severity of hot flashes (R^2 = 0.068, $F_{(6,180)}$ = 2.195, p = 0.046). Interestingly, after controlling for all other independent variables, the regression indicated that relatively, higher intensity of aerobic exercise decreased severity of hot flashes (B = –0.875, β= –0.315, p = 0.003) (**Table 3**).

Spearman rank correlations showed a small relationship between higher consumption of caffeinated soda for

Table 1. Demographics and menopausal characteristics of participants (n = 196).

Characteristic	Description	n* (%)
Age	40-44	21 (10.7)
	45-49	37 (18.9)
	50-54	54 (27.6)
	55-59	50 (25.5)
	60 +	34 (17.3)
Ethnicity	White	187 (95.4)
	African-American	6 (3.1)
	Hispanic	1 (0.5)
	Asian/Pacific Islander	1 (0.5)
Reproductive Stage	Pre-menopausal	32 (16.3)
	Peri-menopausal	28 (14.3)
	Menopausal	11 (5.6)
	Naturally postmenopausal	81 (41.3)
	Post-menopausal due to surgery, chemotherapy, or radiation	44 (22.4)
Currently using alternative therapies	Yes	15 (7.7)
	No	179 (91.3)
Currently taking medications for menopausal symptoms	Yes	13 (6.6)
	No	182 (92.9)
Have experienced menopausal hot flash	Yes	159 (81.1)
	No	36 (18.4)

Table 2. Subjects participation in 30 minutes of physical activity (PA) per week (n = 196).

Characteristic	Description	N (%)*
Aerobic PA		
Frequency	0 times	46 (23.5)
	1-2 times	64 (32.7)
	3-4 times	48 (24.5)
	5-6 times	23 (11.7)
	7-8 times	11 (5.6)
	> 8 times	4 (2.0)
Intensity	Don't participate	38 (19.4)
	Light	47 (24.0)
	Moderate	89 (45.4)
	Heavy	21 (10.7)
Strength PA		
Frequency	0 times	118 (60.2)
	1-2 times	60 (30.6)
	3-4 times	14 (7.1)
	5-6 times	4 (2.0)
Intensity	Don't participate	116 (59.2)
	Light	39 (19.9)
	Moderate	36 (18.4)
	Heavy	2 (1.0)

both frequency (r = 0.17, p = 0.06) and severity (r = 0.19, p = 0.04) of hot flashes. No significant relationship between the other caffeinated or alcoholic beverages and hot flashes was revealed (**Table 4**).

4. DISCUSSION AND CONCLUSIONS

Several studies have examined the effect of lifestyle factors on hot flashes, however, to date; no research has focused simultaneously on the effect caffeine and alcohol consumption and physical activity had on the frequency and severity of hot flashes in women over the age of 40.

Findings from the present study related to frequency of workouts and incidence and severity of hot flashes are congruent with previous research. Whitcomb and colleagues looked at the relationship between physical activity prior to the time of the last menstrual period and hot flashes [6]. This population based study using 512 peri-menopausal and post-menopausal women found highly active women (reported exercising > 16 times per month) were significantly more likely to have moderate to severe hot flashes (OR = 1.70, p = 0.01) and daily hot flashes (OR = 1.79, p < 0.01) than less active women (report exercising 0-15 times per month). Similarly, Thurston, *et al*., found a higher incidence of subjective hot flashes after physical exertion (OR, 1.49; 95% CI, 0.99-2.25; p = 0 .05), although regular aerobic exercisers had fewer hot flashes than sporadic exercisers [7].

Table 3. Ordinary least squares regression analysis of the influence of alcohol, caffeine, and physical activity on frequency and severity of hot flashes (n = 188).

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std.Error	Beta	t	Sig
Frequency of Hot Flashes[1] Constant	2.406	0.360		6.688	0.000
How many times in the last week did you participate in 30 minutes of aerobic physical activity?	0.241	0.111	0.200	2.17	0.031
How intense would you rate your participation in aerobic activity?	–0.423	0.165	–0.261	–2.554	0.011
How many times in the last week did you participate in 30 minutes of strength exercises?	–0.259	0.285	–0.125	–0.909	0.364
How intense would you rate your participation in strength exercise?	0.339	0.257	0.186	1.316	0.19
Total estimated caffeine for the week (mg)	0.000	0.000	0.026	0.327	0.744
Total servings of alcohol for the week	0.019	0.035	0.044	0.550	0.583
Severity of Hot Flashes[2] Constant	4.789	0.611		7.842	0.000
How many times in the last week did you participate in 30 minutes of aerobic physical activity?	0.188	0.190	0.092	0.994	0.322
How intense would you rate your participation in aerobic activity?	–0.875	0.286	–0.315	–3.056	0.003
How many times in the last week did you participate in 30 minutes of strength exercises?	0.004	0.486	0.001	0.008	0.993
How intense would you rate your participation in strength exercise?	0.260	0.443	0.083	0.585	0.559
Total estimated caffeine for the week (mg)	0.000	0.000	–0.047	–0.595	0.552
Total servings of alcohol for the week	–0.055	0.060	–0.074	–0.931	0.353

Note: [1]R^2 = 0.043, $F_{(6, 184)}$ = 1.39, p = 0.221; Dependant variable: Q8 In the last week, how many hot flashes have you had? [2]R^2 = 0.068, F(6,180) = 2.195, p = 0.046; Dependant variable: Q9 In the last week, how would you rate the usual severity of hot flashes?

Table 4. Spearman correlations between caffeinated beverages and frequency of hot flashes.

Frequency of Hot Flashes	n	r	Sig.
Energy drinks (12 fl. oz. serving)	2	NA**	NA**
Caffeinated hot tea (8 fl. oz. serving)	60	–0.005	0.969
Caffeinated iced tea (8 fl. oz. serving)	78	–0.016	0.887
Caffeinated soda (12 fl. oz. serving)	123	0.173	0.055
Hot chocolate or cocoa (8 fl. oz. serving)	19	0.214	0.378
Dark chocolate (8 fl. oz. serving)	99	0.102	0.314
Red wine (5 fl. oz. serving)	51	0.091	0.527
Alcoholic beer products (12 fl. oz. serving)	37	–0.025	0.881
White wine/champagne (8 fl. oz. serving)	55	0.097	0.481
Mixed drinks (1.5-2.0 fl. oz. serving)	30	–0.227	0.227
Severity of Hot Flashes			
Energy drinks (12 fl. oz. serving)	2	NA*	NA*
Caffeinated hot tea (8 fl. oz. serving)	60	0.033	0.804
Caffeinated iced tea (8 fl. oz. serving)	77	–0.087	0.449
Caffeinated soda (12 fl. oz. serving)	121	0.189	0.038
Chocolate or cocoa (8 fl. oz. serving)	20	–0.010	0.967
Dark chocolate (8 fl. oz. serving)	97	0.059	0.563
Red wine (5 fl. oz. serving)	49	0.229	0.114
Alcoholic beer products (12 fl. oz. serving)	34	–0.032	0.858
White wine/champagne (8 fl. oz. serving)	53	–0.015	0.918
Mixed drinks (1.5-2.0 fl. oz. serving)	28	–0.149	0.449

The inverse relationship found between intensity of physical activity and severity of hot flashes supports the findings of Sievert *et al*. [10]. Women who participated in heavy exercise (enough to speed up breathing and heart rate, at least two times per week) were significantly less likely to report both hot flashes and night sweats ($p = 0.05$) compared to those participating in minimal exercise (no exercise, or light exercise less than once per week).

In contrast, Sternfield and colleagues investigated the effects of regular exercise prior to the final menstrual period [8]. There was no association between habitual physical activity and menopausal hot flashes. Research also revealed regular physical activity did not significantly affect the frequency of menopausal symptoms such as hot flashes ($p = 0.291$). Similar observations were reported by Riley *et al*., indicating no significant relationship exists between habitual exercise and frequency or intensity of hot flashes (OR = 1.3; 95% CI = 0.78-2.16) [9].

Limited studies have looked at the effect of caffeine on hot flashes. Even though the present study demonstrated a there was a perceived relationship between caffeinated soda and frequency and severity of hot flashes ($r = 0.17$, $p = 0.04$; $r = 0.19$, $p = 0.04$), Thurston *et al*., found an increased likelihood of objective hot flashes (OR = 1.51; CI = 1.18-3.81; $p = 0.003$) after caffeine consumption [7].

Regression analysis revealed alcohol and caffeine consumption had no influence on frequency or severity of hot flashes. On the contrary, earlier studies have shown significant relationships existed between alcohol intake and hot flashes. Freeman and colleagues [11], found alcohol to be a significant predictor of hot flashes (OR 1.10, $p = 0.002$). Observations were also noted by Sievert *et al*. revealing daily alcohol consumption significantly increased the risk of hot flashes ($p < 0.01$) [10]. Riley and colleagues noted in peri-menopausal women a significant correlation prevailed with consumption of 1-5 drinks per day and bothersome hot flashes (OR = 0.52, CI = 0.31-0.86) [9].

This research was limited to a homogeneous ethnic group of faculty and staff at a Mid-Western University. Recommendations for future research related to hot flashes include: 1) assessment of participants BMI; 2) incorporation of a larger and diverse ethnic group, with varying age and geographical location; 3) comparison of recreational activity to various levels and types of aerobic activity; 4) treatment of hot flashes using complementary and alternative medicine; 5) measurement of actual hot flashes using objective and subjective information; 6) comparison of co-morbidities such as obesity, diabetes, and hypertension and their contributory roles to menopausal symptoms and hot flashes.

Health professionals and scientists need to find a connection to other modifiable behaviors to decrease the occurrence and symptoms of hot flashes. In doing so, a better understanding of the physiological challenges women face can be gained, so appropriate intervention

strategies could be implemented to improve quality of life.

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Appendix: Women's Health Survey

1. Age (years)
   a. 40-44
   b. 45-49
   c. 50-54
   d. 55-59
   e. 60 or over
2. Ethnicity
   a. White
   b. African-American
   c. Hispanic
   d. Asian/Pacific Islander
   e. Other
3. Are you a smoker?
   a. Yes
   b. No
4. Do you currently take any medications to treat menopausal symptoms?
   a. Yes
   b. No
5. Are you currently using any alternative therapies to treat menopausal symptoms (e.g. black cohosh, dong quai root, ginseng, kava, red clover, soy)?
   a. Yes
   b. no
6. What is your current reproductive stage?
   a. Pre-menopausal (regular menstrual cycle)
   b. Peri-menopausal (last menstrual period within the last 3 months)
   c. Menopausal (last menstrual period within the last year)
   d. Naturally Post-menopausal (last menstrual period more than 12 months ago)
   e. Post-menopausal due to surgery or chemotherapy/radiation
7. Have you ever had a menopausal hot flash? (An episode of flushing, sweating, and a sensation of heat, often accompanied by palpitations and a feeling of anxiety, and sometimes followed by chills)
   a. Yes
   b. No
8. In the last week, how many hot flashes have you had?
   a. 0
   b. 1-3
   c. 4-6
   d. 7-9
   e. 10-12
   f. More than 12
9. In the last week, how would you rate the usual severity of the hot flashes? **1** being **very mild** (a warm sensation without sweating or disruption of normal activity) and **10** being **very severe** (heat sensation with sweating that may have interrupted daily activities)?

a. Did not experience hot flashes
b. 1
c. 2
d. 3
e. 4
f. 5
g. 6
h. 7
i. 8
j. 9
k. 10

10. In the last week, how many times did you participate in **30 minutes** of aerobic physical activity (running, swimming, hiking, walking, etc.)?
    a. 0
    b. 1-2
    c. 3-4
    d. 5-6
    e. 7-8
    f. More than 8
11. How intense would you rate your participation in aerobic activity?
    a. Don't participate
    b. Light (don't break a sweat)
    c. Moderate (break a light sweat, heart rate increased)
    d. Heavy (break a sweat, heart rate very increased
12. How many times per week do you participate in **30 minutes** of strength exercises (weight lifting, Pilates)?
    a. 0
    b. 1-2
    c. 3-4
    d. 5-6
    e. 7-8
    f. More than 8
13. How intense would you rate your participation in strength exercises?
    a. Don't participate
    b. Light (don't break a sweat)
    c. Moderate (break a light sweat, heart rate increased)
    d. Heavy (break a sweat, heart rate very increased)
14. In the last week, how many times did you consume caffeinated **coffee** (8 ounce serving)?
    a. Never
    b. 1-3
    c. 4-6
    d. 7-9
    e. 10-12
    f. 13-15
    g. 16-18
    h. More than 18 per week
15. In the last week, how many times did you consume **energy drinks** (e.g. Red Bull, Sobe; 12 ounce serving)?
    a. Never
    b. 1-3
    c. 4-6
    d. 7-9
    e. 10-12
    f. 13-15
    g. 16-18
    h. More than 18 per week
16. In the last week, how many times did you consume **caffeinated hot tea** (8 ounce serving)?
    a. Never
    b. 1-3
    c. 4-6
    d. 7-9
    e. 10-12
    f. 13-15
    g. 16-18
    h. More than 18 per week
17. In the last week, how many times did you consume **iced tea** (8 ounce serving)?
    a. Never
    b. 1-3
    c. 4-6
    d. 7-9
    e. 10-12
    f. 13-15
    g. 16-18
18. In the last week, how many times did you consume **caffeinated soda** (e.g. Coke, Pepsi, etc, 12 ounce)?
    a. Never
    b. 1-3
    c. 4-6
    d. 7-9
    e. 10-12
    f. 13-15
    g. 16-18
    h. More than 18 per week
19. In the last week, how many times did you consume **hot chocolate** or **cocoa** (8 ounce serving)?
    a. Never
    b. 1-3
    c. 4-6
    d. 7-9
    e. 10-12
    f. 13-15
    g. 16-18
    h. More than 18 per week
20. In the last week, how many times did you

consume **dark chocolate** (at least 1 ounce serving)?
   a. Never
   b. 1-3
   c. 4-6
   d. 7-9
   e. 10-12
   f. 13-15
   g. 16-18
   h. More than 18 per week

21. In the last week, how many times did you take **caffeine pills** (e.g. no-doz, vivarin, 1-200 mg pill)?
   a. Never
   b. 1-3
   c. 4-6
   d. 7-9
   e. 10-12
   f. More than 12

22. In the last week, how many times did you take **caffeinated diet pills?**
   a. Never
   b. 1-3
   c. 4-6
   d. 7-9
   e. 10-12
   f. More than 12

23. In the last week, how many times did you consume **red wine** (1 serving, 5 ounce glass)?
   a. Never
   b. 1-3
   c. 4-6
   d. 7-9
   e. 10-12
   f. More than 12

24. How many times per week do you consume **alcoholic beer products** (12 ounce serving)?
   a. Never
   b. 1-3
   c. 4-6
   d. 7-9
   e. 10-12
   f. More than 12

25. How many times per week do you consume **wine** (not red; 5 ounce serving)?
   a. Never
   b. 1-3
   c. 4-6
   d. 7-9
   e. 10-12
   f. More than 12

26. How many times per week do you consume **mixed drinks** (1.5-2 ounce serving)?
   a. Never
   b. 1-3
   c. 4-6
   d. 7-9
   e. 10-12
   f. More than 12

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Effects of cola intake on fertility: a review

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ABSTRACT

The consumption of soft drinks has increased considerably during the last decades. Among them, the cola-based preparations are possibly the refreshments with the largest sales worldwide. During the previous years, important concerns have been raised about the effects of colas on human health. In this review, we introduce the cola effects on reproduction including pregnancy miscarriages, ovulatory and menstrual disorders, and reduced semen quality. Although caffeine intoxication may be thought to play the most important role, a component of cola other than caffeine, or in combination with caffeine, may be associated with increased risk of reproductive hazards in heavy cola (> 1 L per day)-consumers. Cola discontinuation usually leads to an uneventful recovery in the most cases suggesting justification of limitations in the maximum recommended daily dose of these soft drinks. Cola is not an essential beverage, and abstaining from drinking more than 1 L per day is a minor intrusion in one's personal life. Despite these uncertainties, this growing knowledge may alarm the fertility risk of chronic cola intake in peoples of childbearing age.

Keywords: Cola; Soft Drink; Caffeine; Semen; Miscarriage; Ovulation

1. INTRODUCTION

The consumption of soft drinks has increased considerable during the last decades. Among them, the cola-based preparations are possibly the refreshments with the largest sale worldwide. During the previous years, important concerns have been raised about the effects of colas on human health. In addition to the possible detrimental effects of chronic cola consumption (enamel softening [1,2], bone demineralization [3,4], hypokalemic myopathy [5-7], development of metabolic syndrome and diabetes mellitus [8-10], and chronic kidney diseases [11,12]), several lines of evidence suggest that the chronic consumption of large amounts of cola-based soft drinks may increase the risk of reproductive problems such as decreased fetal growth, preterm delivery, and spontaneous abortion [13-16]. However, results from these epidemiological studies suggesting the association between cola consumption and such outcomes have been conflicting and the available information is incomplete and remains controversial. The observations may have important public health implications as, recently, trends of increasing the portion size of these preparations have been noticed. With the aim of evaluating the available epidemiological evidence of the effect of cola consumption on reproductive quality (see **Table 1**), a systematic qualitative review was conductive.

2. PHARMACOLOGICAL EFFECTS OF INGREDIENTS IN COLA

Cola contains many different chemical compounds and no certainty exists as to which ones may be associated with disease risk.

Cola soft drinks may contain large amounts of glucose (up to 11 g of sugar/dL) and high-fructose corn syrup. Highly caloric carbonated soft drinks are often sweetened with high fructose corn syrup, which makes soda inexpensive to produce. While sugary soda may be sold cheaply as a food item and yield a profit, they provide little satiety. Thus, the excessive consumption of theses preparations may lead to glycemic load, resulting in osmotic diuresis and hyperinsulinemia. Fructose itself may cause kidney damage, perhaps mediated by uric acid [17].

Cola contains sufficient amounts of caffeine ranging

Table 1. Main points of the cola (carbonated soft drink) - induced reproductive hazards.

Outcomes	Amount of cola consumption*	Suggested cause	Refs.
Reduced sperm count (30% below the average in non-cola-drinkers)	> 1 L per day during the past week	Constituents in cola other than caffeine	[33]
Ovulatory disorder infertility	> 2 soft drinks a day	Contents in cola other than caffeine or sugar	[30]
Shortened menses duration and cycle	> 300 mg of caffeine per day	Caffeine	[21]
Increased risk of miscarriage (hazard ratio of 2.23)	200 mg of caffeine per day during pregnancy	Caffeine	[45,50]

*Assumed caffeine content of 150 mg/L.

from 95 to 160 mg/L. Caffeine, 1,3,7-trimethylxanthine, is among the most frequently ingested pharmacologically active substances [18,19]. Carbonated soft drinks and coffee are the main sources of caffeine intake.

Other components in cola, such as phosphorus in dark cola, may reveal significant physiological actions.

3. OVULATION AND MENSTRUATION DISOERDERS

Women who consume caffeine may be less likely to have long menses. This phenomena is biologically likely plausible because caffeine is a known vasoconstrictor [20], reducing uterine blood flow. Constriction of uterine blood vessels would be expected to reduce uterine blood flow, which could reduce menstrual bleeding and shorten the duration of menses [21]. Caffeine may alter the duration of menstrual cycle via the effect of caffeine on sex hormones or the hormone receptors [22]. However, the results of studies of caffeinated beverage consumption in relation to fecundity are inconsistent. Several studies in humans have reported an association between caffeine intake and delayed time to conception [23-26], in contrast, others have shown either no association [27,28] or a relation only at very high levels of intake [29]. Most of these studies have retrospectively collected information on alcohol and caffeine intake, making the results susceptible to biases. The study by Fenster *et al.* [21] described the relation between caffeine intake and menstrual function was examined in 403 healthy premenopausal women who belonged to Kaiser Permanente Medical Care Program in 1990-1991. They collected information about caffeinated beverage intake as well as other lifestyle, demographic, occupational, and environmental factors. Subjects collected daily urine samples and completed a daily diary for an average of five menstrual cycles. Caffeine intake was not strongly related to an increased risk for anovulation, short luteal phase, long follicular phase, long cycle, or measures of within-woman cycle variability. Chavarro *et al*. [30] followed 18,555 married women without a history of infertility for 8 years as they attempted to become (or became) pregnant. Soft drinks were the only beverages positively related to ovulatory infertility. Intake of caffeinated soft drinks was associated with a higher risk of ovulatory disorder infertility among women consuming at least 2 or more soft drinks per day. Women consuming 2 or more caffeinated soft drinks per day had a 47% greater risk of ovulatory infertility than women who consumed less than 1 caffeinated soft drink per week. Their analyses suggested that neither caffeine nor fructose was responsible for this association. Extreme comparisons of caffeine and coffee intake suggested no association or an inverse association with ovulatory disorder infertility. Some constituents in cola other than caffeine or sugar may cause ovulatory disorder.

Regarding the risk of ovarian cancer developments, laboratory data suggest that caffeine or some components of coffee may cause DNA mutations and inhibit tumor suppressor mechanisms, leading to neoplastic growth. An increased risk was observed in the multivariate model for women who reported drinking five or more cups/day of caffeinated coffee compared to women who reported drinking none. Decaffeinated coffee, total coffee, and caffeine were not statistically significantly associated with ovarian cancer incidence. A component of coffee other than caffeine, or in combination with caffeine, may be associated with increased risk of ovarian cancer in postmenopausal women who drink five or more cups of coffee a day [31].

4. SEMEN QUALITY DECLINE

A recent Danish study revealed that sperm counts are lower in men who drink cola of 1 L (estimated to contain 100-140 mg of caffeine) or more per day, averaging 31% below the average in control [32,33]. This cola's effect on sperm seems not to be attributable to their caffeine content; caffeine intake of < 800 mg per day and cola consumption of < 14 0.5-L bottles per week is not associated with reduced semen quality. The reduction in semen quality among high-quantity cola drinkers must be attributed to constituents in cola other than caffeine because the caffeine content of cola is not high. They also added that although the cola drinkers' sperm count was still within the normal range, at 35 million/mL, cola might nonetheless dampen their fertility [33]. Among the study participants, those not drinking cola had an aver-

age count of 50 million/mL.

In fact, previous studies on caffeine intake and sperm quality have been contradictory [34-36]. Alternatively, these associations may be attributed to the less healthy lifestyle and diet of high-quantity consumers. Heavy quantity consumers of cola or caffeine had an unhealthier lifestyle, which has previously been associated with poorer semen quality [37-40]. To the extent possible, many researchers considered these factors in the analyses, and they did not appear to explain the caffeine and cola associations. High-quantity caffeine and cola consumers also had a less healthy diet, and previous studies have found reduced semen quality among men who consumed few fruits and vegetables and had a low intake of antioxidant and trace minerals [33]. Cola contains sufficient amounts of many minerals including phosphorus.

High cola and caffeine consumption may be related to in utero exposure to caffeine. Ramlau-Hansen *et al*. [41] studied the association between prenatal coffee and current caffeine exposure and semen quality. There is a tendency toward decreasing crude median semen volume and adjusted mean testosterone and inhibin concentrations with increasing maternal coffee consumption during pregnancy. Sons of mothers drinking 4-7 cups/day had lower testosterone levels than sons of mothers drinking 0-3 cups/day. However caffeine intake had no impact on semen quality.

5. MISCARRIAGES

Caffeine can readily cross the placental barrier to the fetus [42]; its clearance is prolonged in pregnant women, and its metabolism rate is low in the fetus because of low levels of enzymes [43,44]. It may also influence cell development through increasing cellular cyclic adenosine monophosphate (cAMP) concentrations and decrease intervillous placental blood flow via increasing circulating catecholamines [18,19,45]. Caffeine intake during pregnancy has been suggested as a risk factor for adverse reproductive outcomes. Therefore, caffeine could have an adverse effect on fetal development. Indeed, caffeine intake has been reported to increase the risk of miscarriage [15,16,46-48]. Although numerous studies on maternal caffeine consumption and the risk of miscarriage have been published since the 1980s, the effect of caffeine intake on the risk of miscarriage remains controversial because of methodological limitations in past studies. Many studies have relied on retrospective information, which is subject to recall bias [15,16,46-48]. Some had only a small number of participants, which limited their power to detect an effect. Some did not take into account potential confounding factors such as smoking, alcohol consumption, and most importantly, pregnancy-related symptoms including nausea and vomiting. Some recruited women who sought prenatal care at their 13th to 28th weeks of gestation, therefore too late in pregnancy to study miscarriage. Such controversy has led to the uncertainty about the health effects of caffeine consumption during pregnancy among both clinicians and pregnant women alike.

Weng *et al*. [45] demonstrated, in their prospective cohort study, an elevated risk of miscarriage associated with caffeine consumption during pregnancy and a dose-response relationship with most of the risk associated with caffeine consumption at 200 mg (which approximately 1.5 L cola contains) or greater per day. This observed effect was independent of many potential confounders including pregnancy related symptoms such as nausea, vomiting, and aversion to caffeine consumption. Even among women who never changed caffeine consumption pattern during pregnancy, there was an almost 80% increased risk of miscarriage associated with caffeine consumption of 200 mg/day or greater, although it was not statistically significant because of reduced sample size by stratification. The increased risk of miscarriage appeared to be due to caffeine itself rather than other possible chemicals in coffee because caffeine intake from noncoffee sources showed the similarly increased risk of miscarriage. On the other hand, a similar cohort study, published same month, by Svavitz *et al*. [49] demonstrated that this can result in recall bias generating a positive results, whereas when caffeine exposure is ascertained before miscarriage, the findings indicate no effect of caffeine. To data, the literature is still inconclusive regarding the influence of caffeine on miscarriage and the available information is incomplete and remains controversial [47,50].

6. CONCLUSIONS

Although much epidemiological work has been conducted, results from studies investigating the association between cola consumption and outcomes such as reproductive hazards have been conflicting and the available information was incomplete and remained controversial. As summarized in **Table 1**, our reviewing recent reports suggest the association between cola consumption and increased risk of reproductive hazards. In addition to caffeine, cola contains a number of other chemical compounds, and one or more of these could be physiologically active. Further studies might attempt to disentangle a caffeine effect from a noncaffeine effect by comparing different types of beverage drinkers. Cola is not an essential beverage, and abstaining from drinking more than 1 L per day is a minor intrusion in one's personal life.

The growing knowledge may alarm the fertility risk of chronic cola intake in peoples of childbearing age.

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The role of partners in shaping the body image and body change strategies of adult men

——Partners and male body image

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ABSTRACT

The current study examined the relationship between perceived messages about the bodies of adult men from their sexual partners and the actual body image of these men. Interviews were conducted among 38 middle-aged men. Feedback from partners was generally complimentary, and the men were generally positive about their body image. Partners were seen to be more focused on a healthy body rather than a physically attractive body. The implications of these findings for better understanding the social influence on adult men to obtain a healthy body weight are discussed.

Keywords: Men; Body Image; Partners; Qualitative

1. INTRODUCTION

A growing body of literature has demonstrated that the ideal body form for males is slim and muscular [1], and that males receive messages from a range of sources to achieve this ideal [2-4]. Most of this past research has been conducted with adolescent boys, and has demonstrated that adolescent boys are particularly focused on obtaining a lean muscular body [5]. Messages to achieve this body shape come from parents, peers and the media [6]. In a recent review of the nature of the body image of males, and the factors that contribute to males' body image, Gray and Ginsberg [7] found a strong preference for a muscular body among both Western and non-Western males.

There have been limited studies on the sociocultural influences on the body image of adult men. The limited research that is available suggests that they are aware of the sociocultural body ideal for men, and they have similar body image concerns to those of adolescent boys [1]. Grogan and Richards [8] found that boys and men in all age groups drew a relationship between muscularity and masculinity. Rather than focusing on appearance in its own right, they focused more on how their bodies looked in relation to function, fitness and health. Grogan [8] expanded this view further in her review of studies of men's body image and concluded that a large aspect of male body image relates to functionality, rather than appearance.

The above research has not examined if the nature of the messages that men receive about their body, in terms of weight and muscularity, are different according to their weight. Certainly, Luciano [10] suggested that body weight would appear to influence the level of body dissatisfaction experienced by men. Davison and McCabe [1] found that poor body image was related to problems in social and sexual functioning among middle-aged men, and to depression and anxiety among older men. These results suggest that, at least for middle aged men, their sexual partner may play some role in shaping their body image. The current study was designed to explore the nature of the messages from the partner about the man's body, and how these messages varied according to the man's body size.

Previous research has suggested that sexual partners may provide a significant source of appearance-related information to men [11]. Feedback from significant others may contribute to men's perceptions of body image, through criticism or compliments, and partners may provide a point of reference through which men derive their level of satisfaction or dissatisfaction with their bodies [11]. Sheets and Ajmere [12] also found that male and female romantic partners reported telling each other to lose or gain weight. Women were more likely to tell their male dating partners to gain weight, whereas men would typically tell their female partners to either gain or lose weight.

Married men may be more motivated by their own need to possess the ideal male physique than by their wives' opinion of their bodies [13]. A comparison between mar-

ried and single couples found that married couples were far less concerned that their husband or wife possessed the ideal body physique than did single couples [13].

Although the above studies examined the nature of messages from partners, very few studies have investigated the relationship between men's body image and how messages from sexual partners are associated with men's perception of their bodies and how they subsequently try to change their body shape and weight. Studies have shown that men, like women, place a great deal of importance on their appearance [14,15], and may report dissatisfaction with their bodies if they perceive that their current image does not live up to the socially-accepted ideal body for men [16,17]. The use of maladaptive body-change strategies by men, such as steroids and excessive exercise, has increased in recent years, as men attempt to attain and maintain the V-shaped male physique [9,14]. It is therefore important to obtain a better understanding of the types of messages that men receive about their bodies, and how these messages are interpreted.

The aim of the current study was to explore the relationship between perceived messages from sexual partners and body image in men. Of particular interest was the nature of the messages that men receive about their bodies from their sexual partners, and how these messages influenced men to change their weight and shape. A qualitative methodology was adopted to obtain a detailed understanding of the nature of the messages that men reported they received from their partners.

2. METHODS

2.1. Participants

Of the 38 men who participated in this study, 22 were aged 51 to 60 years and 16 were aged between 41 and 49 years. A requirement of the study was that men were currently engaged in a heterosexual relationship. Nine of these men were in a steady relationship, 14 were married for the first time, six were remarried, eight were either divorced or separated, and one was widowed. Twenty two of the participants were employed in a professional/managerial capacity, three worked in education, four were employed in the health sector, five were retired, and two were employed in sales. Twenty five of the participants listed their country of birth as the United States of America, and 13 were born in Australia. Although it was not possible to formally analyze the difference between the responses of men from the United States and Australia, the manner in which the themes clustered for the two groups did not demonstrate any apparent difference in the responses of men from the two countries.

2.2. Materials

The open-ended interviews consisted of eight questions relating to perceived messages about their body received from participants' sexual partners, the impact of these messages on their body image, as well as questions relating to men's perceptions of the sociocultural pressures on males to conform to idealized standards of the male physique. The questions were developed from the literature and were designed to examine the perceived influence of partners on a man's body image and his body change strategies. The questions are listed in the Results section. Men were encouraged to expand on their answers and provide additional information to support their initial response.

2.3. Procedure

Approval to complete the study was obtained from the University Human Ethics Committee. Participants were recruited by placing information about the study on a range of web sites in United States of America and Australia. Fifty websites were located in each of these two countries, information about the study was provided, and men were asked to complete an anonymous questionnaire about the types of messages they perceived their partner's communicated to them about the body. It was a requirement for the study that the men were between 40 and 60 years of age, and were currently engaged in a relationship. Men were asked to answer the open-ended questions on their body image on line. Participants were advised that participation was voluntary and that they would be able to withdraw from the study at any time. There was no reimbursement for participation in the study. Men were asked to provide their height and weight so that their Body Mass Index (BMI) could be calculated. Men also provided information on their age, marital status, occupation, and country of birth.

3. RESULTS

Given the importance of BMI on body dissatisfaction, respondents were organized into three BMI groups: BMI less than 25 (normal weight), BMI 25 to 29.99 (overweight), and BMI 30 + (obese). There were eight participants in the normal weight group, 16 participants in the overweight group, and 14 participants in the obese group. For each of the eight questions, the results were organized into the themes that emerged for respondents from each of the three BMI groups. Responses were read by the two authors, and they independently determined the themes that emerged from each question. The themes from each of the questions were considered separately. A grounded theory approach [18] was used to guide the

interpretation of the data due to the limited previous research on partner influence on male body image. Within this approach pre-coded categories are not used. It is the categories that emerge from the narratives that are explored and subsequently coded. The authors then met to discuss the concordance or otherwise of the themes that they determined had emerged from the data. There was a high level of concordance in the nature of these themes, since the questions largely shaped the nature of the themes that emerged from the data. Discussion between the two authors and some re-reading of the transcripts resulted in an agreement on the themes that are reported in this paper. The main themes for each of the questions are included in **Table 1**.

Question 1: *Do you receive any feedback from your partner regarding the size and shape of your body*?

Normal weight Group

The perceived feedback from partners (both female and male) was mostly positive for the men in this group, with some men perceiving they received complimentary feedback from partners about their bodies, particularly regarding certain aspects of their bodies such as their abdominal muscles, their buttocks, and their overall muscle tone.

I do receive feedback from my partner. *Usually it is positive as I am in good shape and I exercise regularly*. (*PB, Age Range*: 41-50, *Married*)

However, even men who were of normal body weight and who perceived positive feedback from their partners were concerned if they perceived that they did not live up to the muscular male body ideal.

She says that she likes my body and the way I look. She makes jokes sometimes, and that makes it hard. She'll say that she likes this other guy's body, because it is so muscular and he's so strong. When she says this, I feel bad that I'm not as muscular and strong. (*PM, Age Range*: 41-50, *Steady Relationship*)

Overweight Group

Most of the men's partners in this group were perceived complimentary about their partners' bodies, or were encouraging of their partners' strategies to get back into shape (e.g., dieting). The majority of the participants in this group indicated that they were happy with their bodies.

Yes—they think I'm hot. The things I might be most self-conscious about are what turn them on the most (*my beer belly and being very hairy*)! (*MH, Age Range*: 51-60, *Single*)

My wife generally allows me to take care of what's mine with minimal interference (*and appreciates when I do the same*). *Even so, she's commented that she thinks I'm very handsome, likes that I'm tall, has seemed satisfied with my weight at all points in our relationship*. (*CC*: *Age Range*: 51-60, *Married*)

Obese Group

Comments from partners were perceived to be encouraging of good health rather than being directed at achieving the muscular male ideal. Participants reported that even when body change strategies were warranted, partners were perceived to be encouraging of their efforts rather than disparaging.

Yes, my wife of 40 *years tells me I'm in great shape.*

Table 1. Main themes identified by men in different weight categories.

Theme	Normal weight *n* = 8	Overweight *n* = 16	Obese *n* = 14
Positive feedback	6	14	10
Concern about muscular ideal	2	-	4
Negative feedback	-	-	2
Encouragement to lose weight	4	2	8
Encouragement to build muscles	3	6	5
Encouragement to be healthy	5	11	10
Teasing	1	3	7
Encouragement to change shape of body	2	4	4
More pressure on men now compared to previously	7	14	13
Pressure coming from media	7	14	13
Pressure coming from friends	1	4	4
Positive body image	7	13	10
Negative body image	1	3	4

When I start to gain a bit too much, she mentions it, but in a way that she tries to make sound off-hand. (*HM, Age Range*: 51-60, *Married*)

My partner and I often talk about our diet and what we should eat. When I do lose weight my partner is full of praise for me and we both try to stop me from putting on the weight again. We normally attend a gym 3 *times per week.* (*CJ, Age Range*: 41-50, *Steady Relationship*)

However, two men in this category reported perceiving negative feedback about their bodies from their partners, and one participant indicated that his partner did not comment on his body.

I am overweight and she tells me so. (*PB, Age Range*: 41-50, *Remarried*)

Question 2: *Does your partner encourage you to lose or gain weight*?

Normal weight Group

Four participants in the normal weight category indicated that they did not perceive encouragement from their partners to lose weight. Partners of the men in this category were generally perceived to be supportive of the way they looked.

My partner does not complain about my weight and therefore does not encourage me to either gain or lose weight. (*PB, Age Range*: 41-50; *Married*)

Overweight Group

Men in the overweight category reported either perceiving no comments from partners to lose or gain weight, or positive encouragement to maintain a healthy lifestyle, or to lose weight. Men reported that their partners did not apply pressure for them to lose weight, but instead were seen to encourage them to maintain a healthy concern about their body weight that was more related to health and fitness than to the way they looked.

She is encouraging if I say that I plan to stop eating sugar and some carbs (*doughnuts for instance*), *but she does not pressure me if I am not already interested.* (*LC*: *Age Range*: 51-60, *Remarried*)

Obese Group

Men in the obese weight category perceived more encouragement to lose weight than the men in the lower weight categories. This encouragement was focused on health concerns rather than partners wanting their men to achieve the ideal male body.

She's always trying to get me to eat healthier, sometimes with success. I think her main motivation, however, is health, not appearance. (*HM, Age Range*: 51-60, *Steady Relationship*)

Question 3: *Does your partner encourage you to become more muscular*?

For most men in all three weight categories, the incentive to build muscle seemed to come mostly from themselves rather than from their partners. Similar to the encouragement from partners to lose or gain weight, encouragement or perceived encouragement from partners to gain muscle seemed to have more to do with the maintenance of health, than the pursuit of body image ideals.

Normal weight Group

No, never. I have always been drawn to looking more muscular on my own, so no one has ever been inclined to tell me this. (*SJ, Age Range*: 41-50, *Married*)

Overweight Group

No. When I point out various male body types in the media (*more muscular and/or defined than I am*) *and ask if I should attempt to attain that shape, I am usually told I am fine as I am currently, but if I want to change I should do what makes me happy*. (*MA, Age Range*: 41-50, *Married*)

Obese Group

She likes that I have good muscle definition, but has never encouraged me to lift weights or do anything that has the sole purpose of making me more muscular. She says that really muscular men look gross. (*HM, Age Range*: 51-60, *Married*)

Question 4: *Does your partner tease you about the size and shape of your body? Please give details*.

The majority of men, in all three weight categories, reported no teasing from partners regarding the size and shape of their bodies. Overall, partners were perceived to be supportive of their men in terms of body image. More men in the obese group perceived they were teased than in the other two weight groups.

Normal weight Group

My partner's comments about my body are positive, e.g., that I have strong legs etc. She even boasts to her family that while most men my age are becoming soft, that I am in the best shape ever. (*PB, Age Range*: 41-50, *Married*)

Overweight Group

Sometimes she will remind me that I was in much better shape when we were younger, but then she was too. So she does not give me a hard time. (*LC, Age Range*: 51-60, *Remarried*)

Obese Group

Gentle teasing, but we both are in good shape considering our age. We both are fairly attractive physically, according to our friends. (*E, Age Range*: 51-60, *Remarried*)

Question 5: *In what ways does feedback from your partner influence you to change the size or shape of your body*? (*i.e., increasing exercise, eating less, eating more, taking supplements, using steroids, etc.*).

Men generally reported perceiving very little feedback from their partners to change the size or shape of their bodies, and when there was feedback it was mostly posi-

tive. A common theme among the three BMI groups was self-motivation to get into/stay in shape, rather than wanting to change their shape for someone else. However, a number of men reported being influenced by their perceptions of their partners' opinion of their bodies, even if their partner's opinion was not voiced directly. None of the men, in any of the three BMI groups, reported using steroids to change their body shape.

Normal weight Group

Since she says nothing either complimentary or negative, I do what I think I need to do for good health. I do it for myself and to look good among other men. (*HL, Age Range*: 51-60, *Married*)

Overweight Group

It does not influence me, although I think that it is important for a man to feel that he is attractive to/wanted by women. I am happy to receive a positive compliment and my self-esteem gets a boost. (*SB, Age Range*: 41-50, *Steady Relationship*)

Obese Group

I can tell when she's checking me out physically. When she compliments my appearance, that's a big factor influencing me to keep working at it. (*HM, Age Range*: 51-60, *Married*)

Question 6: *Do you think there is more or less pressure on men these days to be slimmer and more muscular than ever before*?

There was a general consensus from the men in all three BMI groups that there is more pressure on men today to conform to a particular body type, *i.e.*, to be slimmer and more muscular or toned. Men generally thought this pressure came from the media: billboards, television, and movies. Some men acknowledged that this pressure had a negative effect on their own body image, in that they felt they had to try to live up to the unrealistic images that they saw displayed in the media. Others indicated that the pressure 'to be perfect' had the effect of portraying those who do not fit the 'profile' as less than competent or stupid. Some of the men even acknowledged that the pressure on men to conform to a body ideal is similar to the amount of pressure on women to conform to a female body ideal.

Normal weight Group

More! (*pressure*). *I noticed today the models that clothing companies use to advertise underwear – absolutely PERFECT in every way! So even though I know it's bullshit, there is still a part of me that compares what I see in the mirror with what I see in the media and thinks that I have to live up to it.* (*NB, Age Range*: 41-50, *Divorced/Separated*)

Overweight group

I feel that due to reality TV shows like Survivor and The Biggest Loser there is a standard of male muscularity and fitness to be achieved, and that those with less, i.e., the more overweight and less fit, or less ability to hit that standard, are viewed as liabilities to be eliminated. (*MA, Age Range*: 41-50, *Married*)

Obese Group

Yes. I think there is more pressure on men, particularly younger men, from movies, TV, and magazines. I know that I feel guilty about being a few kilos overweight and that my dad or my uncles would never have felt that way at all. Overweight people are often portrayed as stupid or incompetent or comic. You can't avoid those enormous outdoor billboards with bulging pecs and bulging briefs. (*RM, Age Range*: 51-60, *Married*)

Question 7: *Where is this pressure coming from?* (*i.e., Men's magazines, Television, Movies, friends, other influences, etc.*)

Most men saw the added pressure on today's males as coming from the media, television, movies, and billboards. Some men also indicated that pressure has seen to come from both same and opposite sex friends. A few participants thought that there was pressure exerted on men to be healthier due to media health messages to reduce obesity. Others felt that indirect pressure was exerted on men via images of perfect male bodies displayed in women's magazines.

Normal weight Group

All of the above (*men's magazines, television, movies, friends, other influences*): *part of a total cultural/generational shift. In my opinion, Western culture has reached something of a hiatus, and values espoused are representative of an increasing shallowness in our culture.* (*BP, Age Range*: 41-50, *Steady Relationship*)

Overweight Group

I think the pressure comes mainly from friends, especially the opposite sex. In previous generations, women were more economically dependent on men, and men could attract the attention of women, and status among men, by being financially successful, even if he wasn't especially fit. Now however, women don't need men as much for financial reasons, and so men are starting to compete for attention and status in other ways, such as physical fitness. (*LM, Age Range*: 41-50, *Married*)

Obese Group

I would say that the companies who use advertising use every avenue they can to put their products in front of men. Everywhere you look there are ads to improve the styles. All the models are young, fit, and good-looking. My observations suggest that companies are wasting their money. Health and fitness, I am afraid, is not winning this contest. (*CJ, Age Range*: 41-50, *Steady Relationship*)

Question 8: *How do you think the pressure on men to*

be slimmer and/or more muscular makes you feel about your body?

Answers to this question were mixed. Societal pressure to conform to an ideal body type left a majority of these men feeling quite negative about their bodies, while a minority of others reported positive consequences. In general, men in the normal weight group felt more positive about their bodies than men in the heavier BMI groups, however, this was not always the case. Some lighter men reported body dissatisfaction, and some heavier men reported being satisfied with their bodies. In some cases, the perceived pressure on men to conform to slimmer/more muscular body ideals had quite a profound, negative effect on men's perceptions of themselves and their bodies.

Normal weight Group

I'm aware that I'm relatively 'skinny' (6 *feet and about* 80 *kilos*) *but I feel quite good about my body.* (*LP*, *Age Range*: 51-60, *Divorced/Separated*)

I sometimes feel 'lazy' that I'm not at the gym 3 *days a week working to get rid of that extra* 4 *or* 5 *pounds. My body is not the 'enemy' but media messages keep reminding me that I'm not doing everything possible to be part of that slim, active, fit demographic.* (*WB*, *Age Range*: 41-50, *Divorced/Separated*)

Overweight Group

I'm more self-conscious that I do not have the 'perfect' male body and women do not see me as attractive, and that makes me feel less needed. It is also a factor in my inability to maintain a relationship with a women. (*DN*, *Age Range*: 41-50, *Steady Relationship*)

It makes me feel like I should be in better shape, and have a washboard stomach, and bigger muscles. (*RB*, *Age Range*: 51-60, *Married*)

Obese Group

I feel that pressure. I feel it in the dating scene. However, for me it is more of a health issue, as I have had a triple by-pass and am in need of losing weight to protect and care for my heart. (*BE*, *Age Range*: 51-60, *Divorced/Separated*)

Just fine. I have never been interested in fads or trends and I have never been fashionable, and being me is about all I have time for. (*MJ*, *Age Range*: 51-60, *Steady Relationship*)

4. DISCUSSION

All three BMI groups of men indicated that they perceived mostly positive feedback about their size and shape from their partners regardless of their body weight. There were no obvious differences in the perception of partner comments in terms of marital status. Men in the normal weight group reported receiving complimentary comments about particular aspects of their bodies (*i.e.*, abdominal muscles, buttocks, muscle tone). Men in the overweight category perceived similar complimentary comments, or partners who were encouraging of men's strategies to get into shape (e.g., dieting). Men in the overweight group were also generally happy with their bodies. Positive and complimentary comments from partners were similarly reported by the men in the obese weight category, with partners perceived to be encouraging good health rather than pushing men to attempt to achieve the ideal male body. These results are consistent with Ogden and Taylor's [11] comments that sexual partners may provide a significant source of appearance-related information to men, and that this feedback is likely to contribute to men's perceptions of their body image through criticism or compliments,

Men in the normal weight group reported that their partners were generally supportive of the way they looked. The men in this group indicated that they did not receive pressure from their partners to lose weight, but instead were encouraged by their partners to maintain a healthy concern about their body weight that was more related to health and fitness than achieving the idealized male physique. Men in the obese group generally perceived more encouragement from partners to lose weight than men in the lower weight groups. However, these men also reported that the focus of comments to lose weight from partners was for concerns about health rather than wanting their men to work towards an unobtainable body shape.

These results are inconsistent with previous research by Grogan [9] who found that men were more focused on health than appearances. Further, Sheets and Ajmere [12], who found that dating partners reported telling each other to lose or gain weight: Women were more likely to encourage their male partners to gain weight, while men were more likely to tell their female partners to lose weight. Perhaps the discrepancy between the results from the present study and past research can be explained by age. The majority of men in the present study were aged over 50 years of age and were likely to be in longer-term, more stable relationships than those in Sheets and Ajmere's [12] study, who were drawn from a college population. Age and length of the relationship may be associated with less concern over appearance and more concern with health and well being. Tom et al. [13] found that having a long-lasting relationship reduced the importance of body image dissatisfaction as well as the impact of unrealistic body image ideals.

Men in all three weight categories reported that the incentive to build muscle came mostly from themselves rather than from their partners. Perceived encouragement from partners seemed to be positive and centered around

the maintenance of good health rather than the pursuit of a muscular body. The majority of men across the three weight categories reported no teasing from partners about their size or shape. Some men, however, reported that they experienced teasing from partners, ranging from gentle teasing comments to more hurtful remarks. Men in the obese weight category reported more teasing comments than men in the other two weight categories. Further research in this area is needed in order to gain a clearer understanding of the extent of partner teasing on body image and the ways is which this may influence men to use body-change strategies to change their shape.

The majority of men in this study, across all three BMI groups, agreed that men are under more pressure now to conform to a particular body type, than in the past. Consistent with a previous review of research examining body image across the lifespan [5], most men thought that this pressure came from the media, advertising, billboards, television, magazines, and movies, but not so much from friends or their partner. However, others implicated same and opposite sex friends as conveyors of social attitudes about male body image. Consistent with findings by Grogan [9], most men acknowledged that this pressure had a negative effect on their own body image, in that they felt that they were expected to live up to unrealistic images that are portrayed in the media. These results are consistent with findings with women that highlight the important role played by the media in shaping their body image [9].

Overall, the perceived influence of sexual partners on male body image in this study was positive. Most partners were perceived to be supportive of men's actual body shape and/or weight and did not actively encourage the pursuit of the idealized, slim and muscular male physique. If partners were perceived to encourage body change, the reasons were generally motivated by concerns for health, rather than appearance, and these messages were perceived to be conveyed through gentle encouragement rather than through teasing. Very few men reported teasing comments regarding their bodies from partners. Men who did pursue a more muscular or toned body did so as a result of their own desire to be more toned or muscular, rather then being actively persuaded to conform to the idealized male physique by their partners. The men in this study almost unanimously agreed that men are generally under a great deal of pressure to conform to the V-shape, muscular male body. However, most men also indicated a positive body image. This is in contrast to research with women that has demonstrated a high level of body dissatisfaction [9].

The results of this study demonstrate the association between perceived messages received by adult men from partners and other sociocultural influences on their body image. The findings indicate that men generally feel quite positive about their bodies, and that strategies to change their bodies are primarily motivated by health related concerns.

This finding has important implications for interventions to address body image concerns among men. Given that men have such a strong focus on health related concerns, and they perceive their partners also to be focused on their health, interventions need to focus on changing men's bodies to be more healthy rather than more attractive. This approach is quite different from women, who are more focused on the appearance, rather than the function of their body. It is important to replicate this study with a larger sample of adult men, across a broader range of cultural groups, and to determine if these findings also apply for homosexual men. Information on the ethnic group of the respondents in the current study, or if they lived in the urban or rural locations was also not provided. It is possible that responses may have varied for men from these different groups. In the current study, men ticked an age category rather than reporting their actual age. Future research should obtain the actual age of participants. The sample in the current study was restricted to men who had access to the internet, and so was not representative of many adult men. Future studies also need to determine the level of muscularity of men, since high BMI may be reflective of high levels of muscularity and not obesity in some men. It is not possible to generalize these findings to adult men more broadly, and so it is important to investigate the role of partners on men's body image with a larger, more representative group of men.

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Beneficial effect of reduced oxygen concentration with transfer of blastocysts in IVF patients older than 40 years old

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ABSTRACT

The aim of the present study was to determine the impact of oxygen concentration on implantation, pregnancy and delivery rates in IVF patients older than 40 year old with transfer of blastocysts. Included were 558 women aged 23-45 years old undergoing IVF/ICSI procedures whose embryos were cultured at blastocyst stage under two different oxygen environments (a bi-gas system: 5.6% CO_2 in air and a tri-gas system: 5.6% CO_2, 5% de O_2 and 89.4% N_2). The main outcome measures of this study are implantation, pregnancy and delivery rates. Implantation, pregnancy and delivery rates are found to be reduced in women older than 40 years old. The implantation and pregnancy rates are significantly higher in women older than 40 years old from the 5% of O_2 group, in comparison to the 20% group (25.00% versus 2.70% and 41.38% versus 5.56%; P < 0.05). The deliveries rates were 13.79% and 5.56% in the 5% and 20% oxygen groups respectively (P: NS). The birthweight was similar in both study groups (P: NS). Gestational age was significantly longer in women from the 5% of O_2 group, in comparison to the 20% (36.87 versus 35.87 weeks, P < 0.05). Results indicated that the embryonic culture with 5% of oxygen and transfer of blastocysts in women older than 40 years old improve the results in the *in Vitro* fertilization/intracytoplasmic injection procedures (IVF/ICSI).

Keywords: ART; Blastocyst; IVF; ICSI; Oxygen

1. INTRODUCTION

Embryos from several mammal species, including human, were exposed in vivo to low oxygen concentrations, ranging from 2 to 8% observed in atmospheric air [1-3]. This probably corresponds to an adaptation mechanism, as it is proven that higher oxygen concentrations may be harmful to the embryo [4] by generating reactive oxygen species (ROS) [5-7].

In Vitro fertilization studies (IVF) in mice [8,9]; cattle [5]; sheep [10]; rabbits [11]; hamsters [12]; rats [13]; cows [14] and pigs [15] have demonstrated that when cultured in oxygen concentrations of 5% present a higher viability and a better development to the blastocyst stage.

However, a pioneer study in human embryos showed that cultures *in Vitro* in atmospheric concentration (20%) or reduced (5%) resulted in similar fecundation and preimplantational embryo development processes [16]. Therefore, in several laboratories of assisted reproduction, the culture of human embryos using oxygen concentration of 20% [17] is now a common practice.

Furthermore, a study in which the effect of oxygen over the 2nd and 3rd day of human embryo development was evaluated was unable to find any differences in the pregnancy and implantation rates when 5% or 20% of O_2 was used [17]. This absence of differences in the results obtained might be because the beneficial effect of low O_2 concentrations happen during the late stages of preimplantational embryo development (day 4-6) [18]; however, the addition of antioxidants to the culture media had as a result better rates of implantation and pregnancy when embryos cultured in 5% of O_2 are transferred in the 2nd and 3rd day [5].

The importance of the transfer in blastocyst stage and the concentration of oxygen have been recently recognized [19,20] These studies reported significant increases in the pregnancy and implantation rates when transfers were done in blastocyst stage and when the cultures were made with 5% of O_2 compared to the culture effect in conditions of 20% of O_2.

Physiologically, the uterus provides a nutritional environment different from than in the fallopian tubes. Therefore, embryo transfer in the cleavage stage would

cause homeostatic stress of the embryo and a reduction in its implantatory potential [21]. Consequently, the transfer in blastocyst stage would allow a better synchronization with rhythm of uterine contractions and the embryo [22,23].

However, there are contradictory results in studies where the human embryos cultured in reduced (5%) or atmospheric oxygen concentrations (20%) are compared. Thus, no improvements in terms of pregnancy and implantation rates were observed if the transfer was performed in the 3rd day of the development in terms of pregnancy and implantation rates [17,20,24]. Similar results have been observed if transfer was done into blastocyst stage (day 5) [24-27].

The maternal age is an important factor to be taken into account in studies on. In fact, there has been a decrease in the women's fertility from 35 years old [28,29], being this reduction significant from the 40 year olds and over, in women attending a processes of assisted reproduction [30,31].

The Latin American Registry of Assisted Reproduction (REDLARA) reported in 2006 a clinical pregnancy rate of 39.6% in patients ≤ 34 years old, 32.8% in patients from 35 to 39 years old and 18.6% in women ≥ 40 years old respectively [32]. In older women there is commonly a reduction in the ovarian follicular reserve and a greater prevalence of chromosomal alterations in the oocyte, which lead to a significant reduction in the implantation rates [33,34] and high rates of miscarriages [35,36].

In the studies comparing the effect of different oxygen concentrations in the embryo cultures, it has not been taken into consideration the maternal age impact when blastocysts are transferred in the programs of assisted reproduction [19,25,27,37].

In a recent publication Kovačič *et al*. [26] did not find improvements in the implantation rates in older women over 40 years of age whose embryos were cultured with oxygen at 5% as compared cultures at 20% of O_2 and embryo transfer in the 3rd day. It is possible that effects of low oxygen concentration may be observed if embryos are transferred in the 5th or 6th days.

We hypothesize that reducing the percentage of oxygen to 5% in the embryo culture systems would have a much more beneficial effect than the usage of oxygen at 20%.

The objective of this study was to evaluate in an IVF/ICSI program, the relationship between the pregnancy and the implantation rates with maternal age whose embryos were cultured in 5% of O_2, compared to those women whose embryos were cultured at 20% of O_2. In addition, the results of pregnancies were assessed.

2. MATERIALS AND METHODS

2.1. Patients

This is a retrospective non randomized study based on secondary analysis of data obtained from 558 cycles of IVF and ICSI at the Laboratories of Assisted Reproduction of Pranor Group (Lima, Peru) between January 2007 and June 2009. This study was approved by the Institutional Review Board (IRB) at the Concebir Clinic (Lima, Peru).

The study group were those gametes and embryos cultured at 37˚C in an atmosphere of 5.6% CO_2, 5% of O_2 and 89.4% N_2 (341 cycles); and a control group of those gametes and embryos cultured at 37˚C and an atmosphere of 5.6% CO_2 in air (20% O_2) (217 cycles). The same kind of incubators (Thermo Scientific, USA) was used for the bi-gas and tri-gas systems.

2.2. Ovarian Stimulation and Oocyte Collection

The patients were submitted to a controlled ovarian stimulation with Leuprolide Acetate (Lupron®, Abbott Laboratories) or Ganirelix (Orgalutran®, Organon) in combination with Recombinant FSH (Puregon®, Organon Laboratories) or HMG (Humegon®, Organon Laboratories) according to the established protocols. The follicular growth was monitored by ultrasound and the ovulation was induced by applying Human Chorionic Gonadotropin (hCG) (Ovidrel® 250 ug, Serono Laboratories or Pregnyl® 10,000 UI, Organon Laboratories). The follicular aspiration was made 34 to 36 hours after giving the hCG. The insemination or ICSI procedure was made-5 hours after the oocyte recovery.

2.3. Semen Samples

The semen samples were obtained by masturbation of every patient's male in aseptic conditions. After the liquefaction process, the motile spermatozoa were recovered from the seminal plasma by centrifugation through Isolate gradients of 45% and 95% (Irvine Scientific, USA) for 10 minutes at 300 × g. the recovered spermatozoa were washed in Sperm Washing Media (Irvine Scientific, USA). In oligospermic samples the spermatozoa were washed in Sperm Washing Media and then placed in 10 µL drops of HTF-Hepes + 10% SSS for the ICSI.

2.4. Fertilization and Embryo Culture

In each one of the evaluated groups, the embryo culture media and mineral oil were prepared and used according to the specifications of the company. The CO_2 concentration in the incubators was of 5.6% and resulting pH

was approximately 7.30 in all the culture media.

The aspired oocytes were washed in a HTF-Hepes medium (IVFonline, Guelph, ON, Canada) supplemented with 10% vol/vol of SSS (Irvine Scientific, USA) and cultured in a 200 µL drop of HTF medium + 10% SSS under mineral oil at 37˚C for 5 hours before the insemination or ICSI procedure.

The insemination was made with 50,000-100,000 motile spermatozoa in 200 µL drop of HTF medium + 10% SSS, where from 1 to 5 oocytes were placed. In the cases of ICSI, the oocytes in metaphase II were injected in every patient by using methods previously described (38). After the insemination or ICSI in 0 day, all the oocytes were cultured up to the evaluation of the fertilization at 37˚C.

The fertilization was evaluated 16-18 hours post insemination or ICSI by the presence of two pronuclei and two polar bodies (day 1). The zygotes with two pronuclei were cultured individually, under mineral oil, in 10 µL drops of Global medium (IVFonline, Guelph, ON, Canada) supplemented with 10% vol/vol of SSS (Irvine Scientific, USA) from day 1 to day 3. On the 3rd day, the embryos were changed to 10 µL drops of fresh Global medium + 10% SSS and cultured 2 or 3 days more up to the transfer day in blastocyst stage. Therefore, the transfer was made in 5 or 6 days.

2.5. Embryo Transfer

The embryos were transferred in blastocyst stage, being the average of 1.96 and 2 embryos transferred in the group of 5% and 20% of O_2 respectively ($P < 0.05$ among the evaluated groups). In the 5% of O_2 group, 16 patients received 1 embryo, 324 received 2 embryos and 1 patient received 3 embryos. In the 20% of O_2 group, 4 patients received 1 embryo, 209 patients received 2 embryos and 4 patients received 3 embryos (**Table 1**).

The embryos that were not transferred were cryopreserved or eliminated according to their morphology. The embryo transfer was made with a Frydman Ultrasoft catheter (CCD Laboratoire, Paris, France) that was previously washed with a culture medium. The catheter was completely filled with culture medium and the embryos filled in the last 10 µL of the catheter medium. All the transfers were made according to the methods previously described by Mansour [39].

The biochemical pregnancy was determined approximately 12 to 14 days after the embryo transfer by measuring the Human Chorionic Gonadotropin beta subunit (hCG-b) in blood. The clinical pregnancy was determined by the presence of the gestational sac and the heart beat which were evaluated by ultrasound at the 21st and 28th days post transfer respectively.

2.6. Statistical Analysis

Data were statistically analyzed using the χ^2 test and Student's t-test as appropriate and differences were considered to be significant at $P < 0.05$. All statistical analysis was carried out using the statistic package Stata 10 (StataCorp, College Station, TX).

In this study, the cycles were organized in 3 segments according to the age of the patient: < 35 years old, 35-39 years old and ≥ 40 years old. The normal fertilization rate was calculated from the number of zygotes with two pronuclei of IVF and ICSI divided by the number of mature oocytes inseminated by 100. The rate of implantation was calculated dividing the number of gestational sacs observed by ultrasound at the 21st day post transfer divided by the total number of embryos transferred by 100. The rate of clinic pregnancy was calculated from the number of patients with at least one gestational sac divided by the total embryo transfers by 100. The abortion rate was defined as the number of pregnancies with total loss of the gestational sacs before the 20 weeks of gestation between the numbers of pregnancies by 100.

3. RESULTS

A total of 558 cycles in which the embryos were cultured under two different O_2 environments were evaluated; embryos of 341 and 217 cycles were cultured in 5% and 20% of O_2 respectively. The age of the patients was similar in both evaluated groups (P: NS). The fertilization rate was similar in the 5% of O_2 group versus the 20% in each age group evaluated in this study (**Table 2**).

Table 1. Characteristics of the two study groups whose embryos were cultured in 5% or 20% of O_2.

	5% O_2	**20% O_2**
Cycles	341	217
Age (y)		
Range	25-45	23-45
Mean ± SE	34.47 ± 0.20	34.33 ± 0.26
Indication[a]		
Tubal Factor	37 (11)	16 (7)
Other female	161 (47)	95 (44)
Male Factor	48 (14)	29 (13)
Multiple Factor	88 (26)	72 (33)
Unexplained	7 (2)	5 (3)
Procedure Class [a]		
Standard IVF	194 (57)	96 (44)
ICSI	147 (43)	121 (56)

Data are Mean ± Standard Error; [a]Values in parentheses are percentages of the total number of patients; P: NS.

Table 2. Implantation rate, pregnancy rate, abortion rate and birth rate by age group in cycles with embryos cultured in atmosphere of 5% and 20% of oxygen.

	< 35 years		35-39 years		≥ 40 years	
	5% O_2	**20% O_2**	**5% O_2**	**20% O_2**	**5% O_2**	**20% O_2**
Cycles	169	108	143	91	29	18
Fertilization rate (%)	83.03	79.78	84.04	78.16	83.52	81.60
Transferred embryos	1.98 ± 0.01	1.99 ± 0.02	1.93 ± 0.02[b]	2.00 ± 0.02	1.93 ± 0.05[a]	2.06 ± 0.01
Implantation rate (%)	34.63	35.81	26.09	26.92	25.00[a]	2.70
Clinical Pregnancy rate (%)	50.89	51.85	42.66	42.86	41.38[a]	5.56
Abortion rate (%)	5.92	7.41	7.69	10.99	17.24[c]	00.00
Birth per transfer rate (%)	42.59	44.44	32.12	31.87	13.79	5.56[d]
Ongoing pregnancy	7	0	6	0	3	0

Data are Mean ± Standard Error; [a]P < 0.05 compared to the average in patients ≥ 40 years old from the 20% O_2 group; [b]P < 0.05 compared to the average in patients of 35-39 years old from the 20% O_2 group; [c]P < 0.05 compared to the average in patients of < 35 years old from the 5% O_2 group; [d]P < 0.05 compared to the average in patients of < 35 years old from the 5% and 20% O_2 group.

The patients ≥ 40 years old from 5% group of O_2 had implantation and pregnancy rates significantly higher compared to those patients from 20% of O_2 group (P < 0.05). Furthermore, these patients older than 40 years old from the 5% of O_2 group received a significantly less number of embryos transferred, compared to the patients from 20% group (P < 0.05). The group of patients < 35 years old and of 35-39 years old had similar implantation and pregnancy rates (P: NS).

Women ≥ 40 years old from 5% of O_2 group had a higher abortion rate compared to women < 35 years old from the same study group (P < 0.05). However, in the group of patients whose embryos were cultured in 20% of O_2, the older women (≥ 40 years old) had a lower delivery rates in comparison to women < 35 years old in both evaluated groups (5% and 20% of O_2) (P < 0.05). In women ≥ 40 years old from 20% of O_2 group there was only 1 pregnancy out of 18 transfers, which resulted in a healthy born baby. In the 5% of O_2 group there were 12 pregnancies from which 5 women had an abortion before the 20th week of gestation, 4 had a normal delivery and 3 pregnancies have a normal development. The delivery rate was similar in women older than 40 years of age in the 5% of O_2 group compared to the 20% of O_2 group (13.79% vs. 5.56%; P > 0.05).

The pregnancy rates according to the kind of procedure of IVF or ICSI were similar among both procedures in the evaluated groups (5% and 20% of O_2) in women < 35 years old, 35-39 years old and older than 40 years old. Less pregnancies were achieved in women ≥ 40 years old when their embryos were cultured in a 20% of O_2 atmosphere, independently from the kind of procedure of IVF or ICSI, in comparison to the group of women < 35 years old and of 35-39 years old (P < 0.05) (data not shown).

The percentage of embryos that reached the blastocyst stage in relation to the total number of fertilized oocytes is show in **Table 3**. There were no differences in the embryonic blastulation rate among patients from the 5% and 20% of O_2 group; these percentages were equally similar in relation to the age of the patients. Furthermore, there was no difference in the pregnancy rates according to the kind of controlled ovarian stimulation in both evaluated groups in this study (**Table 4**).

The data about the gestational age at delivery and birth weight from both groups evaluated in this study is shown in **Table 5**. There were 87 deliveries in the 5% of O_2 group and 78 deliveries in the 20% of O_2 group. However, it was only possible to register information from 39 and 60 in each group respectively. Gestational age was higher in women whose embryos were cultured in reduced concentrations of oxygen, in comparison to those women whose embryos were cultured in atmospheric concentrations of oxygen (P < 0.05) (**Table 5**).

Table 3. Blastulation rate according to age in both study groups.

	5% O_2	**20% O_2**	**P**
Cycles	341	217	
No. of embryos (2PN)	2281	1612	
< 35 years old	39.82%	37.59%	0.487
35-39 years old	36.11%	39.69%	0.348
≥ 40 years old	34.01%	31.37%	0.756
Total	37.97%	37.97%	1.000

P: NS

Table 4. Pregnancy rate according to the protocol of ovarian stimulation with agonist or antagonist from the GnRH (GnRHa –GnRHant) and the recombinant FSH (rFSH) or human menopausal gonadotropin (HMG) in the study groups.

Stimulation Protocol	5% O_2	20% O_2
GnRHa + rFSH	60.00%	36.84%
GnRHa + HMG	50.00%	40.00%
GnRHa + rFSH + HMG	45.45%	53.33%
GnRHant + rFSH	50.43%	41.67%
GnRHant + HMG	55.00%	50.00%
GnRHant + rFSH + HMG	48.21%	60.87%
rFSH	27.27%	43.75%
HMG	57.14%	40.00%
rFSH + HMG	35.71%	38.89%

P: NS

Table 5. Gestational age and birth weight in the study groups.

	5% O_2	20% O_2	P
Biochemical pregnancy	5	4	
Clinical pregnancy	159	96	
Total delivery	87	78	
Deliveries with recorded data	39	60	< 0.05
Gestational age (weeks) (Mean ± SE)[a]	36.87 ± 0.30	35.87 ± 0.37	
Bithweight of newborns (gr.) (Mean ± SE)[a]	2816.26 ± 92.88	2752.96 ± 69.05	NS

[a]Data are Mean ± Standard Error

4. DISCUSSION

Although human embryos can develop successfully in atmospheric concentrations of oxygen (20%), some authors have suggested that low oxygen concentrations (5%) resemble the physiological conditions of the uterus effectively, and thereby improve the quality, viability and embryo morphology [40,41].

An important result of this study was to find that culturing embryos at reduced concentrations of O_2 (5%) is beneficial to patients ≥ 40 years old who perform assisted reproductive procedures with their own oocyte; these are the ones who achieve significantly higher implantation and pregnancy rates compared to those patients of similar age whose embryos were cultured under atmospheric oxygen concentrations (20%) (25.00% versus 2.70%; 41.38% versus 5.56%, respectively, P < 0.05). The implantation and pregnancy rates observed in women > 40 years old were similar to those seen in women < 35 years old and 35-39 years old (P:NS).

Meintjes *et al.* (20) embryos cultured in a 5% O_2 environment consistently resulted in higher rates of live birth implantation and live births when compared with rates among women whose embryos were cultured in an atmospheric O_2 environment.

Furthermore, Nanassy *et al.* [27] cultured human embryos under oxygen atmospheric conditions (20%) until the 3rd day and then cultured the embryos in 5% and 20% of O_2 from the 3rd to the 5th day of development without finding beneficial effects of 5% of O_2 in the advanced stages of preimplantational embryo development. These results suggest that the beneficial effect of hypoxia on embryonic development would be along all stages of *in vitro* cultures even from the oocyte before fertilization up to the blastocyst stage [42], which had previously been observed in mice [8,43] cattle [44], rabbits [45] and pigs [46].

Culturing embryos in 20% of O_2, Karagenc [7] showed damage mainly in the embryonic inner cell mass (ICM). Similarly, Rhesus monkey embryo cultured *in vitro* in 20% of O_2 showed the ICM morphologically disorganized, diffuse, with few vacuolated cells, unlike the blastocysts with large and compact ICM cultured in low concentrations of O_2 [47].

Rho *et al.* [48] culturing bovine embryos has shown that low concentrations of oxygen produce higher rates of cleavage and blastocyst stages compared to embryos cultured in 20% of O_2. Also in mice there is a better development to blastocyst stage, bigger number and size of ICM, and gene expression profile similar to those observed in embryo *in vivo* [49].

The discrepancies between the data obtained in animals and humans could be explained based on the differences in the embryo physiology of each species and a variety of culture conditions and embryo transfer in the laboratory [47]. Beneficial effects of culture in 5% of O_2 have been demonstrated in animals where embryo transfers routinely occur in blastocyst stage [7,50].

Dumoulin *et al.* [18], Pabon *et al.* [8], Quinn and Harlow [43] suggested that the beneficial effect of O_2 in physiological concentrations should be observed in cultures extended to blastocyst, which are nowadays common in assisted reproduction laboratories.

Several studies report increases in pregnancy and implantation in blastocyst transfers compared to embryo transfers on the 3rd day [51] and others report significant increases only in implantation rates [52], which considering the results of this study, would have beneficial effects in those patients over 40 years old.

The culture up to the blastocyst would allow to choose

in a more "natural" way embryos with greater potential for development and implantation, however, this selection would also depend on the O_2 percentage in the systems in which the embryos are cultured [47] and the oocyte origin associated to the patient's age [53,54].

There are many authors who have showed the relationship between chromosomal abnormalities, maternal age and embryo morphology [35,55-59]. Munné *et al*. [54] performed genetic diagnosis for 9 chromosomes [13,15-18,21,22, X, Y) in > 6000 embryos and found that women < 35 years old with good quality embryos had 44% of euploid embryos and that this percentage decreased to 21% in patients ≥ 41 years old. Beside, in patients with poor morphology embryos only 30% and 12% were euploid embryos in the group of women > 35 years old and ≥ 41 years old respectively. It has also been shown that chromosomal abnormalities in human oocytes are common and that these aneuploidies are closely related to maternal age, exceeding 60% in women over 40 years old [60].

Since older women have a higher incidence of oocytes and embryos with aneuploidy [54,60] but with similar rates of embryonic blastulation than young women (≤ 35 years old, 35-39 years old; see **Table 3**) as it has been demonstrated in this study, we should expect similar pregnancy and implantation rates independently from the oxygen concentration under which cultures *in vitro* are performed, but in this study we found that low concentrations of oxygen (5%) are achieved significantly higher pregnancy and implantation rates in patients ≥ 40 years old, compared to the results in conditions of atmospheric concentrations of oxygen.

These results might be caused by high concentrations of oxygen that would affect the embryonic inner cell mass [7], an effect that would be even more noticeable because of the high incidence of aneuploidy observed in oocytes and embryos in older women.

In this study there were 87 births in the 5% of O_2 group and 78 births in the group of 20%, being able to obtain information of their results, in terms of gestational age and birthweight, in 39 and 60 cases respectively.

Within the data obtained, a significantly higher gestational age was observed in the group of patients whose embryos were cultured under 5% O_2 compared to the 20% of O_2 group (36.87 ± 0.30 vs. 35.87 ± 0.37; $P < 0.05$) which could be a consequence of preimplantational embryo development in more physiological concentrations of oxygen to which the embryos were subjected during *in vitro* culture, but we believe that further studies are needed to determinate the possible relationship between the culture in hypoxic conditions and obstetric characteristics of pregnancies.

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Sexual assaults in therapeutic relationships: prevalence, risk factors and consequences

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ABSTRACT

A law has been passed in Germany (paragraph 174c StGB), which prohibits therapists from having sexual contact with their patients. This provides the background for a follow-up survey to the previous study completed by Becker-Fischer and Fischer in 1995. The results of this survey are discussed here on the basis of the current status of research concerning prevalence and risk factors of sexual assaults in therapeutic relationships. The focus of the research lies in determining the specific conditions of sexual assaults in psychotherapy and psychiatry, risk variables of the therapists and patients, the effects it has on the patients as well as the legal consequences it results in. To ensure the comparability of the data, an online version of the Questionnaire about Sexual Contacts in Psychotherapy and Psychiatry (SKPP; Becker-Fischer, Fischer & Jerouschek) was created and a survey of *N* = 77 affected patients was conducted. The majority of the participants in the study reported a serious decline in their overall well being following the incident. However only very few undertook legal steps - only in three cases did it come to a legal procedure. The assumption that sexual contacts in psychotherapy result in extremely damaging consequences to patients, was affirmed. Despite the changed legal situation, therapists in Germany are still not held legally responsible more often than they were 10 years ago. Based on these results a more intensive education of the patients concerning their legal rights is recommended.

Keywords: Sexual Assaults; Psychotherapy; Patient Abuse; Professionale Misconduct

1. INTRODUCTION

1.1. Prevalence

According to the background of the current research situation, it can be assumed that sexual assaults of therapists on patients are not isolated cases. On average 10% of the questioned male therapists admitted to having had sexual contact with a patient at least once [1]. However the prevalence rates fluctuate according to the different definitions of what constitutes sexual assault in therapy. When therapists were questioned on average every second [2-7] up to every fourth [8,9] said that he or she has treated at least one patient that had been exposed to sexual abuse in an earlier psychotherapy. Considering the specific problems inherent in determining the prevalence of professional sexual abuse, Becker-Fischer and Fischer [10] assume that there are at least 300 patients, whom this concerns, per year in Germany alone (not including the forms of therapy not accepted by health insurance).

All previous research on the subject shows that most of the victims of sexual abuse in psychotherapy and psychiatry are women and most of the perpetrators are men [3,11,12]. The therapists are on average 10-15 years older than their female victims [3,11,13-16].

1.2. Risk Factors

Next to their being male, several other characteristics of abusing therapists, which count as risk factors for sexual abusive behavior to patients, are listed in the relevant literature: the therapists are often respected [13,17], professionally experienced [11,18], active in their own private praxis [16,19,20], currently facing difficult life situations [18,21,22], have narcissistic deficits [23-25] and/or have themselves been victims of earlier traumas [27,28]. Based on the results of their survey from the middle of the nineties, Becker-Fischer and Fischer differentiate [10,29] between the abusing therapists - whose personality is determined by decomposition phenomena in the loosest sense according to the authors - according to psychodynamic aspects. Based on the assumption that

sexual assaults are repetitions of traumatic events from the therapist's childhood, differentiation is based on the subconscious motivation for their actions: in the case of wish fulfillment the behavior determining motivation is the denial of the traumatic experience. The denial shows itself in the illusion of a perfect world and the need to be saved by the patient. The actions of the revenge type are motivated by the denial of the traumatic experience and the helplessness experienced in childhood identifying themselves with the former perpetrator. The desire for revenge is then stilled by abusing the patient.

1.3. Consequences for the Patients Concerned

The consequences of professional sexual abuses for the patients are consistent in all international literature: all empirical studies that are available to date show very negative consequences for the victims [14,16,30-35]. Named are symptoms such as stronger distrust, isolation, feeling of shame and guilt, fear, depression and suicidal tendencies, anger and symptoms of posttraumatic stress disorder [36]. Pope [37,38] conceptualizes the consequences of sexual contacts in therapeutic relationships with the term "therapist-patient sex syndrome". According to him the negative effects of the therapeutic assaults take the form of a distinctive clinical syndrome, which is partially comparable to the rape syndrome, the reaction to incest, child molestation and a posttraumatic stress disorder. Becker-Fisher and Fisher [10,29] coined the term "professional abuse trauma" on the basis of their research, which in its course manifests the consequences of the sexual contact in the therapeutic relationship. According to them a disturbance of the ability to love and to have a relationship can be detected in all the victims. The question of which destructive consequences of sexual contacts in therapeutic relationships happen to male victims could not be answered definitively due to the very low number of cases in the available samples. In the thematically relevant literature it is assumed that males suffer from the same consequences of their abuse as women do, but their socialization makes it harder for them to see themselves as victims.

In many cases of sexual abuse by professionals neither the following therapist [4] nor the patient [3,6,31] takes legal steps against the abusing therapist. Even if in some more recent surveys of patients the percentage of those who do initiate legal steps, is higher [14,34], it must still be assumed that many of those concerned either do not know that they can sue [39,40] or shrink back from doing so, because they are afraid of the strain placed on them by such a procedure [31,39]. Furthermore there is proof that lawsuits do have various disadvantages and difficulties for those concerned [31,32,34], which is partially a result of the delinquent orientated nature of the criminal proceedings [41].

1.4. Objective

The aim of this study was to be a follow-up research of the study conducted in the mid-nineties, that was conducted by the federal ministry for family, seniors, women and youths [10,29]. Due to the results of the latter survey, the paragraph 174c of the German criminal code was introduced, which since 1998 has stipulated that sexual contacts between therapists and patients constitute a criminal offense. As in the earlier study [10,29] people were questioned, who had sexual contact with their therapist during the course of their psychotherapy or psychiatric treatment. The focus of the research was on how the individuals concerned experienced it, the consequences of the sexual contact as well as possible coping measures and legal steps.

2. METHODICAL PROCEDURE

2.1. Data Acquisition

For the first survey [10] concerned people were made aware of the study via announcements in newspapers. Currently the research participants are acquired via the internet. The *questionnaire of sexual contacts in psychotherapy und psychiatry* (SKPP; Becker-Fischer, Fischer and Jerouschek) from the earlier study was conceptualized as an internet survey in order to enable a relatively cost-effective access to an otherwise hard to reach sample [42,43]. The internet is a valid survey tool. In the current follow-up study the recommendations and rules for conducting online surveys were implemented in their entirety [44].

Webmasters of 94 thematically relevant internet sites (e.g. information sites for patients, homepages of psychological counseling services, self-help pages) were asked to post the request for participation in the survey with a link to the questionnaire on their site. Additionally the request was posted in 6 forums.

2.2. Description of the Sample

Of the N = 77 patients 66 were female (85.7%) and 11 male (14.3%). At the time of the interview the subjects were on average 34.82 years old (SD = 11.13; range 15-69). The average age at the time of the sexual contact with the therapist was 28.36 years (SD = 11.2; range 6-63 years). However 10 of the surveyed were minors at the time of the sexual contact with the therapist. Between the time of the sexual contact and the survey on average 6.35 years (SD = 7.48) had passed. For 7 of the questioned less than a year had passed at the time of the

survey and the maximum amount of time, which had passed, was 30 years.

Almost half of the questioned (40.0%) were married at the time of the survey or in a permanent relationship. Most of the participants had a high level of education: 54.8% had their Abitur (German entrance qualification for university) and over two thirds had their Fachabitur (German entrance qualification for a university of applied sciences).

The therapy was begun by the survey participants for different reasons; respective their symptomatic they do not differ from the entire population of psychotherapy patients (**Table 1**).

Even though it was not explicitly asked, overall 44.2% of the entire sample said that they had had at least one earlier experience of sexual violence. 29.2% (also) described sexual abuse experiences from their childhood.

3. RESULTS

3.1. Characterization of the Sexual Violating Therapists Age, Sex and Educational Background

According to the statements of the patients concerned the vast majority of the therapists was male (71.2%). Their average estimated age was 46.9 years (SD = 9.05, range 27-65). The female therapists were on average slightly younger (M = 44.4; SD = 10.15) than their male colleagues (M = 47.9; SD = 8.49).

It was also stated that 55.7% of the therapists had graduated in psychology. In 35.7% of the cases they were doctors, who mostly had the practitioner's title for psychiatry and psychotherapy (47.8%).

In each case n = 14 reported a behavioral therapy (BT) or a depth psychologically based psychotherapy (DP). While in the cases of behavioral therapy and client-centered therapy (CCT) according to Rogers, it was mainly psychologically graduated therapists (BT: n = 11; CCT: n = 5), in those cases where depth psychologically based, psychoanalysis or gestalt therapy was used, the patients were treated in equal shares by doctors or psychologists. 23.0% could not say which type of therapy was employed.

Table 1. Prevailing symptoms and complaints at the beginning of the therapy.

Symptoms/Complaints	%
Symptoms of depression	53,5
Fear and panic	36,6
Problems with boundaries (e.g. Borderline personality disorder)	26,8
Self-injury behavior and auto aggression	23,9
Trauma, without a situation being named	22,5
Trauma after experiences of sexual abuse	18,3
Eating disorders	21,1
Suicidal tendencies	16,9

If the distribution of the different types of therapy and the professions of the therapists in the current sample is put into relationship with the number of therapists from the different schools respectively the different professions at the time of the sexual contact, one can determine whether representatives of different career groups or different types of therapy show a more pronounced tendency to sexually assault patients than others [10]. In 1990 over two thirds of therapists had a psychoanalytical orientation. Nearly twice as many doctors as therapists provided psychosocial services, which were accepted by the health insurance companies [10]. In 2001 however 70% of all those therapists participating in the statutory health insurance were psychological psychotherapists, 20% were medical psychotherapists and 10% were child- and youth psychotherapists [45]. The distribution of the different therapy types was also different from that of 10 years before: 40.1% of the treatments were behavioral therapies, 39.6% depth psychology based therapies, 16.0% depth psychologically and analytically based therapies and 4.3% of the cases were analytical psychotherapies [45].

This finding is in accordance with the distribution of therapy types shown in the present study. 71.2% of all therapies began in 1999 or later. Regarding the therapies financed by health insurance (n = 25; there is no comparative data for the therapies not financed by health insurance) 48.0% of these were behavioral therapies, 36.0% depth psychology based therapies and 16,0% analytical psychotherapies [45]. This is almost the same distribution of therapies as the entire distribution of therapies held in this time frame.

Concerning the professions of the abusing therapists the following picture presents itself: Of the treatments, which began before or in 1990, 50% of the cases were treated by medical and 37.5% by psychological psychotherapists. In those therapies, which began in 1999 or later, 66.0% of the cases were treated by psychological and 29.8% by medical psychologists, which is equal to the shift in participation in the overall statutory health insurance coverage. Therefore no indications of a prevalence of a certain type of therapy or profession (doctors vs. psychologists) could be found in the sample of abusive therapists. Risk factors are more likely to be found in situational circumstances and especially in the personality variables of the therapists.

3.2. Problematic Life Situations

In 39.5% of all cases, when asked about their knowledge of the private life of the people, who were treating them, the subjects stated problematic aspects (see **Table 2**).

The therapists sometimes tried to evoke the sympathy of their patients by referring to their own problematic situation (n = 4) or their loneliness (n = 3).

3.3. Impression the Patients Had of their Therapy and their Therapists

The patients concerned were asked to state their personal impression of their therapists and to describe their looks, charm and personality traits in an open text field. In 44.3% of all cases the therapists were described exclusively with positive personality traits. 31.2% stated a very conflicted impression of their therapists and 21.3% of the therapists were described solely by negative traits.

Overall 58.9% of all answers given stated positive and 41.1% negative aspects regarding looks and personality traits of the therapists (see **Table 3**). Based on all the statements of the questioned, the therapists could be classified according to the types wish-fulfilling (74.0%) or revenge (42.1%).

3.4. Consequences of the Sexual Assaults

In almost 80% of the cases the persons concerned stated that the therapists initiated the sexual contact. Overall 86.5% of the people who participated in the survey stated that the sexual contact with the therapist had negative consequences for them, of these 93.3% state problematic consequences and only three respondents gave no as an answer to this question. Therewith the results of this study fall in line with the results of the long list of studies, which in the last decades have proven the negative consequences of sexual assaults of therapists on patients [16,33,35].

3.5. Intensified and New Complaints

60.0% of the questioned stated that after the sexual contact with their therapist, complaints, which they had already had at the beginning of the therapy, intensified. Overall up to 7 intensified complaints were named (M = 2.27; SD = 1.89). In 66.0% of the cases it was stated that after the sexual contacts new complaints appeared. The average number of new symptoms was 1.53 (SD = 1.33) (see **Table 4**).

A vivid image of the traumatic quality of the abusive experience in therapy is delivered by the patient's assessment captured by the Impact-of-Event scale, which

Table 2. Problematic aspects of the therapist's private life.

Problematic Aspects	Frequency
Divorced/Separated	11
Children from an earlier partnership	9
Problematic Marriage/Partnership	4
Loneliness	4
Stressful experiences in previous life history	4
Financial Problems	2
Other Problems	9

Table 3. Most frequent descriptions of the therapists.

	%
Positive Attributes	
(sexually) attractive	34,4
Motherly/vatherly	27,9
Likable, sympathic	26,2
Competent/respectable	23,0
Emphatic/interested	19,7
Charming/jocular	18,0
Self-confident	13,1
Negative attributes	
unimposing	18,0
(sexually) unattractive	14,8
Domineering, scary	14,8
egocentric, narcissistic	13,1
frightened, helpless	9,8
distanced, critical	8,2

Table 4. Intensified and new complaints as consequences of the sexual contact with the therapist.

Most frequent intensified complaints	Most frequent new complaints
Isolation and emotional retreat (34.6%)	Isolation and emotional retreat (30.0%)
Mistrust (23.1%)	Mistrust (30.0%)
Fear and panic (19.2%)	Fear and panic (10.0%)
Shame and guilt (19.2%)	Symptoms of depression (10.0%)
Self-doubt and uncertainty (19.2%)	Lying/dissimulation (10.0%)
Symptoms of depression (10.0%)	Anger and aggression (10.0%)
Psychosomatic complaints (11.5%)	
Self injuring behavior	

captures the psychotraumatic quality of an event that occurred in the last 7 days. The results showed that 89.2% were traumatized by the sexual assaults. In over three quarters of all the cases (83.8%) a medium to high traumatization took place.

If one takes a separate look at the symptomatology of the male patients, the following picture presents itself: According to the clinical diagnostic findings, 2 of n = 6 male patients were traumatized medium severely and 2 were highly traumatized. There were however 2 males in the survey, who were classified as clinically inconspicuous on the basis of the results of the IES-scale.

3.6. Coping and Legal Steps

About half (54.0%) of the patients concerned needed another psychotherapy in order to deal with the massive consequences of the sexual contact to their previous therapist. In those cases, where no need for a follow up therapy was stated, the given reason for this was a general loss of trust in psychotherapists. 25 of the questioned had already completed a follow-up therapy at the time of the survey. It was judged to be very helpful if the following therapist respected borders (professional abstinence, for the basic rules of follow-up therapy see [29]).

Only very few of the victims of professional sexual abuse considered suing the people who had treated them. Over two thirds (68.8%) stated to have never thought of taking legal steps against their therapist. Mostly this was explained by the questioned as being due to their being afraid of taking these steps or of not having enough courage (n = 5). Also the feeling of complicity stopped them from even thinking of initiating legal steps (n = 4). Three people stated that they saw no point in taking legal action, partially due to either weak evidence or lack of it. In two other cases the patients named the statute of limitations as the reason for not having pursued legal options.

Those n = 15 persons, who stated having thought of taking legal steps against their therapists, said in most cases that their follow-up therapist provided the impulse for this. Public information on the topic "Sexual Contacts in Psychotherapy" provided the impulse for others (n = 5). Another relevant factor was the wish to protect other potential victims (n = 5).

In those n = 5 cases, where legal steps were taken, 3 of those cases were criminal lawsuits and 2 were civil law suits. Thus a formal trial only took place or was to take place in three of the cases. At the time of the survey two of the therapists had already been convicted. The third trial has not been held yet.

4. DISCUSSION

In by far the largest share of cases (71.2%) the abusing therapists were male. This corresponds to the results of all previous surveys conducted with patients and/or therapists. It is remarkable that in statutory health insurance the percentage of practicing female therapists is larger than the percentage of male therapists. For example in 2003 about 66% of psychotherapists were female [46]. However in the current study in 28.8% of the cases the therapists were female. This comparatively large share can be seen as an indication of the growing amount of sexually abusive female therapists [47] or respectively the fact that more of these cases are being reported.

The average age of the therapists was 46.9 (SD = 9.05). The average age of the male therapists does not differ greatly from that of the females. Therefore it is reasonable to assume that in both cases, they are not fresh entrants into the field, but therapists with years of professional experience. This corresponds to the results of the international research literature, which states that most of the abusing therapists are experienced practitioners with years of professional experience [18].

An inadequate training of the therapists is not to be discerned in the current sample: in most of the cases the therapists were either university graduated psychologists or doctors (with the relevant practitioner's title). Furthermore the types of therapy that were named most frequently (behavioral therapy and depth psychology based therapy) are all types that are recognized by health insurance. In most of the cases (69.4%) the therapy was also paid for by health insurance, which means that most of the therapists had Approbation (German therapists license necessary for coverage by health insurance). Therefore the scientific literature conclusively shows no indication that abusive therapists have inadequate training. On the contrary it is reported that they are especially well respected and trained [11,13]. Furthermore no indications were found of a prevalence of a certain type of therapy or profession (doctors vs. psychologists).

About 40% of the patients concerned knew of current problematic situations in the private life of their therapists. In accordance with the international research literature it can be summed up that presumably difficult circumstances in a therapist's life heighten the risk of sexual contacts with patients. This risk factor however has limited impact on repeat offenders [26], whose severe personality disorders are the cause of their behavior.

The patients' evaluations of the looks, charm and personality traits of their therapists show very contradictory findings: A large share of the statements are either concentrated on very positive or very negative aspects. In 31,2% of the cases the questioned described very conflicting personality traits of the therapists. It is remarkable that the share of therapists, who are described as having a very ambivalent character, is relatively high.

Abusing therapists often show dissociative traits [10,29], which is shown by the statements of the surveyed. It can be assumed that the conflicting impression, which the questioned have of their therapist, is less determined by the ambivalent feelings of the patients but rather a result of real decompositions in the personality of the therapist. The fact that current difficult life situations and earlier traumatic experiences are important risk factors of therapists is substantiated by the patients' statements.

The different types of therapists discovered by Becker-Fischer and Fischer [10,29] (wish fulfillment and revenge type) can be verified on the basis of the achieved results. While male therapists show an equal share of wish fulfillment to revenge type, most of the female therapists fall into the category of wish fulfillment. This result can be explained due to the background of society's gender stereotypes: The way that people deal with their own traumatic (childhood) experiences is also determined by their gender. It can be assumed that male victims tend more strongly to identify with the perpetrators and thus use their patients to still their desire for revenge [29]. The female stereotype is more compatible with the need to be saved by patients as is characteristic for the wish fulfillment type.

The described resulting complaints are all part of those of the professional abuse trauma with the leading symptomatic being isolation and emotional retreat, mistrust, feeling of fear and panic as well as depression, a syndrome that already showed itself in the first survey. In total 86.5% of the people who participated in the survey said that the sexual contact with their therapists had consequences for them. 93.3% of these reported problematic consequences. The described symptoms are comparable to those, which have been reported in other studies. The basic disturbance of the capacity for love and relationships, which can be determined by those suffering from professional abuse trauma [29], is clearly shown in the named symptoms.

Almost 90% of the questioned achieved scores that show an impact on a traumatic scale - a result which is especially precarious, because of the fact that patients of psychotherapy overall and especially those who are victims of sexual abuse during therapy have already had prior traumatic experiences. Often this was sexual abuse in their childhood. In these cases the professional abuse trauma stems from a retraumatization, which leads to an increase of negative consequences.

Regarding the consequences of the sexual contacts for male patients it can be stated—although only on the basis of a very small sample—that these basically do not suffer less from the abuse than female victims.

In the current study over two thirds of the questioned stated that they never even thought about taking legal steps. Two thirds of those who had thought about initiating legal steps also did nothing. The justification for this was very consistent: Fear of the consequences of such a procedure, the conviction that they would not be believed as well as the assumption that they were complicit in the abuse. These factors were also described by many of those concerned in other surveys of victims [10,31, 39,50]. Furthermore the emotional bond to the abusing therapist in the current results can be seen as a reason for not initiating legal steps. In one case a civil court case was terminated for this reason.

According to the background of the descriptions of those concerned in our study it is reasonable to assume, that it is less the lacking knowledge of the possibility of initiating legal steps, which leads to those concerned not employing their legal options [40], but rather emotional factors such as fear and hopelessness, which are responsible. Despite the existence of §174c in the StGB the questioned in the current sample only initiated legal steps in n = 5 cases, 3 of which resulted in legal proceedings. Obviously the existence of an applicable paragraph in law does not change much in this regard.

It could be understood from the statements of the participants in the survey that they were filled with a deep mistrust regarding the current legal practices in Germany. Even courts of honor and arbitration boards have the reputation of protecting the therapists in the patients' opinion. The feeling of having caused the abuse or to be at least partially to blame for it, which is not only present in those, who were victims of sexual abuse in psychotherapy, but can also be found in many traumatized people, is not corrected by this situation. In the USA legal options are pursued far more often. A possible explanation for this is the establishment of governmental licensing agencies, which the victims in the USA mostly turn to first, because they are closest to their interests [10]. They are comprised of a mixture of representatives from members of the respective professions and patients and are lead by civil servants, who decide whether or not the license to practice will be revoked. A comparable body does not exist in Germany.

5. CRITICAL APPRAISEMENT OF METHODS

Concerning significance and internal applicability of the results, it must be stated critically that both are dependent on the statements of the patients concerned and their subjective assessment. A parallel survey of the relevant therapists however would for obvious reasons meet nearly insurmountable difficulties. With these limitations of the range of significance there is, according to the authors, no further reason not to view the statements of

the patients as reliable sources of information. The effects of suggestion were eliminated as far as possible in the second survey as well as the first. If one assumes that the only reason for participation is to find a neutral place where one can complain about what happened, then this would lead to a very biased constellation of the sample, for example on the issue of a "tendency to complain". This conclusion however would only be justifiable if at the same time it were assumed that otherwise motivated people were prevented from participating in the survey or were repelled by it, for example people who were "content" with the sexual contact. There is no reason to assume this. Why should people, who were "content" with the sexual contact not have participated in the survey? Even if for example the call for participation in the survey also appeared in conjecture with content, which negatively depicted sexual contacts in psychotherapy or warned people about it, then this context could just as well have wakened the contradictoriness of the allegedly "content" group.

Even for those aggrieved, who have already come to terms with the earlier traumatic experience of sexual abuse in therapy at the time of the survey and whose symptoms have subsided, there are reasons to participate in a survey on this subject. For example the need can exist to use one's own experiences to contribute to making the problem public so that other potential victims can be protected from the potentially traumatic consequences of such an event.

Finally the detailed congruence of the results of both surveys can also be seen as a criteria for the internal validity of the first and the follow up survey. The alternative explanation for this congruence must be deduced from factors, which are based on suggestibility or in the questioning itself, for which there are no indications. What reason would the participants of an anonymous survey have in describing their experiences so negatively, if this negative depiction were incorrect?

Even considering the fact that about 300 new cases of sexual abuse in therapy take place every year, it can be said that a sample size of "only" n = 77 is a good precondition for research in a taboo area. It is certainly a sufficient basis for the conclusions, which were drawn in this article. If the number of participants of the first survey is added to that of the current one, then the sample size of n = 138, which is split into 2 separately surveyed partial samples from different points in time, then according to methodological criteria resilient findings have been achieved.

6. CONCLUSIONS

The problem of sexual assaults of therapists on patients and the disastrous consequences for those concerned persists as a constant phenomenon over time, which was shown by the depicted results. The following consequences are all among those found in the professional abuse trauma. The findings concerning the situational circumstances of sexual abuse in psychotherapy and psychiatry, which were arrived at in the first research done in the mid-nineties, were also confirmed [10,29]. The conditions in which the surveys were made differ. Nevertheless the same stereotype patterns of interaction between the therapists and the patients, the same risk factors of the therapists and vulnerability factors of the patients as well as the same consequences for those concerned were found.

The results suggest a need for more effective information, prevention and help for the people concerned. Especially the German legal praxis has to be rethought regarding aspects such as statutes of limitations, the criteria for reality and truthfulness according to the psychology of statements (see [48]) or the perpetrator oriented nature of many criminal proceedings (see [41]).

Of decisive importance for the prevention of sexual abuse of patients by therapists is the *education of experts and the public* about the problems inherent in sexual abuse in a therapeutic relationship [41]. This includes the permanent integration of relevant thematic content into the curricula of psychotherapeutic education and training. For prevention it is at least as important to educate potential victims, namely the patients of psychotherapy. American authors propose for example leaflets with information about patients' rights as well as ethical guidelines, which contain detailed examples of ethical vs. unethical behavior. These procedural methods would also make sense for Germany. A further contribution can be made by the media by communicating a realistic impression of professional goals and aims as well as the borders of psychotherapy [50,51].

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Cervical cancer screening program based on HPV testing and conventional Papanicolaou cytology for jail inmates

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ABSTRACT

Background: To assess the validity of Human Papillomavirus (HPV) testing in a group of women at high risk for developing cervical cancer, a screening intervention was applied to a population of jail inmates in Rome, Italy. This cross-sectional study provided also new insights on the risk factors and on the HPV genotype distribution. Methods: We have invited 350 inmates to the preliminary stage of the screening program and 98 inmates decided to participate to the study and filled out a questionnaire for the history of attendance to previous cervical screening and for the known risk factors for cervical malignancies. HPV DNA test, conventional Pap smear and HPV genotyping were performed. Results: The percentage of women with High Risk (HR) HPV positivity were 19.3%. The inmates with LSIL/HSIL status showed a significantly higher prevalence of HR-HPV positivity (100% vs. 16.3%; $p < 0.001$) and of multiple HPV types (60% vs. 1.2%; $p < 0.001$) compared to women with normal/ASCUS Pap smear. HPV16 was the predominant genotype in either single or multiple infections. Conclusions: The results indicated that HPV DNA-based approach is a strategy useful for incarcerated women which do not have the opportunity or the social and cultural environment to receive preventive care.

Keywords: Cervical Cancer Screening; HPV; Inmates

1. BACKGROUND

During the last few years, cervical cancer screening programs for women in prison have been carried out in the United States and in Canada [1-5]. Although the results of such preventive care interventions are still not complete and require further work and follow-up, these studies have pointed out the interesting profile of a jail inmate population as an high risk group for developing cervical malignancies. In fact, several socio-demographic risk factors including race/ethnicity, lower level of education and drug or alcohol abuse are responsible for the higher incidence rates of cancer in prisoner women [6,7].

Human papillomaviruses (HPVs) of the high risk types are known to play a causative role in cervical cancerogenesis [2]. HPV infection is correlated primarily with the number of sex partners, but is positively associated also with smoking and oral contraceptive use [8-10].

A number of randomized controlled trials have recently shown that HPV testing for cervical cancer screening shows a higher sensitivity compared with the conventional Papanicolaou cytology [11-13].

These studies have indicated that the prevalence of HPV infection in the general population is strictly related to the age of the women attending the screening [14], with a peak incidence of HPV infection occurring at the age of 16-20, as well as to their socioeconomic status [6].

With the aim to contribute to the assessment of the validity of the HPV DNA testing for cervical cancer screening and to increase the knowledge of the role of the risk factors and on the distribution of the HPV types, we have conducted a preventive care program by parallel HPV DNA test and conventional cytology on a popula-

tion of women prisoners in the "Rebibbia" Female Jail of Rome.

2. METHODS

This cross-sectional study was approved by the Second Faculty of Medicine of "Sapienza" University of Rome, by the Regina Elena National Cancer Institute of Rome and by the "Rebibbia" Female Jail of Rome. The protocol was designated following the recommended guidelines of the following italian scientific societies: SICPCV (Italian Society of Colposcopy and Cervico-Vaginal Pathology) and GISCI (Italian Group of Cervical Cancer Screening), adapted in accordance with the experimental arm of the New Technologies for Cervical Cancer (NTCC) [12].

2.1. Procedure and Questionnaire

In the preliminary stage of our study, to invite the inmates to participate to the screening program we first organised three sequential meetings in the prison theatre in order to describe the value of cervical cancer screening, the role of the HPV infection and the routes of viral transmission. To explain the aims of the program and the procedure of the gynecological control we used slides with cartoons and figures to favour the comprehension from a multi ethnical population.

98 out of 350 inmate women decided to participate to the study and filled out a questionnaire by themselves. The questionnaire was written in italian and was translated into English and Spanish. The questions included those required for the history of attendance to previous cervical screening as well as the willingness to be screened in the prison setting and those for the known risk factors for cervical malignancies such as smoke, drug/alcohol and sexual behaviour (**Table 1**). Written informed consent was obtained from all participants.

Table 1. Questions for the inmates enrolled into the screening program.

Characteristics
Patient Code
Age
Race/ethnicity (White, Latino/Hispanic, African, other)
Marital Status (Unmarried without current male partner, unmarried with current male partner, married, widowed, separed-divorced)
Questions
How many sexual partners did you have in your life?
Which was the age of your first sexual intercourse?
Did you never practice oral sex?
Did you never practice anal sex?
Do you smoke? If yes, how many cigarettes/day?
Do you drink? If yes, how much?
Do you use contraceptive methods? If yes, you use condom?
Do you have carried out a PAP smear during the last three years?

2.2. Specimen Collection

The women then underwent a gynecological examination including three different cervical samples: 1) a sample of cervical cells taken by Cervical sampler, (Digene Corporation, Gaitesburg, MD) and collect in standard transport medium for HPV DNA test; 2) an eso-endocervical scraping for conventional Pap Test; 3) a sample of cervical cells taken by Ayre's Spatula and Cytobrush (Cytobrush DOC, Gardening, Genova, Italy) and collect in Transport buffer for HPV DNA genotyping.

2.3. HPV Detection

HPV DNA test was performed by Hybrid Capture II hybridization assay (HC2 Digene Corporation, Gaithersburg, Maryland USA), according to the manufacturer's instructions. HC2 test, was used with the "high-risk" probes designed to detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 positivity. A cervical smear was prepared for conventional Pap test and classified according to the Bethesda System 2001.

HPV genotyping test was performed using a line probe assay for all cervical samples which were positive at the HC2 test. Total DNA was extracted QIAamp® DNA extraction (Qiagen, Hilden, Germany) and appropriate spin columns according to the manufacturer's protocol. The INNO-LiPA HPV Genotyping Extra is a line probe assay designed for the identification of 28 different genotypes of the Human Papillomavirus by detection of specific sequences in the L1 region of the HPV genome. INNO-LiPA identified HPV types 6, 11, 16,18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 74 (INNO-LiPA; Innogenetics Gent, Belgium).

2.4. Cytological and Histological Diagnosis

All the lesions cytologically diagnosed, underwent colposcopic evaluation. The high grade lesions were immediately treated, excisional procedures were performed by a loop electrosurgical excisional procedure (LEEP), or traditional cone-biopsy, in the day surgery of Regina Elena National Cancer Institute of Rome. The histological diagnosis for all treated cases was CIN III according with cytology and colposcopic examination.

2.5. Statistical Methods

Logistic regression analysis was performed to model the association between sociodemographic characteristics, sexual and health behaviours and human papillomavirus (HPV) status or cervical cytology data. Odds Ratios (ORs) was calculated for each potential risk factors. Chi square and Fisher's exact test was used to compare categorical variables and a P for trend was calculated for ordinal categories.

3. RESULTS

As shown in **Table 2**, the epidemiological characteristics of the jail inmates corresponded to those described for a high risk population to develop cervical cancer. The sexual behaviour of the inmate group was also at higher risk because of the increased number of partners and the earlier sex intercourse. In fact, the logistic regression analysis showed that the OR for HR-HPV positivity was significantly associated with lifetime number of sex partners (OR = 2.00 for 5-9 partners; OR = 13.3 for >10 partners; p = 0.036).

Cervical samples from the 98 inmate participants to the screening were analyzed by HPV DNA test using the Hybrid Capture 2 hybridization assay to detect HR-HPV positivity and by conventional cytology. As shown in **Table 3**, the inmates with LSIL/HSIL status showed a significantly higher prevalence of HR-HPV positivity (100% vs. 16.3%; p < 0.001). In addition, the percentage of subjects positive for both HPV DNA testing and cytology was comparable to that reported in other screening programs [12,15], although a high prevalence of high grade pre-neoplastic lesions (HSIL/LSIL) was found among the inmates (**Table 2**). However, the superior sensitivity of the HPV testing compared to cytology was shown by the high percentage of cases positive for HPV DNA but negative at the cytological exam (14.3%) in agreement with several recent studies and new guidelines for cervical cancer prevention [15,16]. The OR for LSIL/HSIL status was significantly associated with the lifetime number of sex partners (OR = 1.8 for 5-9 partners; OR = 4.1 for > 10 partners; p = 0.033) and for the age of first sexual intercourse (OR = 2.0 for age < 15 years; p < 0.05) but not for none Pap smear performed during the past 3 years (OR = 0.8; p = 0.78).

Regard to the distribution of the HR-HPV types in the high risk inmate group and the amount of single and multiple infections, the genotyping assay on the 19 HPV DNA positive samples showed the much higher prevalence of HPV16 compared to the other types in either single or multiple infections (total HPV16 prevalence: 65%; single prevalence: 35%; multiple prevalence: 30%;

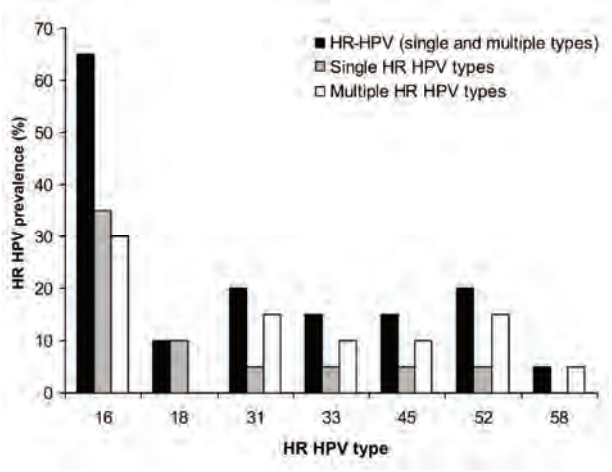

Figure 1. Overview of HR HPV type distribution in the total number of positive cases. HR HPV genotype distribution in order of predominance: HPV 16 (n = 13; 65%), HPV 31 (n = 4; 20%), HPV 52 (n = 4; 20%), HPV 33 (n = 3; 15%), HPV 45 (n = 3; 15%), HPV 18 (n = 2; 10%), HPV 58 (n = 1; 5%). Single HR HPV type distribution: HPV 16 (n = 7; 35%), HPV 18 (n = 2; 10%), HPV 31 (n = 1; 5%), HPV 33 (n = 1; 5%), HPV 45 (n = 1; 5%), HPV 52 (n = 1; 5%). Multiple HR HPV type distribution: HPV 16 (n = 6; 30%), HPV 31 (n = 3; 15%), HPV 52 (n = 3; 15%), HPV 33 (n = 2; 10%), HPV 45 (n = 2; 10%), HPV 58 (n = 1; 5%).

Figure 1), confirming that, as suggested, HPV16 is the most persistent and aggressive HR HPV type [17] and is able to promote secondary infections or co-infections with other genotypes [11]. Moreover, the inmates with LSIL/HSIL status showed a significantly higher prevalence of multiple HPV types (60% vs. 1.2%; p < 0.001) respect to women with normal/ASCUS Pap smear (**Table 2**).

4. DISCUSSION AND CONCLUSIONS

Although the main objective of our study was to assess the validity of the HPV DNA test in a high-risk population, our program was designed also to train the participant inmates about the value of cervical cancer prevention, because only a very low percentage of the incarcerated women has attended for cytological Pap-test screening previously. The number of participants to our study out of the total prisoners (approximately 37%) revealed a general interest to the prevention strategy.

The results of our screening appear to confirm the role of the risk factors taken into account in our study, which characterize the prison population, on the development of higher grade lesions. In fact, although the number of preneoplastic lesions diagnosed by our intervention was comparable to those resulting from larger screenings on the general populations [11,12], the increased percentage of high grade lesions among the inmates confirm the

Table 2. Association between the sociodemographic characteristics, sexual and health behaviours and the HPV status in the jail inmates.

Category	Subjects total no	HPVDNA + no (%)	OR (95% CI)	*P* for trend (chi square)
Age				**0.0046**
< 26 years	12	6 (50)	Reference	
26-35 years	25	4 (16)	0.19 (0.04-0.90)	
36-45 years	42	9 (21.4)	0.273 (0.07-1.05)	
> 45 years	19	0	-	
Race/ethnicity				**0.21**
White	74	16 (21.6)	Reference	
Latino/Hispanic	11	2 (18.1)	0.71 (0.14-3.58)	
African	13	1 (7.7)	0.35 (0.04-2.99)	
Marital status				**0.96**
Unmarried without current male partner	30	6 (20)	1.25 (0.37-4.18)	
Unmarried with current male partner	8	2 (25)	1.66 (0.27-10.02)	
Married	42	7 (16.7)	Reference	
Widowed	8	2 (25)	1.66 (0.27-10.02)	
Separated-divorced	10	2 (20)	1.25 (0.21-7.18)	
Lifetime no of sex partners				**0.033**
0-1	24	3 (12.5)	0.83 (0.18-3.68)	
2-4	41	6 (14.6)	Reference	
5-9	21	5 (23.8)	1.82 (0.48-6.86)	
Over 10	12	5 (41.6)	4.16 (0.98-17.54)	
Age of first sexual intercourse				**0.34**
< 15 years	26	7 (26.9)	2.00 (0.65-6.15)	
15-19 years	58	9 (15.5)	Reference	
> 19 years	11	2 (18.1)	1.21 (0.22-6.55)	
Oral intercourse				**0.34**
Never	64	14 (21.8)	Reference	
Ever	34	5 (14.7)	0.61 (0.20-1.88)	
Anal intercourse				**0.99**
Never	81	16 (19.7)	Reference	
Ever	17	3 (17.6)	0.87 (0.22-3.39)	
Tobacco consumption				**0.60**
Non smokers	23	5 (21.7)	Reference	
1-10 cigarettes	24	6 (25)	1.2 (0.31-4.65)	
> 10 cigarettes	51	8 (15.6)	0.67 (0.19-2.32)	
Alcohol consumption				**0.65**
Never	67	14 (20.8)	Reference	
Mild	23	3 (13.0)	0.56 (0.14-2.18)	
Abuse	8	2 (25)	1.26 (0.22-6.94)	
Condom use				**0.57**
Frequent	20	3 (15)	Reference	
Infrequent or never	78	16 (20.5)	1.46 (0.38-5.61)	
Pap smear during the past 3 years				**0.51**
Yes	15	2 (13.6)	Reference	
No	83	17 (20.4)	1.67 (0.34-8.13)	

Table 3. Detection of HR-HPV DNA and accordance with cervical cytology in the inmates attending for the screening.

Characteristics	Overall no = 98	Normal/ASCUS Pap smear no = 86	LSIL/HSIL Pap smear no = 5	Unsatisfactory no = 7	*P* value
HR-HPV Positive	19 (19.4)	14 (16.3)	5 (100)*	0	< 0.001
Prevalence of multiple HPV types	4 (4.1)	1 (1.2)	3 (60)	0	< 0.001

P values are from Fisher's exact test; ASCUS, atypical squamous cells of undetermined significance; LSIL, low grade squamous intra-epithelial lesions; HSIL, high grande squamous intraepithelial lesions; * Pap smear: 1 LSIL, 4 HSIL.

validity of the risk factors analyzed through our questionnaire.

Moreover, it is well know that most HPV infections spontaneously regress: however, if the infection is persistent, the risk of cervical cancer substantially increases [18]. In natural or organized screening programs, about 80% of the detected low grade lesions spontaneously regressed [18,19], while in a population as the jail inmates that never attended to a screening program or that had not more than a Pap test during the life the lesions are believed to persist and progress to high grade.

Our study was designed evaluating all the cytological scrapings after HR-HPV testing and our results are in accordance with those obtained by the experimental arm of the New Technologies for Cervical Cancer (NTCC) [12]. Since in HPV DNA by HC2 screening strategies it is possible to increase the length of follow up respect to Pap-test based programs (3-5 years if HPV DNA test is negative twice) [20], our approach will be very useful for incarcerated women which do not have the opportunity or the social and cultural environment to receive preventive care.

The results on the distribution of the HR-HPV genotypes among the inmates revealed the much higher prevalence of HPV16 compared to the other types in either single or multiple infections, as expected [11,17]. It is currently known that more than 40% of HR HPV positive cases display multiple viral types [21], although the possible impact of these co-infections on the progression of cervical malignancies has not been determined yet. It is also well recognized that HPV16 represents the predominant genotype [19]. In contrast, the very low percentage of cases positive for HPV18 (10%) in our population is somehow surprising, although Clifford et al. have reported heterogeneity in the prevalence of HPV18 in the general population from 11 countries [22]. However, it is known that HPV18 is under-represented in high grade lesions at the time of diagnosis and that the HPV18 associated lesions rapidly progress [23].

We found a much higher rate of unsatisfactory Pap-smears (7.1%) compared to that reported by Prandi et al. (1.99%) in a screened population in Italy [24], possibly due to the more frequent occurrence of vaginal/cervical infections in inmate women. Because one out of the 7 unsatisfactory cytological tests was HR- HPV positive, we may conclude that HPV DNA testing allows to do not repeat an unsatisfactory cervical sample [20], further assessing the value of the addition of HPV DNA testing for screening of a high-risk population.

Finally, in groups of women do not attending to cervical cancer screenings, such as the population studied in this program, the potential benefit of HPV vaccines are enormous. In fact, it is now well recognized that important additional effects of the vaccines are the cross protections against infections and diseases caused by 16/18 related HPV types, specially against HPV31 and HPV45, which are the other genotypes causally attributed to cervical cancer [25].

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Cytosolic phospholipase A_2 S-nitrosylation in ghrelin protection against detrimental effect of ethanol cytotoxicity on gastric mucin synthesis

——Ghrelin in gastric mucosal protection

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ABSTRACT

Ghrelin, a peptide hormone produced mainly in the stomach, has emerged recently as an important regulator of nitric oxide synthase (NOS) and cyclooxygenase (COX) enzyme systems, the products of which play direct cytoprotective function in the maintenance of gastric mucosal integrity. In this study, using gastric mucosal cells, we report on the role of ghrelin in countering the cytotoxic effect of ethanol on mucin synthesis. We show that the countering effect of ghrelin on mucin synthesis was associated with the increase in NO and PGE2 production, and characterized by a marked up-regulation in cytosolic phospholipase A_2 ($cPLA_2$) activity. The ghrelin-induced up-regulation in mucin synthesis, like that of $cPLA_2$ activity, was subject to suppression by Src inhibitor, PP2 and ERK inhibitor, PD98059, as well as ascorbate. Moreover, the loss in countering effect of ghrelin on the ethanol cytotoxicity and mucin synthesis was attained with cNOS inhibitor, L-NAME as well as COX-1 inhibitor SC-560. The effect of L-NAME was reflected in the inhibition of ghrelin-induced mucosal cell capacity for NO production, $cPLA_2$ S-nitrosylation and PGE2 generation, whereas COX-1 inhibitor caused only the inhibition in PGE2 generation. Our findings suggest that the activation of gastric mucosal $cPLA_2$ through cNOS-induced S-nitrosylation plays an essential role in the countering effect of ghrelin on the disturbances in gastric mucin synthesis caused by ethanol cytotoxicity.

Keywords: Ghrelin; Ethanol Cytotoxicity; Gastric Mucin; CNOS; $CPLA_2$; S-Nitrosylation

1. INTRODUCTION

Acute gastric mucosal lesions, mucosal inflammatory changes, and gastro-duodenal ulceration are well-recognized consequences of alcohol abuse on the health of gastrointestinal tract [1-3]. The gastric mucosal responses to ethanol cytotoxicity are manifested by microcirculatory changes, elevation in proinflammatory cytokine production, enhancement in epithelial cell apoptosis, and the impairment in prostaglandin and nitric oxide signaling pathways [2,4,5]. Furthermore, the disturbances in mucus producing gastric mucosal cells by ethanol affect the synthesis of mucins, the glycoproteins that maintain the integrity and strength of the protective mucus layer which constitutes the pre-epithelial element of gastric mucosal defense [6,7]. Hence, the alterations in the synthesis and processing of gastric mucins induced by ethanol are directly reflected in the impairment of mucus coat function that weakens the inherent resistance of the mucosa to injury, and facilitates the onset of gastric disease [3,6,7].

Recent advances in understanding the nature of factors involved in the maintenance gastric mucosal integrity revealed that the extent of mucosal protection against ethanol cytotoxicity is influenced by ghrelin, a 28-amino acid peptide hormone produced mainly in the stomach [4,8,9]. This endogenous ligand for the growth hormone secretagogue receptor, has been implicated in the control of local inflammations, healing experimentally induced gastric ulcers, and the protection of gastric mucosa against acute injury by ethanol [4,9,10]. Moreover, ghrelin emerged as an important regulator of the cross-talk between NOS and COX enzyme systems [9], the products of which, NO and PGE2, play direct cytoprotective role in maintaining the gastric mucosal integrity under normal physiological conditions [11,12].

Indeed, the literature data support the existence of a functional relationship between the products of NOS and

COX systems, and there are strong indications that the enzyme compartmentalization and substrate availability determines the segregated utilization of the respective products in physiological and pathophysiological processes [13-15]. The stimulation of NO production through NOS induction or the exogenous NO donors leads to up-regulation in COX enzymes activation and the increase in prostaglandin generation [14-17], while the inhibition of NOS decreases prostaglandin formation. Moreover, it has been reported that the NO-induced enzyme protein S-nitrosylation impacts the events of cytosolic phospholipase ($cPLA_2$) activation, and hence influence the processes associated with arachidonic acid release for prostaglandin synthesis [18].

In our previous report, using rat gastric mucosal cells, we have shown that ghrelin protection against ethanol cytotoxicity involves cNOS-mediated $cPLA_2$ activation for the increase in PGE2 production [19]. Here, we examined the influence of ghrelin on the disturbances in gastric mucin synthesis caused by ethanol.

2. MATERIALS AND METHODS

2.1. Cell Preparation and Mucin Synthesis

The gastric mucosal cells, collected from freshly dissected rat stomachs, were suspended in five volumes of ice-cold Dulbecco's modified (Gibco) Eagle's minimal essential medium (DMEM), supplemented with fungizone (50 µg/ml), penicillin (50 U/ml), streptomycin (50 µg/ml), and 10% fetal calf serum, and gently dispersed by trituration with a syringe, and settled by centrifugation [5]. Following rinsing, the cells were resuspended in the medium to a concentration of 2×10^7 cell/ml, transferred in 1 ml aliquots to DMEM in culture dishes containing [^{3}H]glucosamine (110 µCi), used as a marker of mucin synthesis, and incubated under 95% O_2-5% CO_2 atmosphere at 37˚C for 16 h [20]. After washing with DMEM containing 5% albumin to remove free radiolabel, the cells were resuspended in fresh DMEM and incubated for 2 h in the presence of 3% ethanol [5]. In the experiments evaluating the effect of ghrelin (rat, Sigma), cNOS inhibitor, L-NAME, iNOS inhibitor, 1400 W, Src inhibitor, PP2, ERK1/2 inhibitor, PD98059 (Calbiochem), COX-1 inhibitor, SC-560, COX-2 inhibitor, NS398, and ascorbate (Sigma), the cells were first preincubated for 30 min with the indicated dose of the agent or vehicle followed by incubation with ethanol. The viability of cell preparations before and during the experimentation, assessed by Trypan blue dye exclusion assay, was greater than 97%. At the end of the specified incubation period, the cells were centrifuged, washed with phosphate-buffered saline, and the combined supernatants used for mucin assay.

2.2. Mucin Analysis and Ethanol Cytotoxicity Assay

The combined cell wash and incubation medium containing ^{3}H-labeled mucin were treated at 4˚C with 10 volumes of 2% phosphotungstic acid in 20% trichloroacetic acid for 4 h and the formed precipitates were collected by centrifugation. The glycoprotein precipitates were dissolved in 6 M urea and chromatographed on a Bio-Gel A-1.5 column, and the mucin fractions eluted in the excluded volume were subjected to analysis for incorporation of radiolabel and protein content [20]. For the measurement of ethanol-induced cytotoxicity, the aliquots of cell suspension from the control and various experimental conditions were centrifuged at 300 × g for 5 min and the supernatants used for the assay of cytotoxicity using TOX-7 lactate dehydrogenase assay kit (Sigma).

2.3. PGE2 and NO Quantification

The aliquots of cell suspension from the control and various experimental conditions were centrifuged at 1500 × g for 5 min and the conditioned medium supernatant collected. PGE2 assays were carried out using a PGE2 EIA kit (Cayman) and 100 µl aliquots of the spent medium supernatant [5]. To assess nitric oxide production in rat gastric mucosal cells, we measured the stable NO metabolite, nitrite, accumulation in the culture medium using Griess reaction [21].

2.4. $CPLA_2$ Activity Assay

The measurement of $cPLA_2$ activity in the gastric mucosal cells following various experimental conditions was carried out using $cPLA_2$ assay kit (Cayman). The cells were homogenized in 1 ml of 50 mM Hepes buffer, pH 7.4, containing 1 mM EDTA, and centrifuged at 10,000 × g for 15 min at 4˚C. The supernatants were filtered through an Amicon YM30 filter concentrators, followed by 15 min incubation with 5 µM of calcium-independent PLA_2 inhibitor, bromoenol lactone, and the aliquots (10 µl) of such prepared cell lysates were subjected to $cPLA_2$ assay [19].

2.5. $CPLA_2$ S-Nitrosylation

A biotin switch procedure was employed to assess the $cPLA_2$ protein S-nitrosylation [22,23]. The gastric mucosal cells, treated with ghrelin (0.8 µg/ml) or L-NAME (300 µM)+ghrelin and incubated for 2 h in the presence of 3% ethanol, were lysed in 0.2 ml of HEN lysis buffer, pH 7.7, and the unnitrosylated thiol groups were blocked with S-methyl methanethiosulfonate reagent [23]. The proteins were precipitated with acetone, resuspended in

0.2 ml of HEN buffer containing 1% SDS, and subjected to targeted nitrothiol group reduction with sodium ascorbate (100 mM). The free thiols were then labeled with biotin and the biotinylated proteins were recovered on streptavidin beads. The formed streptavidin bead-protein complex was washed with neutralization buffer, and the bound proteins were dissociated from streptavidin beads with 50 µl of elution buffer (20 mM HEPES, 100 mM NaCl, 1 mM EDTA, pH 7.7) containing 1% 2-mercaptoethanol [22]. The obtained proteins were then analyzed by Western blotting.

2.6. Western Blot Analysis

The mucosal cells, collected by centrifugation, were resuspended for 30 min in ice-cold lysis buffer [19], and following brief sonication the lysates were centrifuged at 12,000 g for 10 min and the supernatants subjected to protein determination using BCA protein assay kit (Pierce). The samples, including those subjected to biotin switch procedure, were then resuspended in loading buffer, boiled for 5 min, and subjected to SDS-PAGE using 50 µg protein/lane. The separated proteins were transferred onto nitrocellulose membranes and probed with the antibody (Cell Signaling) against the $cPLA_2$, and after incubation with the horseradish peroxidase-conjugated secondary antibody, the protein bands were revealed using an enhanced chemiluminescence detection.

2.7. Data Analysis

All experiments were carried out using duplicate sampling, and the results are expressed as means ± SD. Analysis of variance (ANOVA) was used to determine significance and the significance level was set at $P < 0.05$.

3. RESULTS

To assess the influence of ghrelin on the disturbances in gastric mucin synthesis caused by alcohol cytotoxicity, we employed rat gastric mucosal cells exposed to ethanol at the dose range (3%) that impairs the mucosal capacity for mucin synthesis and prostaglandin generation [1-3]. We determined that preincubation of the mucosal cells with ghrelin led to a concentration-dependent prevention of ethanol cytotoxicity, and afforded nearly complete protection at 0.8 µg/ml of ghrelin (**Figure 1**). Further, our results revealed that cytotoxicity induced in gastric mucosal cells by 3% ethanol was reflected in a 32.6% decrease in mucin synthesis (**Figure 1**), as well as a 29.8% reduction in PGE2 generation and a 51.7% drop in NO production (**Figure 2**). Ghrelin at its optimal concentration (0.8 µg/ml) for the protection against ethanol

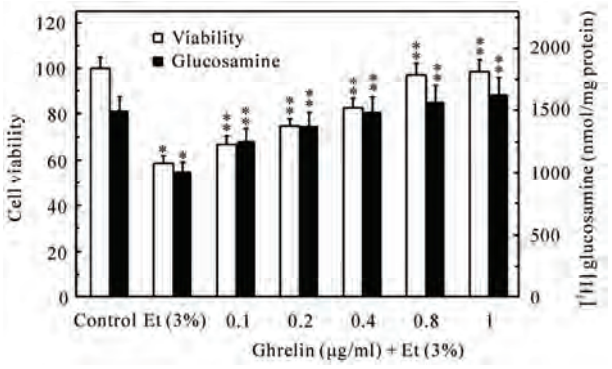

Figure 1. Effect of ghrelin on ethanol-induced cytotoxicity and the synthesis of mucin in rat gastric mucosal cells. The cells, labeled with [^{3}H]glucosamine as a marker of mucin synthesis, were treated with the indicated concentrations of ghrelin and incubated for 2 h in the presence of 3% ethanol (Et). Values represent the means ± SD of five experiments. *$P < 0.05$ compared with that of control. **$P < 0.05$ compared with that of Et alone.

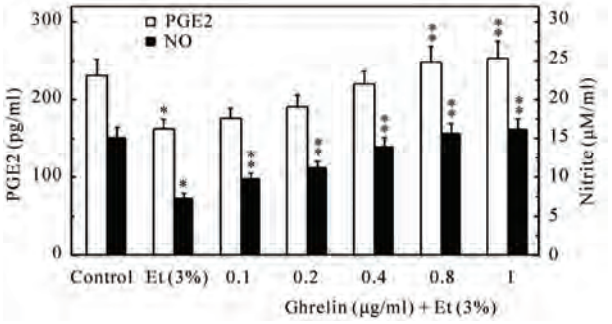

Figure 2. Effect of ghrelin on ethanol-induced changes in the production of PGE2 and nitrite by gastric mucosal cells. The cells were treated with the indicated concentrations of ghrelin and incubated for 2 h in the presence of 3% ethanol (Et). Values represent the means ± SD of five experiments. *$P < 0.05$ compared with that of control. **$P < 0.05$ compared with that of Et alone.

cytotoxicity evoked a 55.4% increase in the mucosal cell capacity for mucin synthesis (**Figure 1**), while NO production increased 2.1-folds and PGE2 generation by 53.1% (**Figure 2**).

Moreover, we found that significant loss in the preventive effect of ghrelin on the ethanol-induced toxicity and the cell capacity for mucin synthesis was attained with cNOS inhibitor, L-NAME as well as specific COX-1 inhibitor, SC-560, while selective iNOS inhibitor, 1400W and a specific COX-2 inhibitor, NS-398 had no effect (**Figure 3**). The effect of L-NAME, furthermore, was reflected in the inhibition of ghrelin-induced mucosal cell capacity for NO production as well as PGE2 generation, whereas the pretreatment with COX-1 inhibitor,

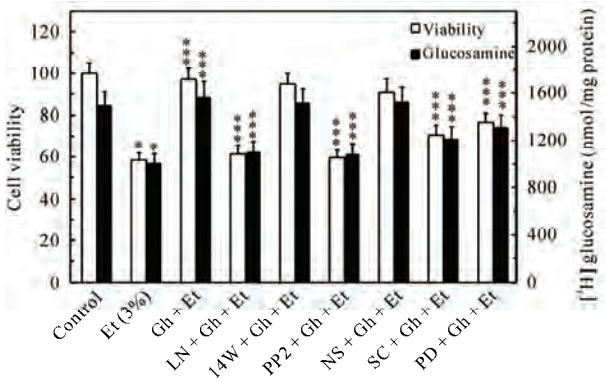

Figure 3. Effect of nitric oxide synthase and cyclooxygenase inhibitors on the ghrelin (Gh)-induced protection against ethanol (Et) cytotoxicity, and the synthesis of mucin in gastric mucosal cells. The cells, labeled with [^{3}H] glucosamine, and preincubated with 300 μM L-NAME (LN), 20 μM 1400 W (14 W), 30 μM PP2, 30 μM NS-398 (NS), 20 μM SC-560 (SC) or 30 μM PD98059 (PD), were treated with Gh at 0.8 μg/ml and incubated for 2h in the presence of 3% Et. Values represent the means ± SD of five experiments. **P* < 0.05 compared with that of control. ***P* < 0.05 compared with that of Et alone. ****P* < 0.05 compared with that of Gh + Et.

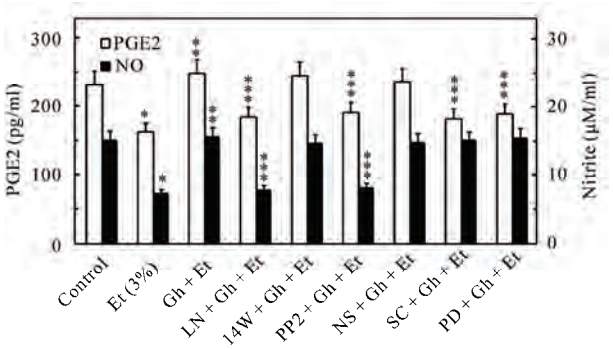

Figure 4. Effect of nitric oxide synthase and cyclooxygenase inhibitors on the ghrelin (Gh)-induced changes in the production of PGE2 and nitrite by gastric mucosal cells in the presence of ethanol (Et). The cells, preincubated with 300 μM L-NAME (LN), 20 μM 1400 W (14 W), 30 μM PP2, 30 μM NS-398 (NS), 20 μM SC-560 (SC) or 30 μM PD98059 (PD), were treated with 0.8 μg/ml Gh and incubated for 2 h in the presence of 3% Et. Values represent the means ± SD of five experiments. **P* < 0.05 compared with that of control. ***P* < 0.05 compared with that of Et alone. ****P* < 0.05 compared with that of Gh + Et.

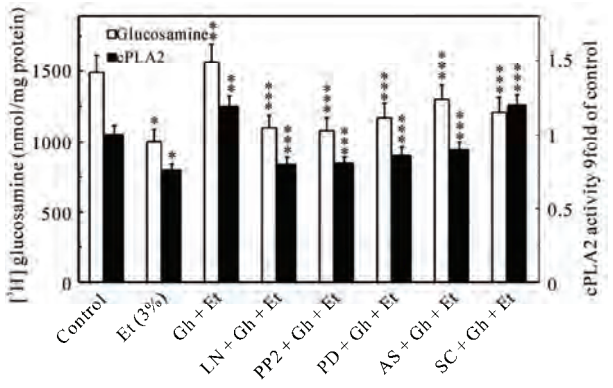

Figure 5. Effect of nitric oxide synthase and cyclooxygenase inhibitors on the ghrelin (Gh)-induced changes in mucin synthesis and cPLA$_2$ activity in gastric mucosal cells in the presence of ethanol (Et). The cells, labeled with [^{3}H] glucosamine, and preincubated with 300 μM L-NAME (LN), 30 μM PP2, 30 μM PD98059 (PD), 300 μM ascorbate (As) or 20 μM SC-560 (SC), were treated with Gh at 0.8 μg/ml and incubated for 2 h in the presence of 3% Et. Values represent the means ± SD of five experiments. **P* < 0.05 compared with that of control. ***P* < 0.05 compared with that of Et alone. ****P* < 0.05 compared with that of Gh + Et.

SC560, led only to the inhibition in ghrelin-induced PGE2 generation (**Figure 4**). The stimulatory effect of ghrelin on the mucosal cell capacity for NO and PGE2 production, however, was not affected by the inclusion of iNOS inhibitor 1400W and COX-2 inhibitor, NS-398 (**Figure 4**). Further, we found that the countering effect of ghrelin on the ethanol-induced changes in gastric mucosal cell capacity for mucin synthesis, and NO and PGE2 production, were subject to suppression by Src kinase inhibitor, PP2, while the inhibitor of MAPK/ERK1/2, PD98059, caused the suppression in mucin synthesis and PGE2 generation, but had no effect on NO production (**Figures 3** and **4**).

As the initial and rate limiting step in prostaglandin production is the liberation of arachidonic acid from membrane phospholipids by highly selective cPLA$_2$ [5,24], we next analyzed the effect of ghrelin on the mucosal cell cPLA$_2$ enzymatic activity. We found that ghrelin countering effect on the ethanol-induced cytotoxicity and the mucosal cell capacity for mucin synthesis was reflected in a 56.5% increase in cPLA$_2$ activity (**Figure 5**). Furthermore, the ghrelin-induced up-regulation in cPLA$_2$ activity, like that of mucin synthesis, was subject to suppression by Src inhibitor, PP2 and ERK1/2 inhibitor, PD98059, as well as the inhibitor of cNOS, L-NAME (**Figure 5**). The pretreatment with COX-1 inhibitor, SC-560, however, while not causing any discernible alteration in the cPLA$_2$ activity, exerted the inhibitory effect on mucin synthesis (**Figure 5**).

Since in addition to phosphorylation, the activation of cPLA$_2$ involves the NO-dependent enzyme protein S-nitrosylation [18,19], we further analyzed the influence of S-nitrosylation on gastric mucosal cPLA$_2$ activity. The results revealed that ghrelin-induced up-regulation in cPLA$_2$ activity and the cell capacity for mucin synthesis was susceptible to suppression not only by the inhi-

bitor of cNOS, L-NAME, but also by ascorbate (**Figure 5**), which is in keeping with known susceptibility of S-nitrosylated proteins to this reducing agent [14,23]. Moreover, Western blot analysis of the mucosal cell lysates subjected to biotin switch procedure, with antibody against $cPLA_2$, revealed that ghrelin prevention of the ethanol-induced cytotoxicity was manifested in the increase in $cPLA_2$ protein S-nitrosylation, whereas preincubation with L-NAME blocked the ghrelin-induced $cPLA_2$ nitrosylation (**Figure 6**).

4. DISCUSSION

Acute gastric mucosal injury and gastro-duodenal ulcerations are the most common consequences of alcohol abuse on the health of gastrointestinal tract [1-3]. Moreover, the disturbances in gastric mucosal cells caused by ethanol cytotoxicity affect the synthesis of mucins, the glycoproteins that maintain the integrity and strength of the protective mucus layer, and hence weaken the inherent resistance of the mucosa to injury and facilitate the onset of gastric disease [6,7]. Therefore, considering the recent evidence for the involvement of ghrelin in gastric mucosal protection against injury by ethanol [4,9], in the study presented herein we examined the influence of this 28-amino acid peptide hormone on the synthesis of gastric mucin.

Using rat gastric mucosal cells exposed to ethanol at the concentration range that impairs mucosal cell capacity for mucin synthesis and prostaglandin generation [1-3], we demonstrated that the protective effect of ghrelin against ethanol cytotoxicity was associated with up-regulation in mucin synthesis, and accompanied by the increase in NO and PGE2 production, and the enhancement in $cPLA_2$ activity. A significant loss in the countering effect of ghrelin on the ethanol-induced cytotoxicity and the cell capacity for mucin synthesis was attained with cNOS inhibitor, L-NAME as well as specific COX-1 inhibitor, SC-560, whereas COX-2 inhibitor, NS-398, and iNOS inhibitor, 1400W, had no effect. These findings are thus in keeping with the results of earlier studies suggesting that the detrimental effect of ethanol on gastric mucin synthesis are associated with the impairment in NO and PGE2 generation [1-3,6,25], and lend further credence as to the role of ghrelin in regulation of the cross-talk between NOS and COX enzyme systems [9].

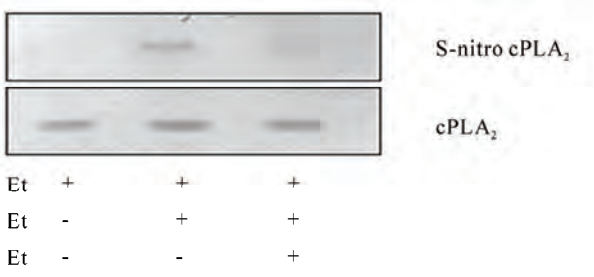

Figure 6. Effect of cNOS inhibitor, L-NAME (LN) on ghrelin (Gh)-induced $cPLA_2$ S-nitrosylation in gastric mucosal cells exposed to ethanol (Et). The cells were treated with Gh or L-NAME + Gh and incubated for 2 h in the presence of Et. A portion of the cell lysate was processed by biotin switch procedure for protein S-nitrosylation and, along with the reminder of the lysates, subjected to SDS-PAGE, transferred to nitrocellulose and probed with anti-$cPLA_2$ antibody. The immunoblots shown are representative of three experiments.

Further, we found that the countering effect of ghrelin on the ethanol-induced changes in mucin synthesis, and NO and PGE2 production, were subject to suppression by Src kinase inhibitor, PP2, whereas the inhibitor of MAPK/ERK, PD98059, elicited suppression in mucin synthesis and PGE2 generation, but had no effect on NO production. Thus the activation of Src appears to be a triggering event whereby ghrelin is capable of affecting the mucosal cell capacity for mucin synthesis as well as NO and PGE2 generation. Moreover, our data on the inhibition of ghrelin-induced mucosal cell capacity for mucin synthesis, and NO and PGE2 generation by L-NAME, and only that of PGE2 and mucin synthesis by COX-1 inhibitor, SC-560, suggest that ghrelin-induced up-regulation in mucin synthesis and PGE2 generation occurs with the involvement of cNOS-derived NO, and requires COX-1 participation. Indeed, the role of NO in modulation of COX enzymes activity in a variety of different systems is well documented [15-17].

As the initial and rate limiting event in prostaglandin production is the release of AA from membrane phospholipids by highly selective $cPLA_2$ [5,24], we further assessed the influence of ghrelin on the processes of $cPLA_2$ activation. We found that ghrelin elicited up-regulation in the mucosal cell cPLA2 activation, which like that of mucin synthesis was subject to suppression by Src inhibitor, PP2, and ERK1/2 inhibitor, PD98059, as well as the inhibitor of cNOS, L-NAME. However, COX-1 inhibitor, SC-560, while not causing discernible alteration in the $cPLA_2$ activity, exerted the inhibitory effect on mucin synthesis. Hence, we concluded that the activation of gastric mucosal $cPLA_2$ by ghrelin for the increase in mucin synthesis to counter ethanol cytotoxicity occurs with the involvement of cNOS and requires Src kinase-dependent MAPK/ERK participation. Indeed, the literature data indicate that MAPK/ERK-dependent $cPLA_2$ phosphorylation on the critical Ser^{505} residue plays a crucial role in Ca^{2+}-dependent translocation of $cPLA_2$ from cytosol to membrane to gain access to AA-rich phospholipid substrates [5,26].

Whilst regulation of the key enzymes for prostaglandin

production through phosphorylation is well recognized, recent evidence indicates that the increase in prostaglandin formation may also result from NO-induced enzyme protein S-nitrosylation [14,17-19]. Indeed, the post-translational modification through S-nitrosylation at the critical Cys^{526} residue has been linked to the NO-induced enhancement in catalytic activity of COX-2 [14], and $cPLA_2$ activation through S-nitrosylation at Cys^{152} was reported to be responsible for up-regulation in arachidonic acid release in human epithelial cells [18]. Therefore, to assess the role of ghrelin in $cPLA_2$ activation, we further analyzed the influence of S-nitrosylation on gastric mucosal $cPLA_2$ activity and the capacity for mucin synthesis. We found that ghrelin-induced up-regulation in $cPLA_2$ activity and the cell capacity for mucin synthesis was susceptible to suppression not only by the inhibitor of cNOS, L-NAME, but also by ascorbic acid, which is keeping with well known susceptibility of S-nitrosylated proteins to this reducing agent [14,23]. Moreover, Western blot analysis of the mucosal cell lysates subjected to biotin switch procedure with antibody against $cPLA_2$ revealed that ghrelin prevention of the ethanol-induced cytotoxicity was manifested in the increased $cPLA_2$ protein S-nitrosylation, whereas preincubation with cNOS inhibitor, L-NAME resulted in the blockage of the ghrelin-induced $cPLA_2$ protein S-nitrosylation. Therefore, consistent with our results, the $cPLA_2$ activation by ghrelin through S-nitrosylation is of direct relevance to the mucosal capacity for mucin synthesis and PGE2 generation, and hence the protection of gastric mucosal cells against ethanol cytotoxicity.

In summary, our study demonstrates that the activation of gastric mucosal $cPLA_2$ through S-nitrosylation plays an essential role in the countering effect of ghrelin on the ethanol-induced disturbances in gastric mucin synthesis.

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Comparative study of breast cancer in Mexican and Mexican-American women

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ABSTRACT

Breast cancer is the number one cause of cancer deaths among Hispanic women in the United States, and in Mexico, it recently became the primary cause of cancer deaths. This malignancy represents a poorly understood and understudied disease in Hispanic women. The ELLA Binational Breast Cancer Study was established in 2006 as a multi-center study to assess patterns of breast tumor markers, clinical characteristics, and their risk factors in women of Mexican descent. We describe the design and implementation of the ELLA Study and provide a risk factor comparison between women in the U.S. and those in Mexico based on a sample of 765 patients (364 in the U.S. and 401 in Mexico). Compared to women in Mexico, U.S. women had significantly ($p < 0.05$) lower parity (3.2 vs. 3.9 mean live births) and breastfeeding rates (57.5% vs. 80.5%), higher use of oral contraceptives (60.7% vs. 50.1%) and hormone replacement therapy (23.3% vs. 7.6%), and higher family history of breast cancer (15.7% vs. 9.0%). Results show that differences in breast cancer risk factor patterns exist between Mexico and U.S. women. We provide lessons learned from the conduct of our study. Binational studies are an important step in understanding disease patterns and etiology for women in both countries.

Keywords: Binational Study; Breast Cancer; Hispanics; Mexico; United States

1. INTRODUCTION

Rates of breast cancer in more developed nations have in the past exceeded those in lower-income countries by a factor of five or more [1]. In Mexico, the breast cancer mortality rate has increased by 84% over the last two decades [2,3] and it is now the most commonly diagnosed cancer among women in Mexico [3]. In the United States (U.S.), age-adjusted breast cancer incidence differs significantly among racial/ethnic groups; rates are higher among non-Hispanic whites (NHWs) and lower among racial/ethnic minorities, including Hispanics [4]. Despite their lower breast cancer incidence rates, Hispanic women are 22% more likely to die of this disease [5]. Published data [6-10], including our own studies in Arizona [11], indicate that Hispanic women present with breast cancer at an earlier age and with larger, more advanced stage disease of higher grade, a profile similar to that observed for African-American women. Relative to other racial/ethnic groups, few epidemiological studies that focus on breast cancer in Hispanic women have been conducted to date. Published reports have provided data on the role of reproductive factors [12,13], body size and obesity [14], physical activity [15,16], contraceptive use [12,17], diet [18], family history [13], and migration history [12, 19], in relation to breast cancer risk in Hispanic women in the U.S.

It is now generally accepted that breast tumors exhibit heterogeneity derived from intrinsic molecular differences that influence their natural history and response to treatment [20]. A number of studies have shown that steroid hormone dependent tumors differ with respect to their biology and their risk factors [21] and that these differences are clinically relevant in terms of treatment selection, response, and patient prognosis [22-24]. In spite of the importance of characterization of breast cancer disease subtypes, no published data exist on the prevalence of the tumor subtypes, such as luminal and basal-like breast tumors in Hispanic women in the U.S. Results of the few population-based studies published to date [25-27], including our own [11], suggest that Hispanic women with breast cancer are more likely to be diagnosed with hormone receptor negative tumors and those that do not express human epidermal growth factor receptor 2 (HER2), compared to NHWs [28,29].

Reasons for breast cancer disparities in Hispanic women likely result from a combination of factors including poor access to health care, less use of mammography screening, genetic susceptibility, and environmental or cultural factors. While there is increasing scrutiny of the validity and theoretical basis of acculturation measures [30-32], adequate and nuanced measurement of acculturation could allow for the identification of variables linked to risk-enhancing or protective behaviors associated with breast cancer and its subtypes.

The ELLA Binational Breast Cancer Study (hereafter referred to as the ELLA Study) was established to compare risk factor patterns, disease phenotypes, and clinical characteristics between the U.S. and Mexico populations. We hypothesized that the distribution of breast tumor subsets differs between women in Mexico and Mexican-American women residing in the U.S. and that these differences are related to reproductive and lifestyle factor profiles indicative of U.S. lifestyle and cultural influences. Here we describe the design and implementation of this binational study and provide a comparison of risk factor characteristics between the two countries.

2. METHODS

2.1. Study Design and Recruitment

The ELLA Study was initiated in 2006 as a pilot effort with the formation of a binational investigative team, comprising three sites in Mexico (Universidad de Guadalajara in Guadalajara, Jalisco; Universidad de Sonora in Hermosillo, Sonora; and Instituto Tecnológico de Sonora in Ciudad Obregón, Sonora) and two sites in the U.S. (Arizona Cancer Center, in Tucson, Arizona, which included recruitment sites throughout the state; and at the University of Texas M.D. Anderson Cancer Center in Houston, Texas, which included two additional recruitment sites) (See **Table 1**). An essential component of the ELLA study was the partnership with clinicians and pathologists from health care settings serving the study participants both in Mexico and the U.S. Health care providers included those from Mexico's nationalized health care system: the Instituto Mexicano del Seguro Social (IMSS) in all three sites and the Hospital Civil and Instituto Jaliscience de Cancerología in Guadalajara. The study was approved by the respective Institutional Review Boards, including the IMSS. Written informed consent was obtained for all participants.

Participants were eligible if they were diagnosed within 24 months of recruitment with invasive breast cancer and were 18 years of age or older. In the U.S., participants were self-identified as being of Mexican descent. Women with carcinoma *in situ* and those with recurrent disease were ineligible. A clinic-based approach was the dominant strategy used for recruitment in the study, although the number of recruitment sites and their tactics

of identifying patients differed by region (see **Table 1**).

2.2. Data Collection

Participation involved in-person (93%) or telephone administration (7%) of a risk factor questionnaire. Three comparable questionnaires were created: English and Spanish versions for the U.S. and a separate instrument for Mexico. No differences existed between the two U.S. versions and only minor differences between the U.S. and Mexico questionnaires (see **Table 2**).

All study personnel were jointly trained prior to the initiation of recruitment and continue to receive training as needed. The instrument takes a median of 45.0 minutes to administer (59.0 minutes for Mexico and 43.2 minutes for the U.S.).

As part of data collection, we abstracted the medical records for clinical and histopathological factors, including predictive and prognostic factors. It is important to note that ER, PR, and HER2 are not uniformly conducted in all IMSS institutions in Mexico; marker status is not conducted at all in the Hospital Civil or the Instituto Jaliscience de Cancerología. The Avon Foundation, one of the study's sponsors, provided additional funding for the breast cancer patients to have consistent tumor marker information that is important for their treatment course.

Figure 1 presents the operational structure of the ELLA Study. The organizational structure includes the five recruitment sites, the research core elements, and the Steering and Advisory Committees. Recruitment, data collection, tissue collection, and DNA extraction are conducted at each site.

2.3. Biological Samples

An essential component of the ELLA Study was the routine collection of formalin fixed paraffin embedded (FFPE) breast cancer tissue including biopsy sample for patients receiving neoadjuvant therapy. FFPE tissue samples were sent to M.D. Anderson Cancer Center for the construction of tissue microarrays (TMAs) (**Figure 1**). The markers being evaluated in the TMAs were selected to characterize basal and luminal subtypes (ER, PR, and HER2, Ki67, epidermal growth factor receptor (EGFR) and basal cytokeratins [CK5 or 6, CK14 and CK17]) for subset delineation by immunohistochemistry as described by Nielsen *et al.* [33]. Prior to initiation of the ELLA recruitment, special trainings were conducted at Ventana Medical Systems (Tucson, Arizona) that included all Mexico pathologists involved in the study. To assure uniformity of tumor marker measures of diagnostic value across community and international laboratories, ER, PR, HER2, and Ki67 analyses were repeated on all tumor samples at Ventana Medical Systems with automation and intra- and inter-batch control. We do not present clinical characteristics or marker data due to their premature nature as data derived from Mexico will need to undergo quality control verification.

Figure 1 also shows the establishment of a deoxyribonucleic acid (DNA) repository with the collection of blood or saliva on all participants. DNA was extracted at each recruitment site from saliva according to manufacturer instruction for the Oragene saliva kit (DNA Genotek©, Ontario Canada) or from blood using the QIAmp Mini Kit (Qiagen©, Valencia CA). A single DNA aliquot was then sent to the Arizona Cancer Center for study banking with each site retaining the remainder of their study DNA.

2.4. Data Management and Tissue Tracking

Data management supporting the risk factor questionnaire and medical record abstraction for the ELLA Study was centralized at the Arizona Cancer Center. We developed web-based databases built with two well-established open-source software systems, providing the capacity to allow access from anywhere via the Internet. Database applications were built around the individual needs

Table 1. Recruitment sites in the ELLA study, March 1, 2007 to June 1, 2009.

Region	Recruitment Sites
Arizona (United States)	Arizona Cancer Center (Tucson); St. Elizabeth of Hungary Clinic (Tucson); University Physician's Healthcare Kino Hospital (Tucson); El Rio Community Health Center (Tucson); Arizona Oncology (Tucson); Maricopa Integrated Health System (Phoenix); Mountain Park Community Health Center (Phoenix); Dr. Edward Donahue (Phoenix); Arizona Oncology (Phoenix); Mariposa Community Health Center (Nogales); Regional Center for Border Health (Yuma).
Houston, Texas (United States)	M.D. Anderson Cancer Center; Lyndon B. Johnson Hospital; The Rose Diagnostic Clinic.
Guadalajara, Jalisco (Mexico)	Instituto Mexicano del Seguro Social; OPD Hospital Civil de Guadalajara; Instituto Jaliscience de Cancerología.
Ciudad Obregon, Sonora (Mexico)	Instituto Mexicano del Seguro Social.
Hermosillo, Sonora (Mexico)	Instituto Mexicano del Seguro Social.

Table 2. Characteristics of the risk factor questionnaire and the medical record abstraction in the ELLA study.

Characteristic	Details
Sociodemographics	Date and place of birth, residence history, age at migration to the U.S. (U.S. only), education, marital status, religious preference, income (Mexico only), parents' place of birth, poverty index (Mexico only).
Occupational history	Longest paid job held for one year or longer; farm work.
Pesticide exposure	Exposure at home, residence in or near agricultural community.
Acculturation/ Westernization	Language use, media language exposure (U.S. only). Westernization questionnaire developed for Mexican patients.
Tobacco Exposure	Cigarette use status (never, past, current), dose, and duration. Second-hand smoke exposure history.
Alcohol History	Alcohol use, type, dose and duration.
Menstrual history	Age at menarche, menstrual cycle regularity. Age at menopause and type.
Pregnancy history	Age at pregnancy, number of full term pregnancies, type of birth, breast feeding, weight gain.
Breast health history	History of breast self exam, clinical breast exam, mammography, biopsies. Method of breast cancer detection, symptoms, delay in care, reason(s) for delay.
Medical history	History of diabetes, hypertension, heart disease, endometriosis, autoimmune disease(s), polycystic ovaries, gallbladder disease, radiation to the chest.
Medication use	Use of aspirin/nonsteroidal anti-inflammatory drugs and oral corticosteroids, dose, and duration.
Birth control and hormone use	Type of birth control and history of use. HRT use, type, and duration.
Family history of cancer	First and second degree family members, type of cancer, and age at diagnosis of affected family member.
Physical activity	Time spent in occupational, housework, and recreational activities and activity type. Sedentary behavior (time spent sitting and sleeping). Activity level at various ages during lifetime.
Anthropometrics	Height, weight, and weight history. Waist and hip measurement.
Medical Record abstraction	Site of recruitment. Age at diagnosis. Stage, histology, tumor markers (ER, PR, HER2).

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HRT, hormone replacement therapy; PR, progesterone receptor; U.S., United States

of the study and tailored to meet its specific tasks. With Secured Socket Layer (SSL) certificate, the password protected online database application was free of intercept by others during data transmission from each study site to the database server. We developed a tissue tracking database in Oracle® (Oracle, Redwood Shores CA), a relational database using caTissue Version 1.0 structure with extensions to track tissue transfer across the ELLA consortium (**Figure 1**). All bodily fluids and FFPE tissue samples were inventoried in the ELLA Station and the data are secure at the M.D. Anderson Cancer Center. The database can be accessed via a browser-based front end using Java Server Faces

3. RESULTS

Data presented are based on 765 study participants recruited as of June 1, 2009 (364 in the U.S. and 401 in Mexico). The majority (78.9%) of participants were interviewed within one year of diagnosis. Response rates were extremely high, ranging from 95 to 99%. **Table 3** presents selected risk factor characteristics for the total population as well as by country.

Women in the U.S. were significantly younger than those in Mexico. Fifty-nine percent of the U.S. patients were born in Mexico and the majority has lived in the U.S. for over ten years. We recruited roughly equal proportions of English and Spanish speakers in the U.S. Women in Mexico had a significantly higher number of live births and rates of breastfeeding. The proportion of post-menopausal women was significantly higher in Mexico than the U.S. Use of oral contraceptives and hormone replacement therapy were significantly higher in the U.S. than Mexico, but use of hormone therapy was low overall (14%). Women in Mexico were less likely to have had prior mammography. Although obesity rates were equally high in both countries, women in Mexico reported a lower BMI at age 30 compared to those in the U.S. Current cigarette smoking was low in both countries, whereas alcohol consumption was significantly higher in the U.S. than in Mexico.

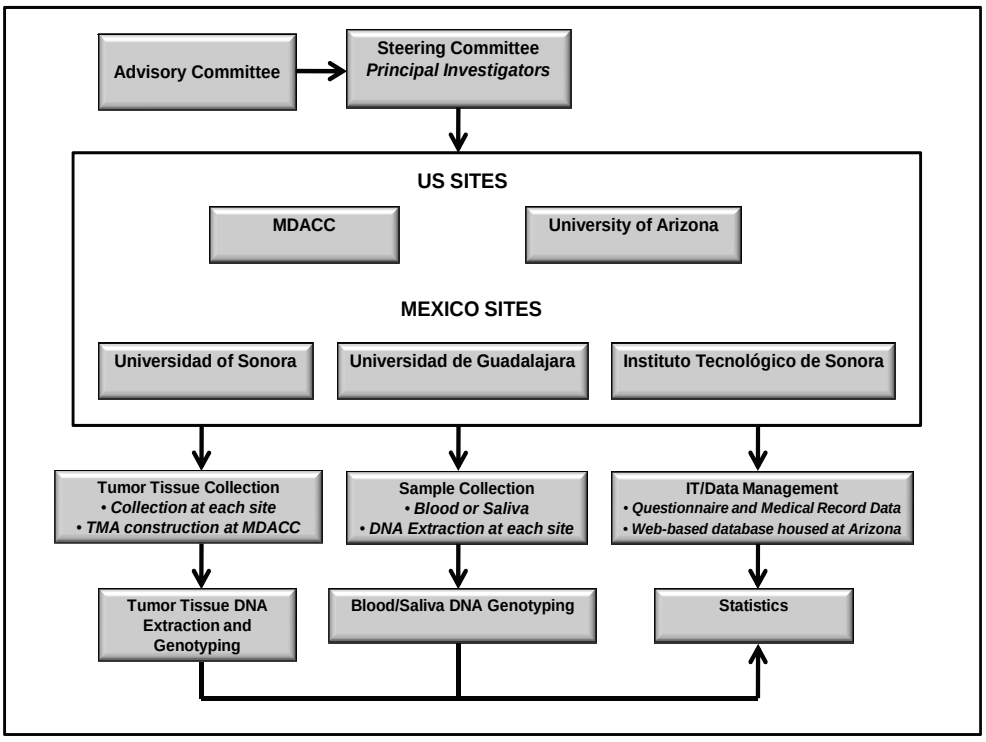

Figure 1. ELLA binational breast cancer study organizational structure. The organizational structure includes the five recruitment sites, the research core elements, and the Steering and Advisory Committees. Recruitment, data collection, tissue collection, and DNA extraction are conducted at each site. Tracking of participants, Risk Factor Questionnaire (RFQ), Medical Record Abstraction (MRA), and biological samples (deoxyribonucleic acid [DNA] and Tissue Microarray [TMA] repositories) are maintained in separate but integrated databases. Databases can be accessed via browser-based front end using Secured Socket Layer certificate.

4. DISCUSSION

In 2006, there were 44.3 million Hispanics (14.8 percent of total) in the U.S., with those of Mexican descent comprising 64 percent of the total. It is projected that the Hispanic population will grow to 102.6 million (24.4 percent of total) by 2050 [34], making it the fastest growing racial/ethnic minority group in the U.S. In addition, foreign born individuals from Mexico comprise the largest immigrant group in the U.S. In spite of the continued growth of the Hispanic population in the U.S. (44.3 million in 2006) [34] and the fact that breast cancer represents the number one cause of cancer deaths in Hispanic women [35], limited data exist on breast cancer risk factors or the breast cancer clinical and tumor marker profile for this underserved population.

The binational nature of the ELLA Study and recruitment of large numbers of women of Mexican descent in the U.S. and Mexico will provide a unique opportunity to explore a variety of risk and protective factors in relation to breast cancer subtypes, including the cultural impact of the adoption of U.S. lifestyles. Pike *et al.* [12] proposed that the lower breast cancer risk among Hispanic women compared to NHWs is almost entirely explained by their younger age at first full term pregnancy, higher parity, low use of HRT, and low alcohol consumption. These authors also observed a lower risk of breast cancer in foreign-born than U.S.-born Hispanics. The large proportion of foreign-born women in the ELLA Study (59%) will provide unique opportunities to assess residency and migration history in relation to risk factor profiles and disease patterns. Our data show that Mexican women with breast cancer tend to have more children, are less likely to use contraceptives or HRT, and less likely to consume alcohol or smoke cigarettes, as compared to their U.S. counterparts. However, no differences were shown for age at menarche, age at menopause, or age at first birth. Data from the South-west Hormone, Insulin, Nutrition, and Exercise (SHINE) Study [15,18] show that a later age at menarche and higher parity were shown to be protective, whereas a later age at first birth was associated with higher risk of breast cancer; however, associations were not all statistically

Table 3. Characteristics of participants in the ELLA study, March 1, 2007 to June 1, 2009.

	Total (N = 765)	U.S. (N = 364)	Mexico (N = 401)
Age at interview, years, mean (SD)	53.6 (12.6)	51.6 (12.2)	55.5 (12.7)*
Highest level of education[a], No. (%)			
Less than high school	376 (53.3)	142 (39.1)	234 (68.4)
High school	187 (26.5)	120 (33.1)	67 (19.6)
Post-high school	142 (20.1)	101 (27.8)	41 (12.0)*
Country of birth[b], No. (%)			
U.S.-born		150 (41.2)	
Foreign-born		214 (58.8)	
Nativity[b], No. (%)			
U.S.-born, living in U.S. ≥ 10 y		137 (37.6)	
U.S.-born, living in U.S. < 10 y		13 (3.6)	
Foreign-born, living in US ≥ 10 y		163 (44.8)	
Foreign-born, living in US < 10 y		51 (14.0)	
Language use[b,c], No. (%)			
English		173 (47.5)	
Spanish		191 (52.5)	
Age at menarche (y), mean ± SD	12.8 (1.6)	12.8 (1.6)	12.9 (1.6)
Parous, No. (%)	700 (91.5)	334 (91.8)	366 (91.3)
Age at first live birth, mean (SD)	22.9 (5.5)	22.7 (5.6)	23.0 (5.5)
No. live births, mean (SD)	3.6 (2.1)	3.2 (1.8)	3.9 (2.4)*
Ever breastfeeding[d], No. (%)	532 (69.6)	210 (57.7)	322 (80.5)*
Up to 9 months	165 (31.0)	87 (41.4)	78 (24.2)
9+ months	367 (69.0)	123 (58.6)	244 (75.8)*
Menopausal status at interview, No. (%)			
Premenopausal	334 (43.7)	186 (51.1)	148 (36.7)*
Post-menopausal[e]	431 (56.3)	178 (48.9)	253 (63.1)
Age at natural menopause, years, mean (SD)	48.4 (5.2)	48.7 (4.7)	48.3 (5.4)
Contraceptive use[f], No. (%)	416 (55.2)	219 (60.7)	197 (50.1)*
HRT use[g], No. (%)	70 (14.3)	49 (23.3)	21 (7.6)*
Prior mammography, No. (%)	470 (61.4)	244 (67.0)	226 (56.4)*
Family history of breast cancer[h], No. (%)	91 (12.2)	56 (15.7)	35 (9.0)*
Recent BMI[i], mean (SD)	29.2 (6.2)	29.6 (6.9)	28.8 (5.4)
Underweight (< 18.5), No. (%)	5 (0.8)	4 (1.2)	1 (0.3)
Normal (18.5-24.9), No. (%)	163 (24.6)	81 (24.7)	82 (24.6)
Overweight (25.0-29.9), No. (%)	232 (35.1)	105 (32.0)	127 (38.0)
Obese (≥ 30.0), No. (%)	262 (39.6)	138 (42.1)	124 (37.1)
BMI at age 30 years[j], mean (SD)	24.4 (4.6)	24.7 (4.9)	24.0 (4.3)
Waist circumference[k], cm, mean (SD)	95.2 (14.2)	94.4 (16.6)	95.6 (12.8)
Waist/hip ratio[l], cm, mean (SD)	0.88 (0.1)	0.88 (0.1)	0.89 (0.1)
Current cigarette smoking, No. (%)	50 (6.5)	29 (8.0)	21 (5.2)
Alcohol use[m], No. (%)	428 (56.1)	228 (62.8)	200 (50.0)*

Abbreviations: BMI, body mass index; HRT, hormone replacement therapy; SD, standard deviation; U.S., United States; *$P < 0.05$ comparing differences between Mexico and the U.S. Differences in means were determined by t test, and differences in proportions were determined by chi-squared tests; *P* values are 2 sided; [a]Includes 705 participants (363 in the U.S. and 342 in Mexico); [b]All Mexican women were born in Mexico had questionnaire administered in Spanish. With the exception of 2 participants, all foreign-born U.S. women were born in Mexico; [c]Based on language use during the interview.; [d]Includes 764 participants (364 in the U.S. and 400 in Mexico); [e]Periods stopped for at least 12 months due to natural menopause, bilateral oopherectomy, or other reason and age at interview older than mean age of natural menopause; [f]Includes 754 participants (361 in the U.S. and 393 in Mexico); [g]Includes women reporting periods stopped for at least 12 months; 488 participants (210 in the U.S. and 278 in Mexico); [h]Family history of breast cancer in first degree relatives. Excludes adopted women who reported not knowing their blood relatives and those who reported not knowing whether any family member had any type of cancer. Includes 746 participants (356 in the U.S. and 390 in Mexico); [i]Weight (kg)/height $(m)^2$. Defined by self-reported weight and height 1-3 years prior to diagnosis. Includes 662 participants (328 in the U.S. and 334 in Mexico); [j]Weight (kg)/height $(m)^2$. Includes 542 participants (296 in the U.S. and 246 in Mexico) and excludes women less than 30 years of age; [k]Includes 603 participants (208 in the U.S. and 395 in Mexico); [l]Includes 599 participants (207 in the U.S. and 392 in Mexico); [m]Includes 763 participants (363 in the U.S. and 400 in Mexico).

significant, and some varied by pre- and post-menopausal status. Data on the protective effect of breastfeeding have been published, including the most recent recommendations from the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) for breast cancer prevention [36]. Given the high proportion of women who breastfed in the ELLA Study (70%), future studies will be able to explore associations among women by molecular subtype with higher rates of breastfeeding than those reported in the literature.

Our data on obesity show that prevalence is equally high in both countries. Obesity in Mexico is a recent major epidemic; in 1988, 33.4 % of adults were classified as overweight/obese and this figure has risen to 71.9% in 2006 [37], with rates higher in women than men. In the U.S., Hispanics suffer disproportionately from obesity [38], albeit foreign-born Hispanics have a lower likelihood of being overweight/obese compared with those born in the U.S. [39]. Although results of the SHINE study showed no deleterious effect of obesity for risk of breast cancer in pre- or post-menopausal Hispanic women [15], a better understanding of its potential effects on the distribution of breast cancer specific phenotypes is needed. Results of two studies found that a higher BMI and/or a high waist-to-hip ratio was associated with an increased risk of basal-like breast cancer [40,41] or triple negative tumors [42]. Data from the ELLA study will allow us to elucidate the relation of obesity measures to disease phenotypes for women of Mexican descent who are disproportionately affected by high rates of overweight and obesity.

Reports in the literature show that Hispanic women tend to be diagnosed at a younger age than NHWs [6, 7,43]. Our data based on age at interview are consistent with these findings. Whether or not this lower age primarily reflects the age structure of the population is unclear. In the U.S, Hispanics have a lower median age compared to NHWs (25.8 vs. 38.6 years) [34] and is similar to the median age in Mexico (25 years) [44]. Data on tumor stage show that women in Mexico are diagnosed less frequently with early stage tumors, which is consistent with published reports from Mexico [45]. Results of our study show lower rates of mammography screening in Mexican participants compared to those in the U.S., albeit these are much higher than those reported in national surveys for Mexico [46].

Limitations of our study include the lack of a population-based sample. Both countries included largely a clinic-based recruitment. Given that Mexico does not have a population-based cancer registry, recruitment for the study occurred in the IMSS hospitals in all three sites. IMSS covers approximately 60% of the Mexican population by providing care to all formally employed individuals and their family members. In Guadalajara, two additional hospitals that serve the unemployed, poorer segment of the population were used for recruitment. Thus, in Mexico, patients seeking care in the private hospital setting are not captured through our recruitment strategies; however, this is likely to represent only approximately 5% of the population [47]. In the U.S., recruitment took place at M.D. Anderson, a tertiary referral center, a county hospital, and a clinic serving indigent patients and underserved women. In Arizona, cases were ascertained at the Arizona Cancer Center, county hospitals in Tucson and Phoenix, community health centers, and other facilities that serve the Hispanic population in the state.

Results of the ELLA Study show that differences exist in breast cancer risk factor patterns between Mexico and the U.S. women. Our experience working with Mexico has shown that logistics related to recruitment, collection of medical record data, and tissue retrieval is less challenging in this country compared to the U.S. Given that our study includes well-characterized populations, with comprehensive epidemiological data linked to well-annotated tumor samples and clinical data, future studies will be extremely valuable in understanding the breast cancer burden in Mexican and Mexican-American women.

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Destruction of an advanced malignant tumour by direct electrical current-case report

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ABSTRACT

We carried out a study on the effect of low-level direct current on cancer by using it to treat a woman who had a large malignant squamous cell carcinoma of the sinus cavity. We used a device that produced low-level direct current and passed the current through the tumour via a 4 × 4 cm flat aluminium foil and a needle electrode that was insulated along its entire length except for the portion actually inserted into the tumour. The treatment was eight hourly daily and lasted for eight weeks. The therapy resulted in the total remission of the tumour and a feeling of wellness by the patient. This finding implies promising therapeutic potential for the use of direct electrical current as a simple, effective, low cost alternative for the treatment of cancer.

Keywords: Destruction; Malignant Tumour; Human Patient; Direct Electrical Current

1. INTRODUCTION

Many clinical cases of cancer do not respond to the conventional approaches of surgery, chemotherapy, radiotherapy, hormone therapy and biological therapy. The disadvantages of these cancer treatment modalities include damage to healthy cells and the resulting significant side effects, such as hair loss, fatigue, hormonal changes that may affect fertility and libido, blood clots, flu-like symptoms, and/or complex, risky, expensive surgical procedures. Consequently, a new cancer treatment modality, which uses a device that is minimally invasive, and provides effective treatment without major side effects, is on demand. One of the newer techniques that researchers and clinicians are investigating for its potential role in clinical therapy is electrotherapy, [1-5] Eaton [6] suggested as early as 1776 that electricity might have a role in the treatment of tumours. He reported the case of a patient with a breast tumour whom lightning struck and her tumour retarded. Other investigators of that era used the available electronic techniques to treat tumours with electricity and narrated positive results [6]. Humphrey and Seal [7] reported that a growing foetus or a growing uterine tumour would cause the uterus to be electronegative with respect to the abdominal surface. In the guinea pig and mouse, the tumour is also negative. This supports the many findings that a growing region is electronegative with respect to a slower growing or non-growing region in the same organism, irrespective of plant or animal [7]. The presence of direct current (DC) surface electro-potentials that a microvolt meter can detect and measure, characterizes living tissues [8]. Humphrey and Seal [7], Schauble et al [8] and Habal [9] described the necrosis and retardation of tumour growth in three solid tumour models when they passed low-level direct current through the tumours. According to Weber [10], a cell must replicate its deoxyribonucleic acid (DNA) strand for it to divide. The building blocks of this strand are four bases that are in short supply in a healthy, resting cell. On the other hand, the building blocks of a related molecule, ribonucleic acid (RNA), are always in great abundance because many cellular functions need RNA. When a cell is ready to divide, an enzyme called ribonucleotide reductase (RR), converts building blocks of RNA into those of DNA. The enzyme RR is therefore pivotal for cell growth. The activity of this enzyme is thus tightly linked, much more than that of any other enzyme, to neoplastic transformation and progression. Kulsh [11] promulgated the hypothesis that a novel way of arresting the activity of this all-important enzyme in cell growth lies in the fact that the active site of RR contains a stable tyrosyl free radical, which is essential for its activity [12]. Kulsh [11] surmised that free-floating electrons, which are easily available in the form of direct electric current, could neutralize or destroy such free radicals. Direct current

electrotherapy should therefore result in inhibition of RR and cessation of malignant cell proliferation. Low-level surface DC electrotherapy would act selectively on cancerous growth since the concentration of the target enzyme RR is exponentially higher in cancerous cells, as compared to healthy quiescent cells [10]. Metastasized cancer should also be treatable by direct current electrotherapy since even in the metastatic state, the biochemical mechanism of cell division involving the enzyme RR remains the same notwithstanding the organ microenvironment.

In view of the fact that both the direction and the spread of electric current can be controlled, thereby limiting the effects to a defined area, we believe that the exploration of this phenomenon holds great promise. It should also be possible to deliver the current to areas of the body that are not accessible to surgery.

A conspicuous and essential point in this article is that the case we present is not a laboratory experiment but a stage IV cancer of the TNM (Tumour, Node, Metastasis) system involving a poor woman in dire condition because of the advanced nature of the disease and because she could not afford the cost of conventional surgery. Furthermore, this is the first time in West Africa that a human patient received direct current therapy.

The aim of this study was to examine the effect of the application of low-level direct current on patients with large malignant oral tumours. We are of the opinion that this novel method of cancer treatment is important in a developing country because it is low-cost, non-toxic, non-invasive, site specific, and easy to administer.

2. CASE REPORT

A 60-year-old Nigerian woman presented to our oral and maxillofacial clinic in 2007. She complained of swelling of the right face and the palate that began three years earlier. In addition, she had hearing problems, especially of the right ear. The tumour has steadily been increasing in size (**Figure 1**). She also complained of blockage of the right nostril, difficulty in breathing and in swallowing, speech impairment and discomfort when chewing. The swellings were tender to touch and the tumour on the palate bled occasionally. The patient's medical history was benign—she denied fever, chills, weight loss, vomiting and nausea. She admitted that she had hitherto patronized unorthodox medical practitioners before a medical doctor referred her to our maxillofacial unit.

Clinical examination showed an elderly, nervous and talkative woman with a facial mass on the right side of the face in moderate respiratory distress. There was also a large swelling (diameter = 4.5 cm) on the palate (**Figure 1**). The lesion did not affect the facial nerve. The

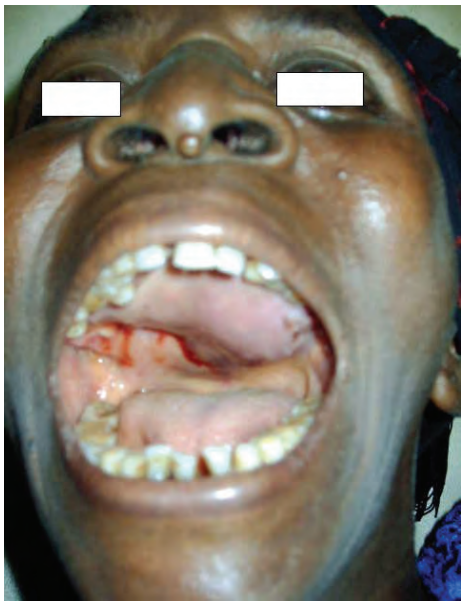

Figure 1. Tumour of the palate before treatment.

radiograph (jug handle or occipitomental view) revealed lytic bone destruction of the right sinus cavity. The biopsy result reported squamous cell carcinoma. We therefore made a diagnosis of squamous cell carcinoma (SCC) in stage IV of the TNM (Tumour, Node, Metastasis) system.

After obtaining ethical clearance from the health institution where we treated the patient; informed consent from her and extra/intraoral photographs, we started treatment using the GEIPE[a] device and the following materials: custom electrodes, needle electrodes, electrode gel, and sandpaper strips. The device was battery-powered and provided constant electrical current. In accordance with the treatment protocol of the GEIPE Cancer Treatment, we prepared the skin by removing dead skin cells from the area with very fine sand paper; cleaning with water; drying and finally rubbing conducting gel on the skin surface. We connected the wires first to a passive surface electrode, which was an aluminium foil plate that measured 4 × 4 cm (extra oral electrode) and then to an active needle (intra oral electrode). This was a 14-gauge stainless steel injection needle, whose hub was removed. It was insulated with tight-fitting silicon tubing that covered the needle except for the 5 mm tip. The exposed tip had a diameter of 2.25 mm, and a surface area of 0.35 cm^2.

We placed the electrodes in such a way that the cancerous tissue fell in the path between them by visualizing a straight line going through the body and through the tumour. We taped the surface electrode to hold it down (**Figure 2**). After sterilizing the tip of the needle, we inserted it into the tumour so that the exposed tip was

Figure 2. Placement of the electrodes.

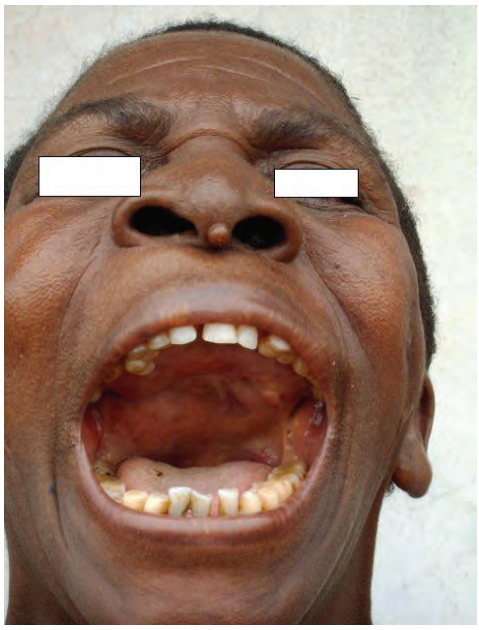

Figure 3. The palate-eight weeks after onset of treatment.

within the tumour and switched the device on. Initially, we passed a current of 2 mA and a voltage of 3 V through the tumour for one hour. After examining the skin and establishing that there were no adverse reactions, we increased the time of treatment slowly to a maximum of eight hours at a time. The GEIPE device, which was equipped with two ON positions, required that the patient or a health worker activate the first ON switch and then the second ON switch after every five minutes. This was necessary in order to avoid electrolysis and dissolution of electrodes. Since we found this procedure stressful, we consulted an engineering firm, GODIAC[b] (Nig.) Ltd that modified the GEIPE device to perform the switching function automatically.

At the end of each treatment session, we gently washed the skin with soap and water and placed a moisturizer on the area. After the first three days of treatment, we observed oedema and discoloration of the lesion in the palate. Seven days later, there was marked necrosis of the lesion. We carefully removed the necrotic tissue and the colour of the palate mucosa normalized in the course of treatment. The extra oral as well as the intra oral tumour flattened after eight weeks of treatment (**Figure 3**), and the patient's accompanying ailments disappeared. She left the hospital against our advice explaining that she felt well and had neither the money nor the need for further medical attention. This was the reason why we could not execute final radiographic examinations and biopsy. We saw the healthy-looking patient eighteen months later in the marketplace where she sold vegetables. She refused our request for her to come for a check-up.

3. DISCUSSION

The case that we reported here suggests that direct electric current can destroy an advanced malignant tumour within a relatively short span of time (eight weeks). Many possible mechanisms may account for tumour destruction by direct current. Kulsh [11] postulated the enzyme-mediated mechanism for the first time. He, however, suggested that voltage between 1.2-3 V would be most beneficial for the disabling of RR through free radical interactions. Kulsh [11] was of the opinion that higher voltage for this mechanism would be undesirable because more and more electrons would engage in electrochemical processes leaving less and less electrons free as free radicals, and the concentration of toxic electrochemical species would increase steadily. In our case, we used 3 V.

Yen *et al*. [13] applied direct current of 400 µA at 3 volts for 208.4 minutes (*i.e.*, 5 Coulombs in 5 mL or 1 Coulomb/mL), to a human cancer cell culture. The pH at the anode decreased to 4.53 and increased to 10.46 at the cathode. The effect of pH alteration on cells is thus likely one of the mechanisms of tumour cell destruction.

A 1994 study by Berendson *et al*. [14], showed that the main reactions at the anode are the formation of oxygen, acidification due to liberated hydrogen ions, and, if platinum is used as anode material, the formation of chloride. At the cathode, hydrogen is formed and hydroxide ions are liberated. Based on calculations, the authors concluded that the liberated hydrogen ions de-

termine the extents of the locally destroyed zone around the anode and that the destructive effect of chlorine probably occurs in an inner zone close to the anode.

Though Marino *et al*. [15] employed a current of 2 mA and a voltage of 3 V in their experiment, they opined that the pH changes in the tumour tissue—to the extent that they overcome the body's buffering capacity—might be responsible for the observed effects on tumour growth. They reasoned that the absence of an effect on tumour growth with AC (alternating current) supports this view because in this case, the electrochemical events at each electrode are identical but they do not produce a pH gradient.

According to Harguindey [16], tumour hyperacidification might activate cytolytic mechanisms through increased activity of lysosomes, resulting in the destruction of tumour tissues. Low pH also inhibits glycolysis and protein synthesis upon which malignant tissues are dependent.

A positive potential on metal electrodes leads to corrosion of the metal with the release of metal ions from the electrode and possible resultant necrosis and metal toxicity. At the anode, the stainless steel electrode is corroded with the ferrous ions going into solution [8]. In their experiment with mice, Morris *et al*. [17] detected that DC-induced tumour necrosis was polarity specific. At the anode, it was coagulative and at the cathode, it was ischemic. They surmised that this was further evidence that the mechanism for tumour destruction was electrochemical in nature. Taylor *et al*. [18] noted that vascular occlusion by thrombosis can be reliably produced by the passage of an appropriate quantity of electrical current.

It must, however, be noted that DC therapy has its limitations, namely, its inability to effect a complete remission in many cases. The percentages of total and partial remissions vary from case to case as evidenced by the works of Turler *et al*. [19] Kirson *et al*. [20] and Barbault *et al*. [21]. Other drawbacks of this therapy its stationary nature (in the case presented here, the patient was in the supine position for eight hours daily) and its duration (again, in our case, the treatment lasted for eight weeks).

All the papers referenced here agree on one issue, namely, that DC destroys tumours. There are, however, diverse views of the way it works. It appears, therefore, that there is yet no clear understanding of the underlying mechanisms of action. Nonetheless, we share the belief of Taylor *et al*. [18] that there is a great therapeutic potential for the development of this new technology. If further studies can confirm the beneficial effects of direct electrical current on malignant tissues, as we have seen in our study and as shown in other studies, then the application of relatively small amounts of direct electrical current using a variety of purpose-designed delivery electrodes, could produce an innovative low cost treatment alternative for patients with malignant diseases. We urge governments and stakeholders in the health care delivery to encourage and support researchers to continue these studies.

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Interactive effect of combined exposure to ethylene glycol ethers and ethanol on hematological parameters in rats

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ABSTRACT

The study of the interaction of three glycol ethers, *i.e.* 2-methoxyethanol (ME), 2-ethoxyethanol (EE) and 2-butoxyethanol (BE) administered subcutaneously for 4 weeks and ethanol simultaneously given as 10% w/v solution for drinking in male rats, was carried out from a toxicodynamic point of view. Administered alone, ME (2.5 and 5.0 mM/kg), EE (2.5 and 5.0 mM/kg) or BE (0.75 and 1.25 mM/kg) resulted in a decrease of red blood cells (RBC), packed cell volumes (PCV), and hemoglobin concentration (HGB), as well as an increase in mean corpuscular volume (MCV) and reticulocyte count (Ret). In the rats co-exposed to ethanol and EGAEs, a significantly less pronounced hematological changes in comparison with animal exposed to these ethers alone were seen. The rats simultaneously exposed to ethanol and both ME and EE at the lower dose demonstrated mainly protection from the alterations in leukocyte system. In contrast, in the rats which consumed ethanol and were simultaneously treated with the higher dose of ME or EE (5.0 mM/kg) the amelioration of same hematological parameters were displayed. The intake of ethanol along with BE treatment at both doses resulted in markedly ameliorated hematological parameters, compared to those which were changed by BE alone. In conclusion, the decrease of the hemolytic effects of EGAEs is ethanol dependent. Ethanol is a substrate of alcohol dehydrogenase (ADH), and affinity of this enzyme to ethanol is greater than that to glycol ethers. It is possible that ethanol results in the change in EGAEs metabolism.

Keywords: Ethylene Glycol Alkyl Ethers; Ethanol; Repeated Exposure; Toxicodynamic Interactions

1. INTRODUCTION

Ethylene glycol alkyl ethers (EGAEs), *i.e.*, 2-methoxyethanol (ME), 2-ethoxyethanol (EE), and 2-butoxyethanol (BE) are extensively used as water-miscible organic solvents in industrial and household applications. Ingestion, inhalation, and/or dermal absorption of these compounds may lead to adverse testicular, teratogenic and hematological effects in animals and humans. These ethers may cause local toxicity, *i.e.*, skin irritation and sensitization in susceptible humans [1] and also, systemic toxicity. The primary systemic toxicity of these chemicals in animals include reproductive, developmental and hematological effects [2-6]. Similar effects have been observed in exposed workers. The hematological toxicity of ME resulted in marrow depression, leukopenia, pancytopenia, and decreased red blood cells count (RBC), hemoglobin concentration (HGB), and platelet count [7,8]. Shih *et al.* [9] showed that the HGB, packed cell volume (PCV), and RBC in male workers exposed to ME for 2.6 years were significantly lower relative to controls. Also, the frequency of anemia in the exposed group (26.1%) was significantly higher, with respect to their corresponding control group (3.2%). Moreover, RBC was significantly negatively associated with air concentrations of ME, whereas HGB, PCV, and RBC were negatively correlated with urinary concentrations of methoxyacetic acid (MAA), a metabolite of ME. These hematological effects were reversible, and they had returned to normal after reduction in ME exposure [10].

Other survey of male shipyard painters exposed to mixed solvents containing EE acetate suggests that this compound might be toxic to bone marrow [11], whereas in female workers chronically exposed to high concentrations of EE, average RBC and HGB levels were nor-

mal [12]. In male workers exposed to low concentration of BE, a statistically significant decrease in PCV value and an increase in mean cell hemoglobin concentration (MCHC) value were observed [13].

EGAEs can produce toxicity following oxidation to the corresponding aldehyde and alkoxyacetic acid by alcohol dehydrogenase (ADH; EC 1.1.1.1) and aldehyde dehydrogenase (ALDH; EC 1.2.1.3), respectively. Both the alkoxyacetaldehyde and alkoxyacetic acid metabolites of the EGAEs are considered to be toxic agents [14,15].

The metabolic activation of EGAEs by ADH and ALDH leads to the appearance of their toxic effects [16,17] . Butoxyacetic acid (BAA), a metabolite of BE, is thought to be a more efficacious hemolytic agent than the parent compound *in vitro*, and causes similar hemolytic changes *in vivo* [17,18].

The inhibition of ADH by pyrazole or 4-methylpyrazole is known to decrease the hemolytic effect of BE [17,19] and the urinary excretion of BAA [16,17]. The simultaneous administration of BE with ethanol, n-propanol or n-butanol to rats almost totally inhibited the hemolytic effect of this chemical, and decreased the urinary excretion of BAA. In contrast, the co-administration of ethanol with ME did not modify the urinary excretion of MAA, but induced accumulation of this metabolite in rats [20,21]. These data mainly arise from acute experiments.

This study presents the results of an investigation into hematological changes in peripheral blood of rats simultaneously exposed to EGAEs and ethanol for 28 days. Ethanol and other primary alcohols are used in industrial solvent compositions containing glycol ethers and are metabolized by ADH and ALDH.

In addition, the excessive consumption of ethanol by a large part of the workers may lead to interactions with EGAEs.

2. MATERIALS AND METHODS

2.1. Chemicals

ME, EE, and BE were purchased from Sigma-Aldrich Ltd, Poland. Other chemicals were obtained from POCh (Poland). ME, EE, and BE solutions were prepared in saline, immediately before dosing, and administered to rats by subcutaneous injections in a fixed volume of 2.0 ml/kg body weight, regardless of a dose.

2.2. Experimental Animals

Experiments were performed on 12-week-old male Wistar rats (Krf: (WI)WUBR), with an initial body weight of 319 ± 22.4 g, and obtained from Jagiellonian University Faculty of Pharmacy Breeding Laboratory (Kraków, Poland). The animals were kept under standard laboratory conditions (temperature 21 ± 2˚C; relative humidity 50 ± 10%) with a 12 h:12 h (light:dark) cycle and had free access to drinking water free of ethanol or 10% w/v ethanol solution, and standard pellet Murigran chow (Agropol, Motycz, Poland) during the experimental period.

2.3. Experimental Design

The rats were randomly divided into fourteen groups of five animals each. The rats (six groups) had free access to drinking water and were treated with ME, and EE at doses of 2.5, and 5.0 mM/kg or BE at doses of 0.75, and 1.25 mM/kg, 5 days per week, for 4 weeks. Control rats (one group) received drinking water *at libitum*.

An ethanol groups (six groups) had free access to an aqueous solution of ethanol (10% w/v solution of rectified spirit POLMOS, Poland) as the only drinking fluid and were treated by subcutaneous injections with ME, EE or BE at the doses mentioned above. Control rats (one group) drank 10% w/v ethanol, but were not exposed to ME, EE or BE.

The rats were observed daily and were weighed once weekly, whereas the consumption of an aqueous solution of ethanol and food was measured daily during the whole experiment.

Before experiment, during exposure and after its termination, *i.e.*, at 0, 4, 11, 18, and 29 day, blood samples from the tail vein of rats were collected for hematological analyses.

The study was accepted by the Local Ethical Committee for animal experiments in Kraków. Procedures involving the animals and their care conformed to the institutional guidelines, in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research.

2.4. Hematological Analyses

Heparin-added whole blood samples, immediately after collection, were used for hematological analyses. RBC, PCV, mean corpuscular volume (MCV), HGB, MCHC, and mean cell hemoglobin (MCH) were analyzed by means of a COBAS MICROS (Roche, Palo Alto, CA, USA) analyzer. Reticulocyte count (Ret) was evaluated after staining blood samples (without anticoagulant) with brilliant-cresol blue. White blood cells (WBC) were counted by a hemacytometer after diluting the fresh blood samples (without anticoagulant) by Türk's reagent solution. The differential white cell count was evaluated after Pappenheim-stained blood films.

Hematological analyses were systematically checked by means of standard human blood CBC-3D Hematology Control (R&D System Inc., Minneapolis. MN, USA).

A day-to-day precision of RBC, PCV, and MCV measurement (n = 30) in blood was 4.2, 4.5, and 4,4%, respectively.

2.5. Statistical Analyses

Results are expressed as the mean ± SD. Data were analyzed by two-way analysis of variance with repeated measurements on one factor and evaluation of simple effects (MANOVA). The analysis was performed with the SPSS 12.0 statistical packet (SPSS Inc., Chicago, IL, USA). For comparison of exposed groups with control group and exposed groups to ME, EE or BE alone with groups simultaneously treated with these compounds and ethanol at each point of time, one-way analysis of variance (ANOVA) followed by Dunnett test was used. Probabilities lower than 0.05 were considered significant.

3. RESULTS

Ethanol intakes in the ethanol alone and co-exposed to ethanol and EGAEs groups were similar, when expressed in mM/kg b.w./24 h (**Table 1**).

Control rats consumed in average 59.6 ± 9.3 g/kg b.w./24 h of Murigran food during the 4 week experiment. The food consumption in the ethanol group and in the co-exposed to ethanol and ME at a dose of 2.5 mM/kg group was similar to that of the control rats. In other co-exposed groups, simultaneously treated with ethanol and EGAEs, the food intake was significantly diminished by mean 31% in comparison to the control group (**Table 1**).

Body weight gain in both control and ethanol-drinking groups was similar. An increase in the body weight, at the end of exposure, in ethanol-drinking rats and simultaneously treated with ME, and EE at a dose of 2.5 mM/kg or exposed to BE at a dose of 0.75 mM/kg was significantly lower in comparison to both control and ethanol groups. The rats co-exposed to ethanol and ME or EE at a dose of 5.0 mM/kg and BE at a dose of 1.25 mM/kg lost weight by 42.7 ± 5.1, 28.0 ± 6.2, and 11.0 ± 0.6 g, respectively (**Table 1**).

3.1. Hematological Changes

The consumption of ethanol alone for 4 weeks had no effect on hematological parameters. ME administration at doses of 2.5 and 5.0 mM/kg resulted in a decrease of RBC, PCV, and HGB that occurred from 11th day of exposure to its termination and in an increase in the Ret exclusively at the end of exposure. At the end of experiment, *i.e.*, on day 29, the greatest changes of RBC, PCV, HGB, and Ret were observed (**Figure 1**). These hematological parameters demonstrated dose, both dose and time, and time dependence.

In the rats co-exposed to ethanol and ME, significantly less pronounced hematological changes were seen, mainly in HGB, PCV and MCH (**Figure 1**).

Hematological changes in the peripheral blood of rats treated with EE alone were less pronounced than those after ME administration. The lower dose of this compound (2.5 mM/kg) resulted only in a decrease of HGB and an increase of MCV on days 11 and 18 of experiment. The higher dose of EE (5.0 mM/kg) led to a significant decrease in RBC, PCV, HGB, as well as to an increase in MCV and Ret for most of the exposure time. These hematological changes demonstrated dose dependence and both dose and time, and time independence (**Figure 2**).

In the rats simultaneously treated with EE at the higher dose (5.0 mM/kg) and ethanol, RBC, PCV, and

Table 1. Effects of ethylene glycol alkyl ethers on an average ethanol and food consumption and body weight gain in rats.

Group	**Ethanol intake** (mM/kg b.w./24 h)	**Food intake** (g/kg b.w./24 h)	**Body weight gain** (g/4 weeks)
Control[1]		59.6 ± 9.3	+24.3 ± 0.9
10% Ethanol	123.1 ± 18,9	52.3 ± 6.2	+23.0 ± 0.8
ME (2.5 mM/kg) + 10% ethanol	90.3 ± 20,0	55.5 ± 8.7	+2.4 ± 0,2*†
ME (5.0 mM/kg) + 10% ethanol	115.0 ± 23.9	36.6 ± 3.9*†	–42.7 ± 5.1*†
EE (2.5 mM/kg) + 10% ethanol	136.7 ± 1.9	42.7 ± 3.4*†	+4.0 ± 0.4*†
EE (5.0 mM/kg) + 10% ethanol	145.3 ± 16.0	39.2 ± 5.8*†	–28.0 ± 6.2*†
BE (0,75 mM/kg) + 10% ethanol	130.0 ± 3.1	42.3 ± 3.7*†	+4.0 ± 0.1*†
BE (1,25 mM/kg) + 10% ethanol	124.0 ± 6.2	43.2 ± 4.1*†	–11.0 ± 0.6*†

[1]The rats had free access to drinking water. Values are the means ± SD of 5 animals/group. $p < 0.05$ compared to control (*) and ethanol (†) group.

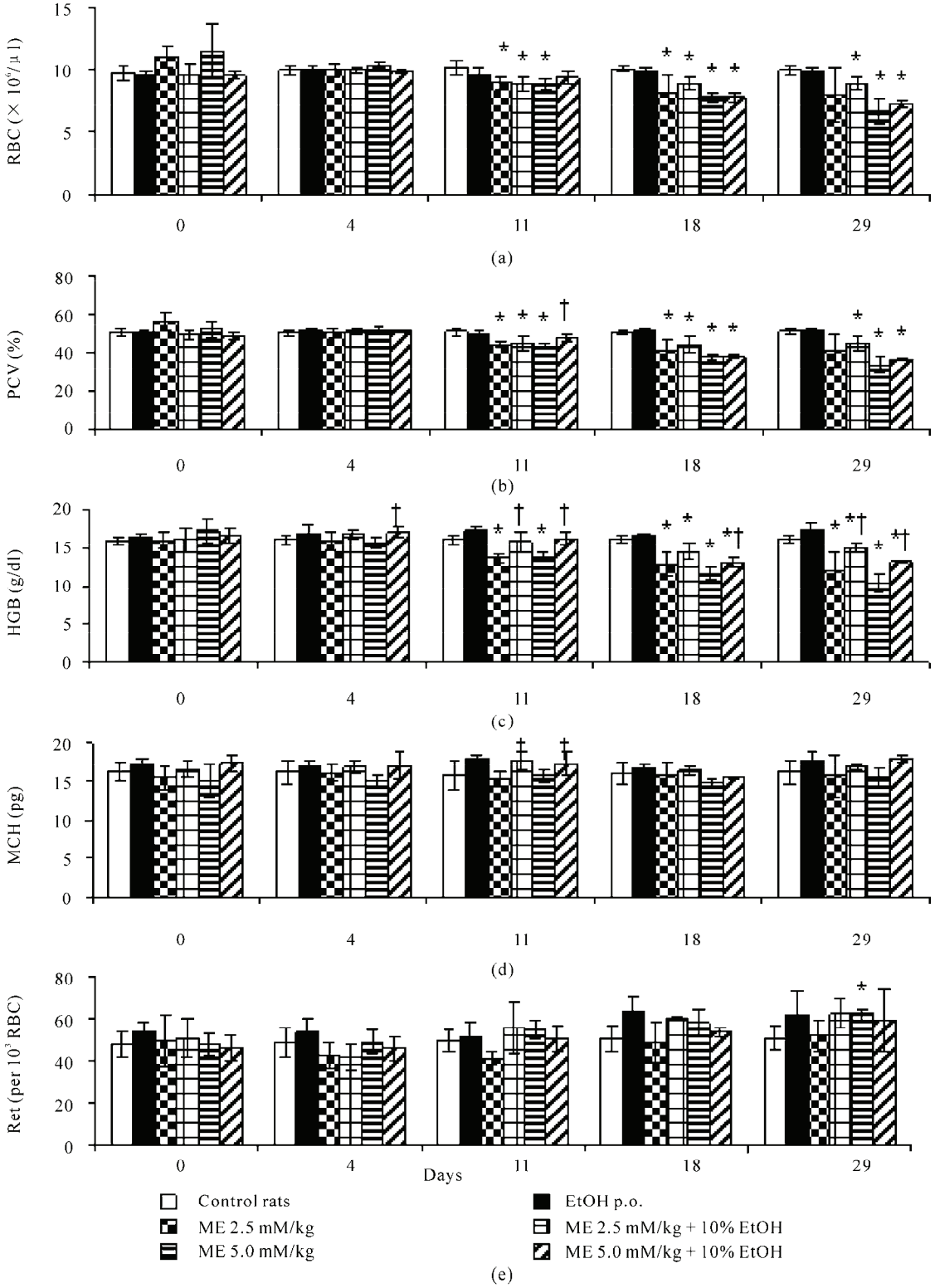

Figure 1. Effects of ethanol (EtOH) consumption on RBC (a), PCV (b), HGB (c), MCH (d) and Ret (e) values of the peripheral blood at the designated time points of male rats treated with methoxyethanol (ME) at doses 2.5 mM/kg b.w. or 5.0 mM/kg b.w. The values are means ± SD of five rats, *—$P \leq 0,05$ significantly different from control rats; †—$P \leq 0,05$ significantly different from rats treated with ME alone.

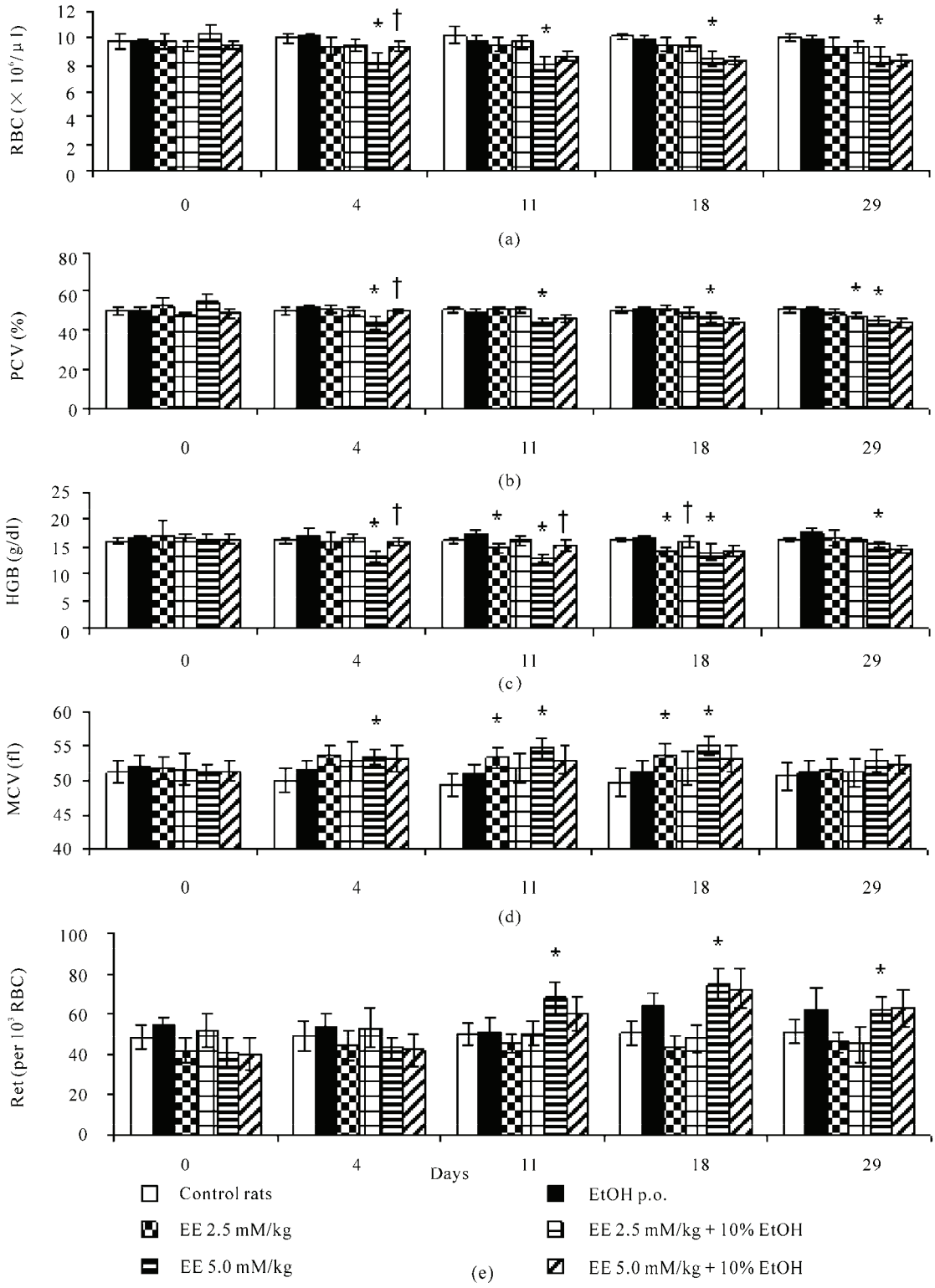

Figure 2. Effects of ethanol (EtOH) consumption on RBC (a), PCV (b), HGB (c), MCV (d) and Ret (e) values of the peripheral blood at the designated time points of male rats treated with ethoxyethanol (EE) at doses 2.5 mM/kg b.w. or 5.0 mM/kg b.w. The values are means ± SD of five rats, *****—$P \leq 0.05$ significantly different from control rats; †—$P \leq 0.05$ significantly different from rats treated with EE alone.

HGB at day 4, and HGB at day 11, were significantly higher than in the rats exposed to EE alone. These parameters did not differ in comparison to the control group (**Figure 2**).

BE administration at doses of 0.75 and 1.25 mM/kg resulted in a decrease in RBC, PCV, and HGB and an increase in MCV, and Ret. The greatest changes of RBC, PCV, and HGB were observed at day 4 of experiment, whereas other hematological alterations (MCV, and Ret) showed a maximum on day 11 of the exposure. The changes in RBC, MCV, and Ret during exposure period were persistent, whereas alterations in PCV, and HGB independently on duration of exposure, were reversible (**Figure 3**).

In the rats co-exposed to BE and ethanol, the values of RBC, PCV, and HGB were significantly higher, whereas MCV, and Ret were markedly lower in comparison to the rats treated with BE alone (**Figure 3**). Some of hematological parameters (HGB, MCV, and MCHC) in rats exposed to BE at a lower dose (0.75 mM/kg) and ethanol were similar to those in the control group.

3.2. Leukocyte Alterations

The leukocyte counts were markedly reduced at each dose of ME from the 4th day of exposure to its termination. These alterations were caused by a decrease mainly in the number of lymphocytes. Also, reductions in number of neutrophils in rats treated with ME at a dose of 5.0 mM/kg at days 11 and 18 of the experiment were observed. Both leukocyte and lymphocyte alterations at a dose of 5.0 mM/kg demonstrated dose, dose and time, and time dependence (**Figure 4**).

The rats simultaneously exposed to ethanol and the lower dose of ME (2.5 mM/kg) showed a lack of changes in both leukocyte and lymphocyte counts in comparison to control group at days 11, and 18 of the experiment. The number of lymphocytes in those animals at days 18 and 29 of the experiment was significantly lower than in the control group, but statistically higher in comparison to the rats treated with ME alone. In the rats co-exposed to ethanol and the higher dose of ME (5.0 mM/kg), the leukocyte and lymphocyte counts were significantly diminished in comparison to the control group, but very similar as in the group exposed to ME alone. Also, the reduction of the number of neutrophils at days 11, 18, and 29 was observed (**Figure 4**).

No significant leukocyte and lymphocyte alterations in rats exposed to EE at a dose of 2.5 mM/kg were observed. In the rats treated with EE alone at the higher dose (5.0 mM/kg) the leukocyte counts at day 4 and 18 of the experiment were statistically lower than in the control group. The number of lymphocytes in these rats was reduced in comparison to the control group during the whole of exposure period (**Figure 5**).

The number of both leukocytes and lymphocytes in the rats co-exposed to EE at a dose of 2.5 mM/kg and ethanol only at day 4 of the experiment was significantly higher in comparison to group treated with EE alone. In the rats simultaneously exposed to ethanol and EE at the higher dose (5.0 mM/kg) the leukocyte counts on day 4, and the number of lymphocytes on days 4, and 29 were significantly lower than in the control group (**Figure 5**).

BE alone had no effect on leukocyte and lymphocyte counts in peripheral blood in rats treated at the dose of 0.75 mM/kg and 1.25 mM/kg for 28 days.

In the rats simultaneously exposed to ethanol and BE at the dose of 0.75 mM/kg and 1.25 mM/kg the number of both leukocytes and lymphocytes in peripheral blood were similar as in the control group.

4. DISCUSSION

The aim of the present work was to assess the effect of ethanol drinking on hemolytic action of EGAEs in rats. The experimental protocol applied in this experiment aimed at the constitution of a model of conditions that may take place in human life.

The treatment with ethanol may be tantamount to its misuse in man. The daily consumption of ethanol in the rats drinking 10% (w/v) water solution of ethanol was equivalent to about 0.7 l/day of 40% vodka in men [22]. Since the rate of ethanol oxidation in rats (0.3 g/kg/h) is three times faster than in humans, these animals needed a higher dose of ethanol to produce comparable toxic effects.

The rats simultaneously treated with EGAEs and ethanol consumed similar quantity of ethanol as in the control group. Food intake in these animals was diminished in each experimental group in relation to both control and ethanol groups. The reduction in food intake was most pronounced at the highest dose of ME, EE, and BE, *i.e.*, 5.0, 5.0, and 1.25 mM/kg, respectively. The growth retardation in rats simultaneously exposed to EGAEs and ethanol was observed. It was most likely to be caused by the reduced food consumption observed in these animals. Although ethanol has been reported to cause anorexia and weight loss [23,24], it seems that EGAEs alone may be one of the main reasons for retardation of body weight gain. The body weight alterations observed previously in rats exposed to EGAEs alone [6] seem to confirm of this suggestion.

In the present study, it was found that subcutaneous repeated administration of each of three EGAEs led to distinct hematological alterations. These alterations were evidenced by reduction in RBC, PCV, and HGB, and also by an increase in MCV value and Ret in peripheral

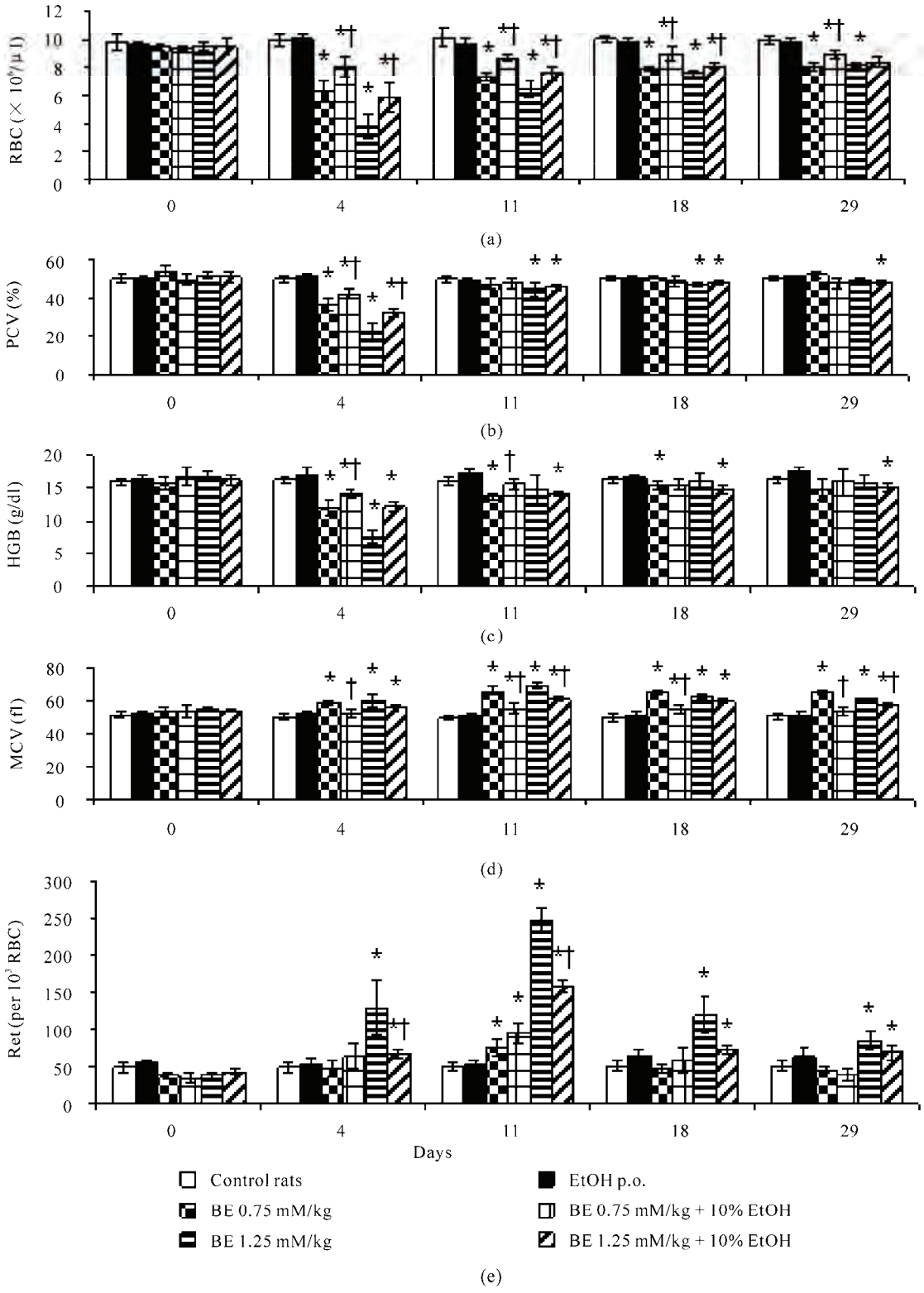

Figure 3. Effects of ethanol (EtOH) consumption on RBC (a), PCV (b), HGB (c), MCV (d) and Ret (e) values of the peripheral blood at the designated time points of male rats treated with buthoxyethanol (BE) at doses 0.75 mM/kg b.w. or 1.25 mM/kg b.w. The values are means ± SD of five rats, *—$P \leq 0{,}05$ significantly different from control rats; †—$P \leq 0.05$ significantly different from rats treated with BE alone.

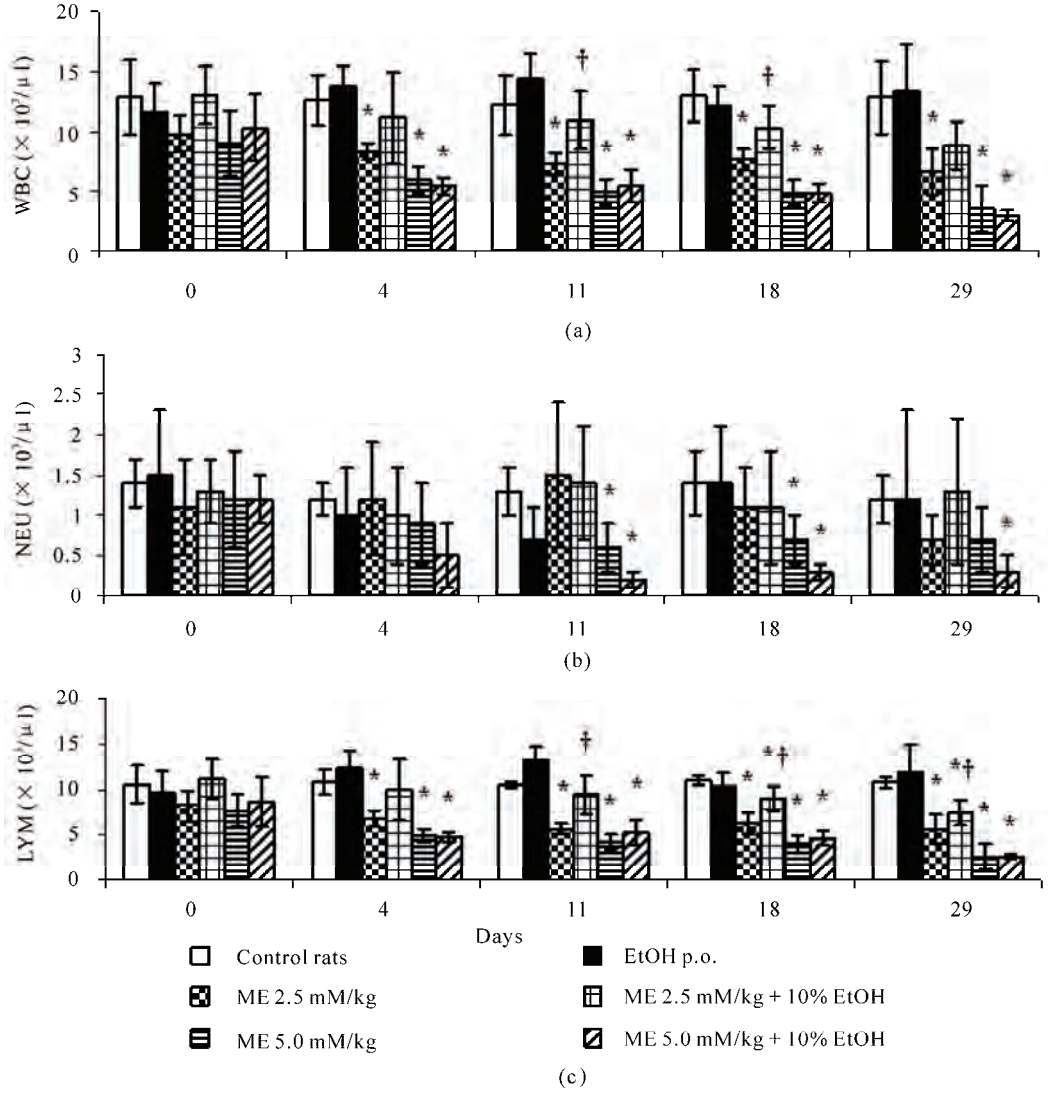

Figure 4. Effects of ethanol (EtOH) consumption on WBC (a), NEU (b) and LYM (c) values of the peripheral blood at the designated time points of male rats treated with methoxyethanol (ME) at doses 2.5 mM/kg b.w. or 5.0 mM/kg b.w. The values are means ± SD of five rats, *—P ≤ 0,05 significantly different from control rats; †—P ≤ 0,05 significantly different from rats treated with ME alone.

blood. While in rats treated with ME these changes were strongly pronounced and progressively increased with exposure time beginning from the day 11, those in animals treated with EE were less pronounced and rather persisted at low constant level for the whole exposure period. On the contrary, the rats treated with BE demonstrated the distinct intravascular hemolysis resulted in hemolytic anemia at the beginning of exposure (on day 4). Independently of exposure duration, these alterations were regressed, although the decrease in RBC and the increase in MCV were more persistent, probably due to the selective hemolysis of the aged erythrocytes [25], leaving a population of young red cells. Hemoglobinuria observed only in the first day of exposure to BE at dose of 1.25 mM/kg seems to confirm of this suggestion. The various hematological changes observed during exposure to EGAEs are typical of hemolytic anemia with an associated reticulocytosis and hyperplasia of both bone marrow (erythroid elements) and spleen (extramedullary hemopoiesis) [26].

The results presented in this paper confirm previous observations that continued exposure to BE, contrary to ME and EE, resulted in significantly less pronounced hematological changes [6]. While the majority of hematological effects were dramatic at the beginning of the exposure, later these alterations clearly regressed despite continued weekly exposure to these compounds. It was suggested that the gradual recovery from the haemolytic

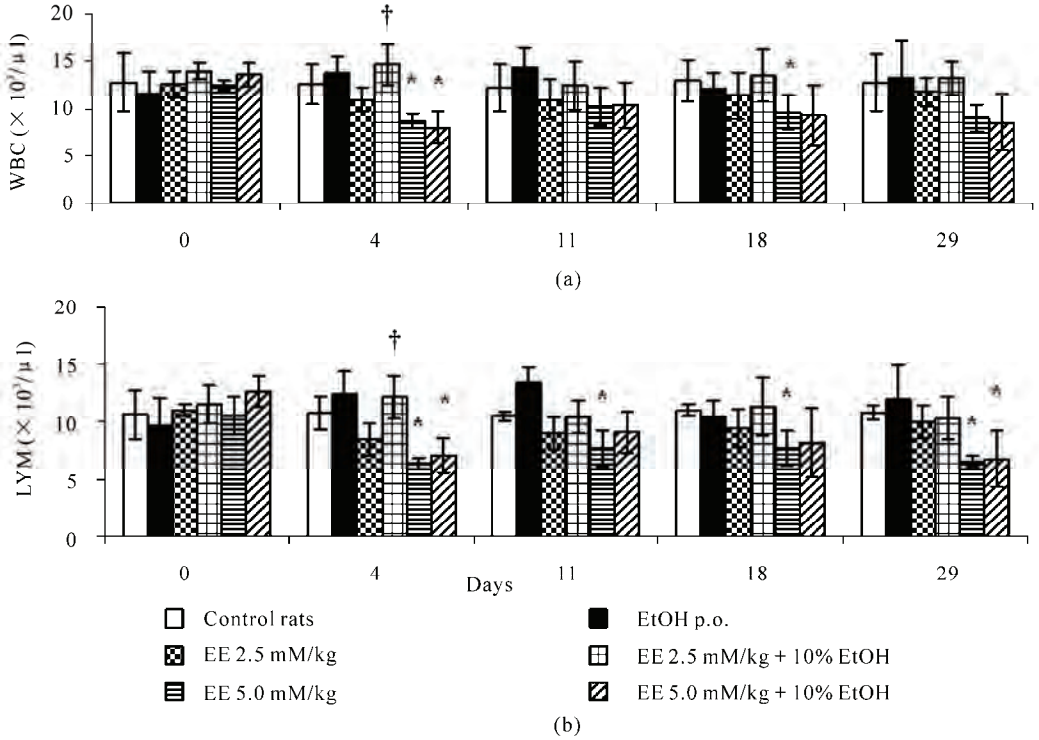

Figure 5. Effects of ethanol (EtOH) consumption on WBC (a) and LYM (b) values of the peripheral blood at the designated time points of male rats treated with ethoxyethanol (EE) at doses 2.5 mM/kg b.w. or 5.0 mM/kg b.w. The values are means ± SD of five rats, *—$P \leq 0.05$ significantly different from control rats; †—$P \leq 0.05$ significantly different from rats treated with EE alone.

anemia may be associated with tolerance development to the hemolytic effect of BE. This tolerance was characterrized by a progressive removal of hematological changes, manifested in an increase of RBC, PCV, HGB, and a decrease in Ret value in peripheral blood. The tolerance to BE-induced hemolytic anemia was also observed in other studies [27,28]. Moreover, our studies demonstrated that the effects of repeated exposure for 28 days to BE were less pronounced than the effects of single doses of this ether at the same or comparable doses [29,30].

The changes in the leukocyte system of rats were produced by two of three EGAEs, but there were marked quantitative differences in the responses. While ME strongly suppressed this system causing leukopenia, lymphocytopenia, and neutrocytopenia, EE at the higher dose (5.0 mmol/kg) only, exerted the middle inhibitory effect resulted in the occasional reduction of leukocytes with a simultaneous decrease in the number of lymphocytes at each time point of the exposure. In contrast, BE caused no changes in the leukocyte system. These results are inconsistent with the data of Grant *et al.* [26] which observed inhibitory effects of ME and BE on leukocyte system in rats. This inconsistency concerning BE only, may be due to considerably higher doses of this compound administered to rats, reported in the paper cited. The literature data [26,31] indicate that ME is immunotoxic. Dermal exposure of rats to this compound at dose levels in a range of 2-16 mM/kg/day for four consecutive days produce droplets in thymus and spleen weight, enhanced the lymphoproliferative responses to mitogens, and a reduction in the antibody plaque-forming cell response to either trinitrophenyl-lipopolysaccharide or sheep RBC. Miller *et al.* [32] observed lymphoid tissue atrophy after inhalation of ME in male New Zealand white rabbits. Also Smialowicz *et al.* [33,34] reported a marked immunosuppressive effects of ME and both MAA and methoxyacetaldehyde, the metabolites of ME, on thymus weight and lymphoproliferative functions in rats.

There is still too little knowledge on the effects of ethanol on EGAEs-toxicity, especially on hematological changes. Morel *et al.* [21] did not observed the effects of three aliphatic alcohols, *i.e.*, ethanol, n-propanol, and n-butanol, at dose of 10 or 30 mmol/kg, on the urinary creatine/creatinine ratio, the testicular toxicity or the 24 h urinary excretion of MAA in rats after simultaneous treatment with a single dose of ME (10 mM/kg) by gavage. On the other hand, the simultaneous administration of 30 mM/kg of above mentioned alcohols almost totally inhibited the hemolytic effect of BE (5 or 1 mM/kg), and

reduced the urinary excretion of BAA, metabolite of this compound, by 31-43%. It was suggested that competitive inhibition of ADH by alcohols results in the change in BE metabolism.

The results of the present study indicate that ethanol alone consumption did not have any effect on examined hematological parameters. However, ethanol intake along with EGAEs, only partially protected the rats against hemolytic effects and the alterations in leukocyte system induced by these ethers. The preventive effect was seen at both lower and higher doses of ME (2.5 and 5.0 mM/kg) and BE (0.75 and 1.25 mM/kg), as well as at the higher dose of EE (5.0 mM/kg), while in rats simultaneously exposed to ethanol and both ME and EE at the lower dose (2.5 mM/kg), mainly protection from the alteration in leukocyte system was observed. In contrast, the rats which consumed ethanol and simultaneously were treated with the higher dose of ME or EE (5.0 mM/kg), demonstrated the amelioration of these hematological parameters (RBC, PCV, and HGB) in relation to the animals exposed to these compounds alone. On the other hand, ethanol intake along with BE treatment (both 0.75 and 1.25 mM/kg) markedly ameliorated hematological parameters, especially RBC, PCV, HGB, MCV, and Ret in comparison with the group exposed to this ether alone.

The results presented in this paper clearly demonstrate that ethanol modifies the hematological effects of continued exposure to EGAEs. The explanation the biological basis of the interactions between ethanol and EGAEs may be related to the metabolism of these compounds. The glycol ethers, especially EE, BE, and 2-phenoxyethanol, are metabolized in vitro by rat hepatic and cutaneous cytosolic preparations in the reaction involving ADH and ALDH in both tissues [35]. Hepatic cytosol metabolizes ethanol in preference to intermediate chain-length EGAEs, whereas the skin cytosol preferentially metabolizes the glycol ethers. It was found that EGAEs oxidation is performed predominantly by ADH3, and ALDH1 isoenzymes. Repeated exposure to EGAEs results in induction of these enzymes. The rates of ADH oxidation by rat liver cytosol were the greatest for ethanol followed by EE and BE. In contrast, the order of metabolism by rat skin cytosolic fraction was changed to BE > EE > ethanol [35]. Although skin contains enzymes that have the capacity to biotransformation EGAEs localized in the basal layer of the epidermis [36], the physicochemical properties of these compounds result in rapid penetration and distinct reduction in dermal metabolism during their percutaneous absorption [37]. Thus, EGAEs are mainly metabolized in the liver similarly as ethanol.

In conclusion, the decrease of the hemolytic effects due to EGAEs is ethanol dependent. Ethanol is a substrate of ADH, and the affinity of this enzyme is higher than those of glycol ethers. It is possible that ethanol results in the change in EGAEs metabolism. The difference between EGAEs metabolism may be one of the reasons for the differences observed between ME, EE, and BE in the interaction of ethanol.

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P53 pseudogene: potential role in heat shock induced apoptosis in a rat histiocytoma

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ABSTRACT

The p53 tumor suppressor gene is either non-functional or highly and frequently mutated in majority of cancers. In our study towards understanding cellular adaptations to stress using a rat histiocytic tumor model, we have identified mis-sense mutation in p53 that led to premature termination of translation at the carboxyl-terminus. Further, the cDNA isolated from heat stressed cells producing two amplicons with cDNA specific primers (N-terminus) suggested occurrence of possible pseudogene(s). A comparative analysis between different tumor cell lines of rat origin and rat genomic DNA using p53 gene specific primers resulted in the amplification of a processed pseudogene and its positive interaction with wild type p53 probe on Southern blot analysis. The genomic DNA sequence analysis, and sequence comparison with cDNA discovered that the processed pseudogene lacks DNA binding domain and nuclear localization signal, however, contains the ribosomal entry and stop signals. Rat genome BLAST analysis of the pesudogene suggested chromosome-18 localization which was in addition to 14, 13, 10, 9 localization of the cDNA. In the interest of unraveling hidden dimensions of p53 tumor suppressor gene, our study explores the probability of p53 functional pseudogenes in rat histiocytoma.

Keywords: Pseudogene; P53; Tumor; Rat Histiocytoma

1. INTRODUCTION

The tumor suppressor p53 is a multifunctional protein that is involved in a variety of biological processes such as growth arrest, apoptosis, differentiation and senescence [1,2]. Aberrant expression of this gene results in either a gain of transforming potential or a loss in tumor suppressor activity [3,4]. The p53 gene mutation, deletion, insertion or protein sequestration etc are often found in many cancers [5,6] and these mutations affect the p53 binding to DNA [7]. Analysis of the degeneracy of p53 DNA-binding site suggests that there may be as many as 200-400 p53 target sequences or perhaps more [8]. Despite the high frequency with which p53 is mutated during tumor development, a substantial proportion of tumors still express the wild type p53 [9]. This could be the reason in spite of exhaustive information on p53 modifications the corresponding role of p53 modification in experimental animal tumor models is poorly understood.

We are investigating the role of p53 in heat stress-induced rat histiocytic tumors models. In the process of elucidating heat stress induced cell death pathways and evaluating the functional significance of p53 in heat shock induced cell death in tumor cells, we have identified mutated form of p53 with two functional alleles by reverse transcriptase polymerase chain reaction, and the deletion and addition of nucleotides had resulted in C-terminal deletion of 50 amino acids. We demonstrated that Fas/CD95 induced apoptosis requires p53, and hypothesized that C-terminal deletion and loss of oligomerization domain and nuclear localization signal probably are responsible for p53-transcritpion independent apoptosis as suggested [10,11]. In the present study we show that there are two processed pseudogenes for p53 in this tumor model and one of them also has ribosomal entry site. A comparative genome analysis further revealed that the processed pseudogene is predominantly present in all the rat and mouse species but absent in humans.

2. MATERIALS AND METHODS

2.1. Animal Handling

All animal maintenance and handling was accomplished as per the institutional ethical committee approval at Centre for Cellular and Molecular Biology, Hyderabad, India.

2.2. Tumor Growth and Cell Culture Maintenance

AK-5 tumor cell line is established from i.p injections of cell-free ascites fluid of a chemically induced and established rat liver tumor, Zajdela ascetic hepatoma (ZAH). These cells possess typical characteristics of macrophages. Single clone of AK-5 tumor, called BC8, was adapted to grow in culture for several generations in Dulbecco's Modified Eagle's Medium (DMEM) with 10% heat inactivated fetal calf serum (FCS) in the presence of penicillin (100 U/ml) and streptomycin (50 µg/ml) is used in the present study. Rat fibroblasts (F111) was procured from ATCC and maintained similar to BC8 as mentioned above. BC-8 cells (8×10^6 cells) were used for injection either for s.c. or i.p. of six-week-old naïve male Wistar rats and tumor growth was monitored. The i.p tumor development approximated by the mean total cell mass calculated from the percentage of packed cells and the total ascites weight.

2.3. Genomic DNA Isolation

For normal rat live genomic DNA, six month old male Wistar Rat was scarified as per institutional animal ethics recommendations and genomic DNA was isolated from the liver by phenol: chloroform method and used in the present experiment.

2.4. RNA Isolation and cDNA Library Construction

The control and heat stressed tumor cells are subjected to single step total RNA isolation using Trisol reagent, the integrity of RNA was examined by 1% agarose gel, and 5 µg total RNA was used for cDNA preparation by reverse transcriptase system containing the MMLV reverse transcriptase enzyme and oligo d(T) primer and the cDNA prepared was used for further experiments.

2.5. Primers Used for the Polymerase Chain Reaction

PCR primers were designed for the cDNA clone spanning the coding sequence of rat wild type p53 (Acc. No. X13058). Four sets of primers for the amplification of full length as well as partial cDNA amplification were made and used in the present study (**Figure 1(a)**). Primer set II, P1: Forward-5' atggatccatggaggattcacagtcg 3', P2: Reverse-5' atgaattcgcacagggcatggtcttc 3'; Primer Set III, P3: Forward-5' atggatcctctgccagctggcgaagacat 3', P4: Reverse-5' atgaattcggacaggcacaaacacga 3'; Primer Set IV, P5: Forward-5'atggatcctgaggttcgtgtttgtgc 3', P6: Reverse-5' atgaattctgtcagtctgagtcaggc 3'; and the Primer set I is the combination of primers P1 and P6. Care has been taken while designing the PCR primes to have 50% GC content. The PCR conditions are as follows, 94˚C—1 min followed by 94˚C—1 min, 55˚C—1 min, 72˚C—1 min × 30 cycles unless otherwise indicated.

2.6. Southern Blot Analysis

The full length wild type p53 cDNA (1.2 kb) was radiolabeled using α^{32}P-dATP by random primer labeling. Hundred nanograms of the template DNA was incubated

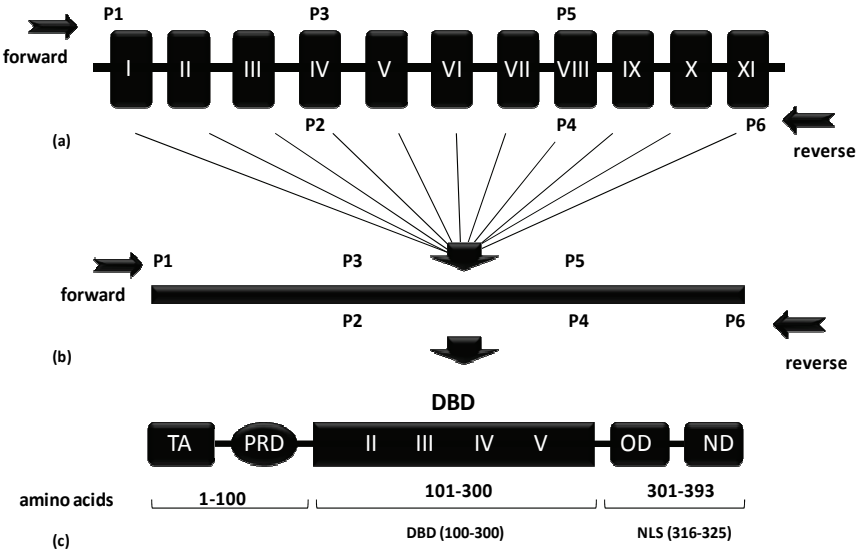

Figure 1. Structural organization of p53. (a) Genome organization depicting the exons of p53 and indicating the primers P1-P6 matching regions; (b) Schematic representation of coding region (cDNA) indicating primers P1-P6; (c) Functional domains showing the conserved regions of p53, appropriate amino acid lengths (1-393) are mentioned. DBD: DNA binding region; NLS: nuclear localization signal.

(37˚C, 15 min) with dNTPs exempting dATP and in the presence of 10 µci of α^{32}P-dATP, random primer, Klenow enzyme (5 U) and reaction buffer. After the reaction, labeled template was purified through sephadex G-50 column, probe containing 1×10^8 µci per microgram DNA was used for hybridization. PCR amplicons first run on 1% agarose gels were vacuum transferred to N+ nylon membrane (Amersham), UV cross-linked and hybridized with radiolabeled probe for overnight. Blots were washed under stringent conditions (sodium phosphate buffer + SDS) and exposed to X-ray film, and photographed.

2.7. Cloning and Sequence Characterization

The PCR amplicons are purified using PCR Wizard purification system (Promega, USA) either cloned in TOPO cloning vector and or taken to automated DNA sequence analysis (Model 3730, M/s Applied Biosystems, USA). The obtained DNA sequences were subjected to blast analysis (Entrez at http://www.ncbi. nlm.nih.gov) and the deduced amino acid sequences were analyzed at http://www.expasy.ch, and http://www. isrec.isb-sib.ch.

3. RESULTS

3.1. Heat Stress Induces p53 Transcription

In continuation of our interest to know the functional significance of p53 in rat histiocytic tumor models, we compared control cells with heat stress and found that heat stress enhanced p53 transcription (**Figure 2(a)**). Interestingly when heat stressed samples were subjected for partial PCR analysis we found that primer set II gave two prominent amplicons, while primer sets III and IV giving single amplicon (**Figure 2(b)**). The PCR amplicons obtained by all the primer sets were excised from agarose gel, purified using PCR product purification kit (PCR Wizard, Qiagen) and re-amplified using same set of primers. All the amplicons showed significant re-amplification suggesting that these amplicons are p53 gene specific (**Figure 2(c)**). However to confirm and avoid ambiguity with p53 sequence specificity, all the products hybridized with wild type radio labeled p53. Except the lower band of the amplicon with primer set II, all other amplicons showed signficant binding to the radiolabeled probe (**Figure 2(d)**). The amplicons were cloned in TA cloning vector (Promega) and subjected to automated DNA sequencing. The sequences obtained were aligned with wild type p53 cDNA sequence and found to be homologous (data not shown). While full length did not show any duplication, only primer set II showing such amplicon suggested presence of possible pseudogenes.

3.2. BC8 Genome Contains a Processed Pseudogene

In addition to the two alleles reported [10] the additional amplicons obtained may be related to processed p53 alleles originating from the genomic DNA. Therefore the genomic DNA from the tumor cells was isolated and subjected to genomic PCR using p53 cDNA specific primer sets I, II, III, and IV. While primer sets I, II, and IV were giving a single amplicon, primer set III did not yield any amplification (**Figure 3(a)**). Genomic southern however identified only the full length amplicon amplified using the primer set I (**Figure 3(b)**). These results therefore suggested a processed pseudogene of p53 in these tumor cells.

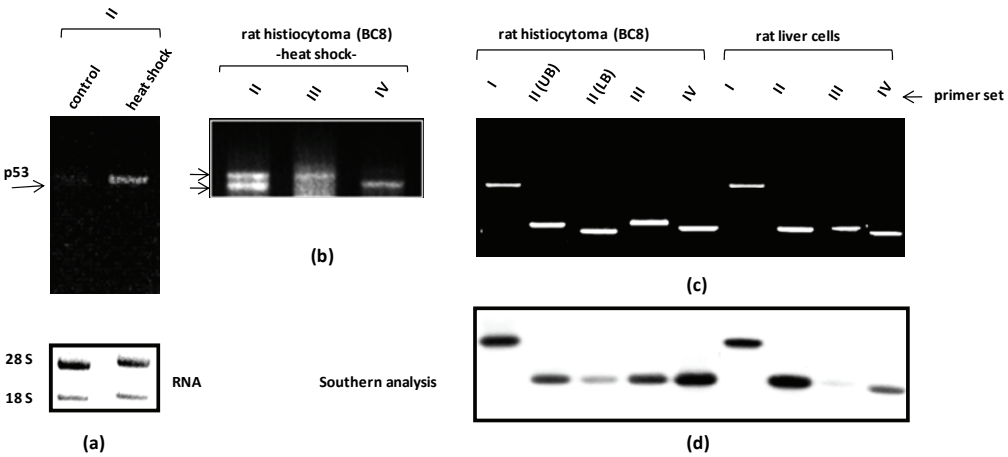

Figure 2. Reverse transcription and polymerase chain reaction. (a) The total RNA from control and heat shocked BC8 tumor cells was isolated and subjected to RT-PCR analysis with primer set I. The RNA loading control was also shown with intact 28S and 18S RNA; (b) The cDNA of heat shocked BC8 cells was used as a template to amplify p53 with primer sets II, III, and IV. Note only the primer set II showing two amplicons; (c) Re-amplification of first round PCR products after gel elution with appropriate primer sets mentioned; (d) Southern blot analysis of re-amplified PCR products.

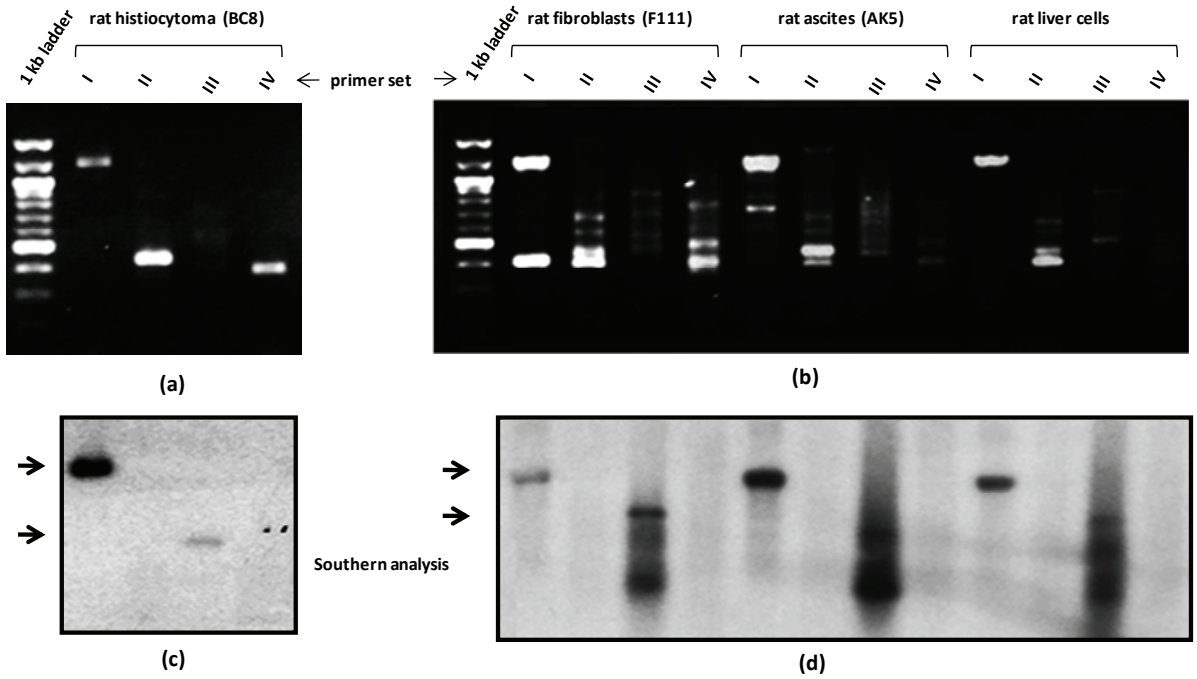

Figure 3. Genomic PCR and Southern analysis. (a) BC8 genomic PCR analysis with four primer sets, I, II, III, and IV; (b) Genomic Southern analysis of PCR products obtained from **Figure 3(a)**; (c) Genomic PCR analysis of rat fibroblasts, rat histiocytoma (AK5), and rat liver cell; (d) Genomic Southern analysis of PCR products obtained from **Figure 3(c)**.

Processed pseudogenes arise through a mechanism whereby a spliced mRNA is reverse transcribed and subsequently inserted into the genome [12]. If pseudogenes are formed in this way during evolution, these pseudogenes should present in the rat genome and should coexist with all the cell types. To examine this, transformed rat fibroblast cells (F111), parental ascites rat histiocytoma (AK5) were compared with the normal rat genomic DNA.

3.3. Blast and In-Silico Translational Analysis

We went ahead of cloning p53 pseudogene and sequence alaysis of cloned product using automated DNA sequencing. From the sequence analysis we found that there is indeed a processed pseudogene having a potential to provide two gene products with different reading frames. A comparative sequence alignment of processed pseudogene with full length RT-PCR product of rat histiocytoma additionally showed high sequence homology. Analysis of pseudogene revealed loss of DNA binding region (nt 700-860) and nuclear localization signal (nt 1030-1080) of cDNA (**Figure 4**). Whole rat genome Blast analysis with cDNA sequence identified its chromosome localization on chromosomes 14, 13, 10, 9 and 2, and the pseudogene sequence Blast identified its additional localization at chromosome 18 (**Table 1**).

4. DISCUSSION

The p53 gene is frequently lost or rearranged in a large variety of cancers, and most of the alterations in p53 are found in the core domain that interfere with p53 DNA-binding activity [5]. Although p53 has been a wonder molecule and the guardian of genome, mutation of p53 affects its native functions including the antiapoptotic function. Several p53 mutant cells are reported to have lost apoptotic functions but not the cell cycle inhibition [13,14]. While our earlier study suggesting that loss of C-terminal 50 amino acids could have played a role in p53-transcription independent apoptosis via Fas/CD95 translocation from golgi to plasma membrane [11,15], a report from Zhu *et al*. [16] indicated that the N-terminal 43-63 amino acid are more than sufficient to activate p53 transcription dependent apoptosis. Further, induction of pro-apoptotic factor Bax, a known transcriptional client for p53 [17], and subsequent activation of intrinsic apoptotic death pathway through mitochondrial dysfunction [18] directed us to look for possible processed genes in the tumor genome.

By definition, pseudogenes lack a function. However, the classification of pseudogenes generally relies on computational analysis of genomic sequences using complex algorithms [19]. It has been established that quite a few pseudogenes can go through the process of

transcription, either if their own promoter is still intact or in some cases using the promoter of a nearby gene; this expression of pseudogenes also appears to be tissue-specific [20]. Pseudogenes are often referred to in the scientific literature as nonfunctional DNA. Failure to observe pseudogenes coding for a product under experimental conditions is no proof that they never do so inside an organism. Homologous recombination between the intact functional p53 gene and the p53 pseudogene is thought to have occurred in such a perturbed intracellular environment with genomic instability, thus inactivating the intact allele of the functional p53, therefore the persistence of pseudogenes is in itself additional evidence for their activity. Natural selection would remove

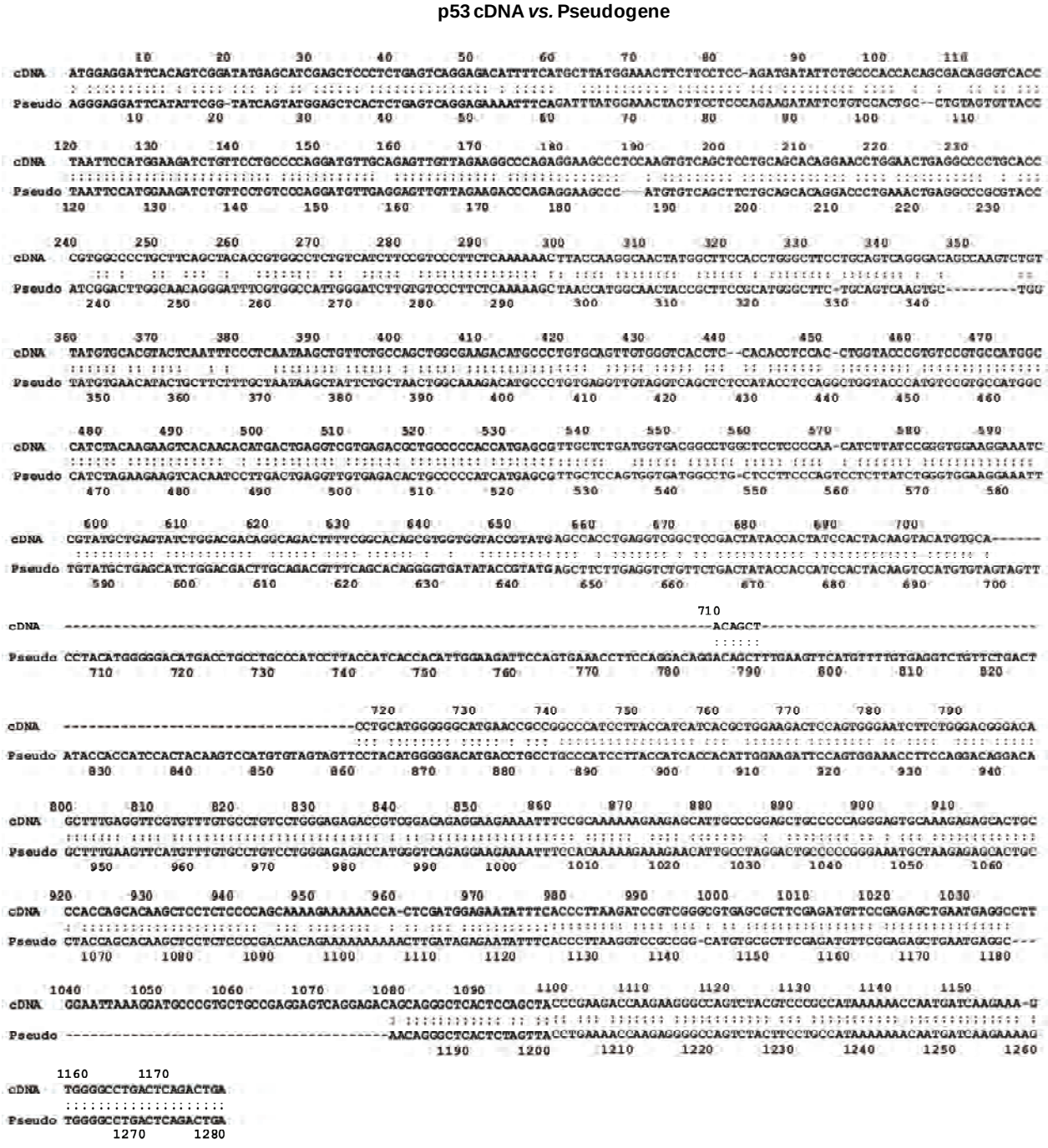

Figure 4. Blast analysis of p53 cDNA and pseudogenes showing the loss of DNA binding domain (DBD) and nuclear localization signal (NLS) in the pseudogene.

Table 1. Genome blast analysis showing the chromosome localization of cDNA and pseudogenes.

S. No.	Accession number	chromosome	E value	% identity
cDNA	NW 047430.2 NW 001084694.1	14	0.0	84
	NW 047390.2 NW 001084680.1	13	0.0	78
	NW 047334.2 NW 0010884656.1	10	1e-140	100
	NW 047813.2 NW 001084694.1	9	0.0	84
	NW 047627.2 NW 001084680.1	2	0.0	87
pseudogene	NW 047518.2 NW 001084740.1	18	0.0	99
	NW 047430.2 NW 001083694.1	14	0.0	84
	NW 047390.2 NW 001084680.1	13	3e-23	76
	NW 047334.2 NW 001084656.1	10	1e-42	81
	NW 47813.2 NW 001084880.1	9	5e-86	95

this type of DNA if it were useless, since DNA manufactured by the cell is energetically costly. As the function of more pseudogenes is being uncovered by testable and repeatable science, it is evident that these genetic elements, which are copiously spread in the genomes of different organisms, have been created with purpose.

In addition, and in contrast to previously believed information that pseudogenes are non functional copies of genes [21,22], growing evidence suggests that at least some pseudogenes are functional. It has been demonstrated that pseudogenes notably arise from seemingly absent or disabled promoters, premature stop codons, splicing errors, frameshift-causing deletions and insertions, etc., and do not necessarily abolish gene expression [23,24]. McCarrey *et al*. [25] have suggested that pseudogenes can be functional in terms of the regulation of the expression of its paralogous genes, otherwise antisense to pseudogenes should not interfere with cellular functions. In support of this earlier we have used N-terminal siRNA to p53 and could inhibit its functions [10]. With respect to the evolution of regulatory functions of pseudogenes we must now conclude that transcribed pseudogenes are not necessarily without function. Indeed, they would appear to be especially suited to roles involving the antisense regulation of the active genes to which they are related [24]. In summary we report a processed pseudogene and additional translational products for p53 in a rat histiocytoma that differ from the parental tumor and from the rat genome may have function roles upon stress and tumorigenesis.

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Reduced bile duct contractile function in rats with chronic hyperglycemia

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ABSTRACT

The incidence of gallstone is higher in patients with diabetes mellitus than in general population. It is generally attributed to hypomotility and lowered emptying function of the gallbladder. In this study, we investigate if chronic hyperglycemia is correlated with reduced contractile function of the bile ducts in rat. Hyperglycemic rats were induced by streptozotocin-nicotinamide treatment. Hyperglycemic rats were sacrificed eight months after induction and bile ducts were removed for the subsequent studies. The bile duct contractility of the normal rats is consistently higher than that of the hyperglycemic rats. The contractities were measured to be 5.5 ± 0.2 mg vs. 4.2 ± 0.1 mg without CCK stimulation, and 5.5 ± 0.3 mg vs. 7.9 ± 0.4 mg with CCK stimulation, respectively for hyperglycemic and normal rats. There was no significant difference in plasma CCK concentration in hyperglycemic rats and normal rats. The expression of CCK-A receptor protein in the bile duct tissue was decreased in hyperglycemic rats compared with that of the normal rats, and it may, at least in part, responsible for a reduced contractility. A reduced bile duct motility may cause bile retention, and may be one of the factors predispose to gallstone formation in type 2 diabetes patients, which is characterized with chronic hyperglycemia.

Keywords: Hyperglycemia; Bile Duct; Contractile

1. INTRODUCTION

Cholelithiasis is one of the most prevalent gastroenterologic diseases in humans. It is a complex metabolic disorders, and its exact pathogenic mechanisms have not been fully elucidated. Gallstones represent a serious burden for the health care systems: over 10% of Europeans and Americans carry gallbladder stones [1], and the prevalence of gallstone disease seems to be rising as a result of longer life expectancy [2]. Many gallstones are silent, but symptoms and severe complications ensure in around 25% of the cases, necessitating surgical removal of the gallbladder [3]. Mortality rates following cholecystectomy range from less than 0.1% in clinical studies [4] to 0.8% (as documented for all cholecystectomies performed in Germany in 2002) [5]. In the US about 3,000 deaths (0.12% of all deaths) per year are attributed to complications of cholelithiasis and gallbladder disease [6]. Nonsurgical approaches, including gallstone dissolution by ursodeoxycholic acid and extracorporeal shockwave lithotripsy, have increasingly lost their impact on therapy and are performed only for uncomplicated symptomatic cholecystolithiasis in a very small number of selected patients.

Bile formation enables the removal of excess cholesterol, either directly or after catabolism to bile salts, and it is a key function of the liver. Bile is an aqueous solution of lipids, in which bile salts (67% of solutes by weight), phospholipids (22%) and cholesterol (4%) representing the three main lipid species [7]. More than 80% of gallstones consist mainly of cholesterol and are formed within the gallbladder [8]. Three major mechanisms contribute to the formation of cholesterol gallbladder stones: cholesterol supersaturation of bile, gallbladder hypomotility and destabilization of bile by kinetic protein factors. Cholesterol-supersatu-rated bile contains more cholesterol than can be solubilized by mixed micelles (cholesterol saturation index > 1). It contains multilamellar vesicles (liquid crystals), whose

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fusion and aggregation precede the formation of solid cholesterol crystals. As illustrated in the classic triangular phase diagram, solid crystals occur in bile at high relative bile salt and low phospholipids concentrations and at cholesterol: phospholipids ratios > 1 [7]. An excess of biliary cholesterol in relation to bile salts and phospholipids can result from hypersecretion of cholesterol, or from hyposecretion of bile salts or phospholipids. Cholesterol hypersecretion is the most common cause of supersaturation [8]. It might be caused by increased hepatic uptake or synthesis of cholesterol, decreased hepatic synthesis of bile salts, or decreased hepatic synthesis of cholesteryl esters for incorporation in VLDL. Accordingly, any enzyme, transporter or regulator involved in hepatic cholesterol metabolism could potentially affect the formation of cholesterol gallstones [9]. In humans, most gallstone cholesterol is of dietary origin, consistent with the observation that hepatic biosynthesis contributes less than 20% of the biliary cholesterol [2]. The hepatic uptake of cholesterol is mediated by the scavenger receptor B1 for HDL, which contributes most of the biliary cholersterol under physiologic conditions. The inverse correlation between serum HDL levels and gallstones suggests that cholesterol cholelithiasis is associated with an induced reverse cholesterol transport and hepatic catabolism of HDL [2]. The rate-limiting enzymes of hepatic cholesterol and bile salt synthesis are 3-hydroxy-3-methylglutary-coenzyme A reductase and cholesterol 7α-hydroxylase, respectively. These enzymes are regulated by the sterol-regulatory-lement-binding protein (SRBP) and nuclear receptor signaling pathways [10,11]. Stasis of bile in the gallbladder favours stone formation, as indicated by stone formation during pregnancy, rapid weight loss or total parenteral nutrition. Postprandial gallbladder volumes are increased and gallbladder emptying in response to cholecystokinin (CCK) is impaired in patients with gallstones [8], probably as a result of absorption of cholesterol from supersaturated bile by gallbladder wall. Excess cholesterol in smooth-muscle cells stiffens sarcolemmal membranes and decouples the G-protein-mediated signal transduction of the CCK, thereby paralyzing gallbladder contractile function [12].

Among the diabetes patients in China, about 10% of them also bear gallstones. The impaired emptying function owing to hypomotility of the gallbladder is considered an important factor for the development of cholelithiasis [13-17]. Because of hypomotility and lowered emptying function, the incidence of gallstone is higher in patients with diabetes mellitus than in general population, however, its underlying mechanism has not been well understood.

Gallbladder mortility is regulated by cholinergic and gastrointestinal hormone [18,19]. Postprandial gallbladder emptying is triggered mainly by plasma CCK from small intestine. CCK interacts with CCK receptor-1 (CCK-R) in gallbladder smooth muscle cells, which in turn elicits the contraction of gallbladder by the activation of post-membrane signaling passway [20]. We hypothesize that abnormal gallbladder contraction in response to CCK, may play an important role in the development of cholesterol gallstone in hyperglycemic patient. The purpose of this study was to examine if there are differences in gallbladder motor function, plasma CCK concentration, and the CCK-R activity in response to CCK in hyperglycemic and normal rats.

2. MATERIALS AND MATHODS

2.1. Animals

Male SD rats, age 8-10 week, obtained from the National Laboratory Animal Center, Taiwan, were used for the study. Streptozotosine (STZ), which selectively destroys the pancreatic β-cells that secrete insulin, was used to induce insulin dependent DM, and nicotinamide was used to attenuate STZ effect to induced insulin-deficient diabetic rats [21]. STZ-nicotinamide DM rats were induced in overnight fasted animals by a single intravenous injection of STZ (60 mg/kg body weight), and nicotinamide (120 mg/kg body weight) (Sigma Chemical Co., St Louis, MO) was administered intraperitoneally 15 min after STZ. Combined administration of STZ and nicotinamide leads to the development of a diabetic syndrome, which is characterized by moderate and stable hyperglycemia and reduced pancreatic insulin stores [22].

Hyperglycemia was confirmed by elevated plasma glucose levels, measured on days 3 and 7 after drug injection. STZ-nicotinamide treated rats exhibited a fasting plasma glucose concentration of 13.4 ± 0.8 mmol/L and a plasma insulin level of 95.0 ± 0.2 pmol/L (n = 24). In contract, the fasting plasma glucose and insulin levels of the normal rats were 4.8 ± 0.05 mmol/L and 168.5 ± 4.8 pmol/L, respectively (n = 24). All studies were carried out with animals two weeks after the induction of diabetes. Blood samples were taken from overnight fasted rats at 0, 2, 4, 6 and 8 months after the confirmation of hyperglycemia.

2.2. Bile Duct Contractile Response

Bile ducts were isolated from hyperglycemic rats and normal rats, respectively, and placed in Kreb's solution containing (in mmol/L) 118.4 NaCl, 25 $NaHCO_3$, 11.66 glucose, 4.75 KCl, 1.18 $MgSO_4 \cdot 7H_2O$, 2.5 $CaCl_2 \cdot 2H_2O$, 1.19 KH_2PO_4, 0.02 EDTA. The solution was maintained

at pH 7.4 and continuously bubbled with 95%O_2-5%CO_2. Bile ducts were carefully mounted on the isometric force transducer in the organ chamber (95%O_2-5%CO_2, at 37˚C), and were equilibrated for 90 minutes in an organ bath with a resting tension of 1.8 mg. CCK (from 10^{-9}-10^{-5} M) was used to induce bile duct contraction.

2.3. Determination of Plasma CCK Concentration

Animals were fasted overnight and anesthetized by pentobarbital (30 mg kg^{-1} body weight, i.p.). Blood samples (0.1 mL) were collected from femoral vein using a chilled syringe that contained 10 IU heparin. Plasma CCK levels were measured by enzyme immunoassay of 50 μL aliquots of plasma with a CCK octapeptiode rat ELISA kit (Harbor Boulevard, Belmont, California). The immunoplate in this kit is pre-coated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody binds to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in sample. The biotinylated peptide is able to interact with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the conversion of 3,3',5,5'-tetramethylbenzidine (TMB) to produce a blue colored solution. The reaction was stopped by adding acid and the reaction solution turned yellow and was read spectrophotometrically.

2.4. Determination of Plasma Glucose

Animals were fasted overnight and anesthetized by pentobarbital (30 mg kg^{-1} body weight, i.p.). Blood samples (0.1 mL) were collected from femoral vein using a chilled syringe that contained 10 IU heparin. The samples were centrifuged at 13,000 rpm for 3 min, and aliquot (15 μl) of plasma was added to 1.5 ml of a Glucose Kit Reagent (Biosystems S.A., Barcelona, Spain) and incubated at 37˚C in a water bath (Yamato-BT-25, Tokyo, Japan) for 10 min. Plasma glucose was determined by a glucose analyzer (Quik-Lab, Ames, Miles Inc., Elkhart, IN).

2.5. Determination of Plasma Insulin

Plasma insulin levels were measured by enzyme immunoassay of 25 μL aliquots of plasma with a Rat Insulin ELISA kit (Mercodia, Uppsala, Sweden). During incubation, insulin in the sample reacted with peroxidase-conjugated anti-insulin antibodies which were bound to the plastic surface of the microtitration well. The bound conjugate was detected by reaction with 3,3',5,5'-tetramethylbenzidine. The reaction was stopped by adding acid to give a colorimetric endpoint that was read spectrophotometrically.

2.6. Western Blot Analysis

Bile ducts were homogenized by mechanical homogenization using a glass/Teflon homogenizer. Protein content was determined using the BCA™ protein assay kit (Rockford, USA). A total of 50 μg of tissue protein was fractionated on 10% sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE). Following transfer, the membrane was washed with phosphate-buffer saline (PBS) and blocked for 1 h at room temperature with 5% (w/v) skimmed milk in PBS. Blots were then incubated overnight at 4˚C with the polyclonal antibodyies against rat bile duct CCK-A or CCK-B (both at 1:1000) (abcam Littleton, CO). β-actin was also blotted with mouse monoclonal antibody (1:1000) against β-actin and served as internal reference. After removing the primary antibody, the blots were extensively washed with PBS/Tween 20, and incubated for 1 h at room temperature with the appropriate peroxidase-conjugated secondary antibody. Following removal of the secondary antibody, blots were washed as described and developed by autoradiography using the ELC-Western blotting system (Amersham Corp.). Densities of the obtained immunoblots at 50 KDa for CCK-B, 48 KDa for CCK-A and 42 KDa for β-actin were quantified using a laser densitometer.

2.7. Statistical Analysis

Data are expressed as mean ± SEM (standard error of the mean). Repeated measures of analysis of variance (ANOVA) were used to analyze the changes in plasma glucose and other parameters. The Dunnett range of post hoc comparisons was used to determine the source of significant differences where appropriate. A p value of 0.05 or less was considered as significant.

3. RESULTS

3.1. Measurement of the Plasma Glucose and Insulin Levels

Plasma glucose and insulin levels were measured from blood samples taken from overnight fasted rats. The blood glucose concentrations measured at 0, 2, 4, 6 and 8 months after confirmation of the hyperglycemia were 13.4 ± 0.8, 14.4 ± 0.6, 15.6 ± 0.2, 16.2 ± 0.1, 16.6 ± 0.2 mmol/L, respectively (**Figure 1**). The plasma insulin concentrations measured at the same time intervals after confirmation of hyperglycemia were 95.0 ± 0.2, 99.5 ± 0.4, 115.2 ± 0.3, 125.6 ± 0.6 and 128.4 ± 0.8 pmol/L, respectively (**Figure 1**). During the same period of time, the plasma glucose and insulin levels of the normal rats were 4.8 ± 0.5 mmol/L and 168.5 ± 4.8 pmol/L, respectively (n = 24).

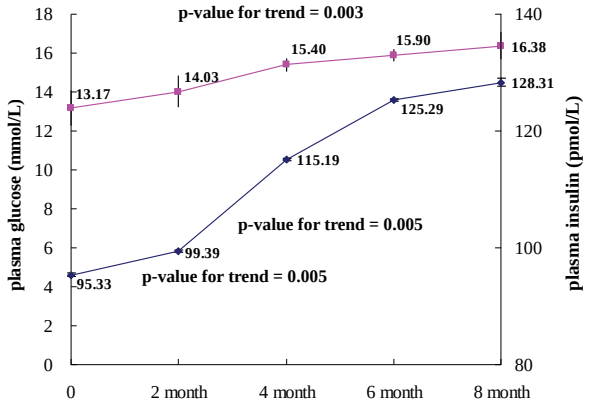


Figure 1. Plasma glucose concentrations and plasma insulin concentrations in STZ-nicotinamide induced hyperglycemic rats. Data are presented as Means ± Std.

3.2. Measurement of the Plasma CCK Levels

No significant difference in plasma CCK levels between normal and hyperglyvemic rats were observed. In the fed normal and fed hyperglycemic rats, the plasma CCK levels were ranged from 0.70 ± 0.05 to 0.79 ± 0.04 ng/ml, and 0.74 ± 0.06 to 0.78 ± 0.04 ng/ml, respectively (**Figure 2(a)**). In overnight fasted normal and hyperglycemic rats, the plasma CCK levels were ranged from 0.41 ± 0.06 to 0.43 ± 0.04 ng/ml, and 0.42 ± 0.04 to 0.44 ± 0.05 ng/ml, respectively (**Figure 2(b)**).

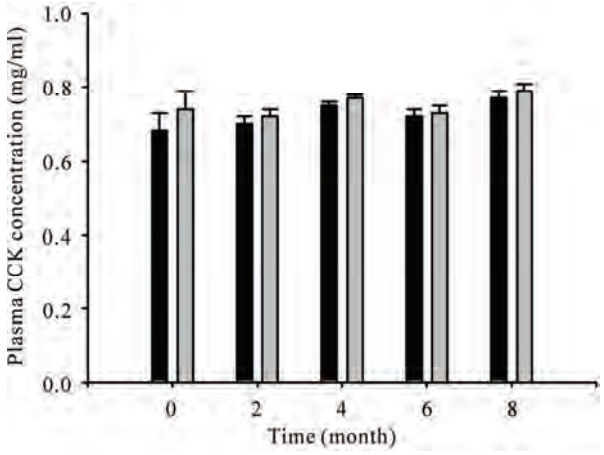
3.3. Effect of CCK on Bile Duct Contraction

Bile duct contraction in response to graded doses of CCK was measured in an organ bath as described. Bile duct rings obtained from normal rats contracted in response to CCK in a dose dependent manner. At 10^{-5} M CCK, a 20% increase of the ring tension was observed (**Figure 3**).

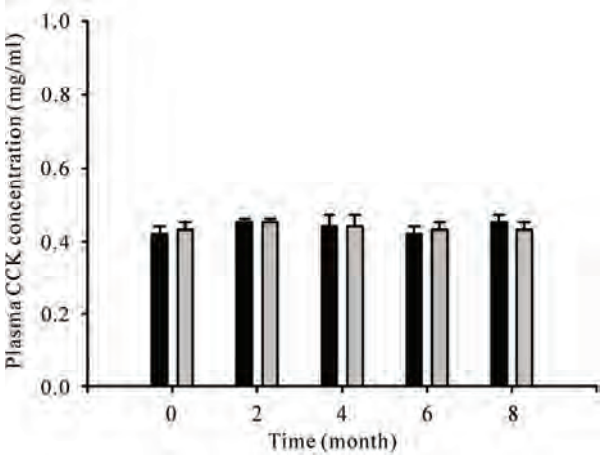
Bile duct rings prepared form hyperglycemic rats at 8 months after confirmation of hyperglycemia symptom exhibited lower contractile tension under the same CCK dose range (**Figure 3**). The contractile response to CCK was somewhat dose responsive, however, at the highest CCK concentration (10^{-5} M) used, the tension generated was still lower than that of the normal without CCK stimulation.

3.4. Expression of CCK Receptor in Bile Duct

Total bile duct tissue lysates were prepared from normal and hyperglycemicrats. Proteins were fractionated on a 10% SDS-PAGE, transferred to cellulose nitrate membrane and blotted for CCK-A receptor. **Figure 4** is a representative blot shown that the protein content of the CCK-A receptor in hyperglycemic rats was reduced by about 50% compared to that of the normal rats.



(a)



(b)

Figure 2. Plasma CCK levels in STZ-nicotinamide induced hyperglycemic rats. (a) during fed stage; (b) during fast stage. Data are presented as means ± SEM, n value was 8 for each experimental group. ■, Normal SD rats; □, STZ-nicotinamide induced hyperglycemic rats.

4. DISCUSSION

Epidemiological studies have shown that obesity, hyperinsulinemia, and diabetes are important risk factors for the development of the gallstones [23,24]. Hyperinsulinemia, or insulin resistance, and obesity are among the metabolic syndrome cluster, and people with metabolic syndrome have been shown to predispose to diabetes, cardiovascular disease, hypertension and gallstone disease [25]. Metabolic disorders may affect the biochemical characteristics, especially the lipid composition of the

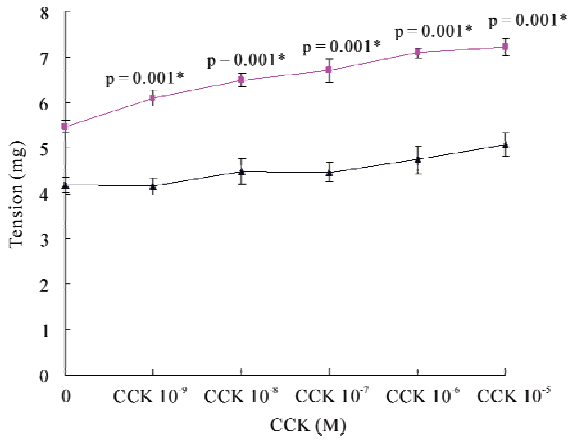


Figure 3. Dose effect of CCK on the contraction of bile duct isolated from normal (■) and STZ-nicotinamide induced hyperglycemic rats (▲). Bile duct of the STZ-nicotinamide induced hyperglycemic rats was isolated at 8 months post iduction.

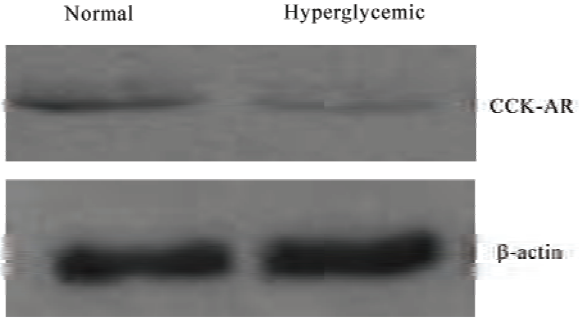


Figure 4. CCK-A receptor protein expression in the bile duct of the normal and hyperglycemic rats.

bile. In fact, cholesterol supersaturation or an increase cholesterol to phospholipids ratio has been suggested to be one of the determinants for cholesterol gallstone formation [26]. One other possible influencing factor is the contractile function of the gallbladder. The major hormonal regulator of gallbladder contraction in the intestinal phase of digestive function is CCK [27,28]. Reduced gallbladder contraction function due to defect in CCK signaling has been shown to impair gallbladder empting and enhance gallstone formation [29]. From a recent study we know, gallbladder dysmotility may have accelerated sludge and gallstone formation in male and female CCK-1(A) receptor-deficient mice, but its contribution was limited [30]. Since diabetes patients, which are characterized by chronic hyperglycemia have been reported to have a higher incidence of gallbladder stone disease, we therefore, sought to examine the possible effects of the hyperglycemia on the gallbladder contractile function in response to CCK in rats.

We found that bile duct contractility of the chronically hyperglycemia rats with and without CCK stimulation was consistently lower that of the normal rats. Further examination by Western blotting showed that the expression of CCK-A receptor protein in the bile duct of the hyperglycemic rats was reduced by 50% compared with that of the normal rats. It is therefore concluded that reduced bile duct response to CCK contractile stimulation in hyperglycemic, and perhaps, diabetic rats is due, at least in part, to a reduced expression of CCK-A receptor. Interestingly, the plasma CCK contents of the normal and hyperglycemic rats were not significantly differed. Thus, the reduced bile duct contractility may be one of the important influencing factors of the high coincidence of gallbladder stone disease and diabetes.

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High-sensitivity C-reactive protein as a marker of cardiovascular risk in obese children and adolescents

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ABSTRACT

Background and aim of the work: High-sensitivity C-reactive protein (hsCRP) is a marker of low grade inflammatory state, which characterises an atherosclerotic process. The metabolic syndrome is associated with insulin resistance and a systemic low-grade inflammatory state. These disorders may arise at a very early age in obese children. We aimed to assess the utility of (hsCRP) as a marker of cardiovascular risk in obese children and adolescents. Patients and methods: This study was conducted on 100 obese child and adolescents (6-16 years). 50 apparently healthy children of matched age and sex served as control. All patients and controls were subjected to: 1-complete history taking. 2-anthropometric measurements and clinical examination including body height, weight, waist circumference, body mass index and blood pressure. 3-laboratory investigations including fasting glucose, lipid profile, apolipoprotiens and (hsCRP) were assessed. Metabolic syndrome patients had to meet three out of five criteria: concentration of triglycerides (TG) ≥ 110 mg/dL, high density lipoprotein cholesterol (HDL-C) ≤ 40 mg/dL, waist circumference ≥ 90th percentile, glucose concentration ≥ 110 mg/dL, and systolic or diastolic blood pressure ≥ 90th percentile. Results, height, weight BMI and blood pressure were significantly higher in the obese than the control. Obese group had significantly higher (hsCRP) levels than control group, (p < 0.01) and significantly higher LDL-C, triglyceride (TG), and lower HDL-C than the control group. Log (hsCRP) showed a positive correlation with BMI (p < 0.001), blood pressure, and TG. The prevalence of the metabolic syndrome was 24%. Mean concentrations of (hsCRP) were higher among patients who had the metabolic syndrome. Among whom, 35% had a concentration of (hsCRP) > 3.0 mg/L, a concentration considered to place adults at high risk for cardiovascular disease. In multiple logistic regression analysis only abdominal obesity was significantly associated with (hsCRP). Conclusion: metabolic syndrome and abdominal obesity among our patients predispose to cardiovascular disease later in life through early low grade inflammation. (hsCRP) is one of the inflammatory markers that can be easily estimated in these patients.

Keywords: HsCRP; Cardiovascular Risk; Obesity

Aim of the work: We aimed to assess the utility of the high-sensitivity C-reactive protein (hsCRP) as a marker of cardiovascular risk in obese children and adolescents.

1. INTRODUCTION

The prevalence of childhood obesity has more than doubled in the last 15 years in many regions of the world [1]. This phenomenon is associated with a rapidly increasing trend in cases of type 2 diabetes in childhood. Obesity in childhood also seems to harbor a number of risk factors for cardiovascular disease (CVD) in adult life, but is not yet clear whether these are determined by glycemia, degree of obesity, or other demographic, clinical, or biochemical features of the obese child [2]. Childhood obesity seems to contribute to the development of vascular inflammation and the progression of arterial wall changes. (hsCRP) has recently emerged as a useful biomarker for vascular inflammation associated with atheRosclerosis [3]. Of novel risk factors for cardiovascular disease currently under investigation, high-sensitivity C-reactive protein (hsCRP) is the most promising. To date, more than 20 prospective epidemiologic studies have demonstrated that (hsCRP) independently predicts vascular risk [4]. CRP is a major inflammatory cytokine that functions as a nonspecific defense mechanism in

response to tissue injury or infection. Synthesized mainly in the liver, CRP activity is stimulated by other cytokines, especially interleukin (IL)-6, IL-1β, and tumor necrosis factor-α (TNF-α). Accumulating evidence suggests that CRP, which is also found within macrophages of atheromatous plaques, is causally or mechanistically related to atherothrombosis [5]. The metabolic syndrome has generated a great deal of interest in recent years. Comprised of a constellation of anthropometric, physiologic, and biochemical abnormalities, the metabolic syndrome is a risk factor for cardiovascular disease and diabetes among adults. However, research about the metabolic syndrome among children and adolescents and the implications of having the metabolic syndrome is limited [6]. The metabolic syndrome has been defined as a cluster of risk factors for atherosclerotic cardiovascular disease that includes insulin resistance, dyslipidemia, abdominal adiposity, and often hypertension [7].

2. PATIENTS AND METHODS

This study was conducted on 100 obese child and adolescent (simple obesity) their ages ranged from (6-16 years) enrolled from pediatric outpatient clinic, Prince Sultan Armed Forces Hospital, Saudia Arabia. 50 apparently healthy children of matched age and sex served as control.

Exclusion Criteria:

1) Smokers.

2) Under any regular medication.

3) Family history of premature vascular disease.

4) Any symptoms of infection during the 2 weeks before the study.

5) Underlying etiology (secondary obesity).

All patients and controls were subjected to:

1) Complete history taking

2) Anthropometric measurement and clinical examination: All anthropometric measurements were taken with stress on body height and weight were measured in light clothes using a portable standiometer. Body mass index (BMI) was calculated as weight divided by the square of the height (kg/m^2). In children, > 95th percentile for BMI growth chart is considered obese (4), so we used BMI > 30 as our prospectively defined criterion for obesity, and BMI < 25 was defined as the non-obese control level [8].

Waist circumference was measured at the high point of the iliac crest to the nearest 0.1 cm at the end of normal expiration with a steel measuring tape. Blood pressure measurements were obtained for each participant in the study. Participants were seated with their right arm resting at the level of the heart. Blood pressure was measured with a mercury-gravity sphygmomanometer. Child, adult, and large arm-cuff sizes were available.All measures were evaluated according to appropriate centiles. Characteristics of studied groups was shown in **Table 1**, There was found that height, weight and BMI were significantly higher in the obesity group than in the control group. BMI was 27.20 ± 12.30 kg/m^2 in the obesity group and was 16.68 ± 2.00 kg/m^2 in the control group. Obese children had significantly higher systolic blood pressure (SBP) (115.0 ± 9.95 mmHg vs. 95.0 ± 7.82 mmHg) and diastolic blood pressure (DBP) (75.85 ± 5.03 mmHg vs. 68.28 ± 7.45 mmHg).

3. LABORATORY INVESTIGATIONS

Fasting glucose: concentration was measured using the glucose hexokinase method [9].

Serum lipids and apolipoprotein: Venous blood samples were taken in the morning after overnight fasting (10-12 hr). Serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were measured by standard enzymatic methods with the use of Boehringer Mannheim GmbH and a fully automatic analyzer. Serum apolipoprotein A-1 (Apo A-1), apolipoprotein B (Apo B), and apolipoprotein E (Apo E) were measured by immunonephelometry (Behring Nephelometer II, Dade Behring, Inc., Newark, DE [10].

High-sensitivity C-reactive protein was assessed with the immunonephelometric assay method using company reagents (assessments made using high sensitivity method with a latex intensification). High-sensitivity C-reactive protein A BN II Nephelometer Analyzer (Dade Behring, Inc.) was used to measure serum (hsCRP) values using a high sensitivity latex-enhanced immunonephelometric assay method. The detection limit of this assay was 0.17 mg/dL. Because the 90th percentile of normal CRP distribution is 0.3 mg/dL, we included only patients with

Table 1. Comparison of characteristics between obesity and control groups.

	Obesity (N = 100)	Control (N = 50)	P
Age	11.82 ± 0.6	11 ± 0.2	NS
Height	134.23 ± 4.0	125.13 ± 6.0	< 0.01
Weight	68.05 ± 14.40	31.56 ± 7.28	< 0.01
BMI	27.20 ± 12.30	16.68 ± 2.00	< 0.01
Systolic BP	115.0 ± 9.95	95.0 ± 7.82	< 0.01
Diastolic BP	75.85 ± 5.03	68.28 ± 7.45	< 0.01

NS: not significant.

CRP values below 0.3 mg/dL to avoid the influence of acute infection [11]. Metabolic syndrome, Patients had to meet three out five criteria: concentration of triglycerides ≥ 110 mg/dL, HDL cholesterol ≤ 40 mg/dL, waist circumference ≥ 90th percentile (sex specific), glucose concentration ≥ 110 mg/dL, and systolic or diastolic blood pressure ≥ 90th percentile (age, height, and sex specific) [6].

Statistical analysis: Data was collected and expressed in tables. SPSS version computer program was used for all statistical calculations. Results were expressed as mean ± standard deviation. Geometric mean and standard error were calculated. Comparisons between two groups were performed by the independent student-t test. Univariate and multivariate regression analyses were used to delineate the relationships between components of metabolic syndrome and (hsCRP). Due to the skewed distribution of (hsCRP) levels, (hsCRP) values were logarithmically transformed prior to regression analyses. For all analyses, probability (p) values below 0.05 were considered statistically significant tests were performed to compare mean and log-transformed concentrations of hsCRP. We examined the independent contribution of the five components of the metabolisc syndrome to (hsCRP) concentration in multiple linear regression analyses. For regression analyses, (hsCRP) concentration was log transformed to improve the distribution of this variable [12].

4. RESULTS

Results of our study were expressed in the following tables:

Table 2 shows comparison of metabolic parameters between obesity and control groups: Obese group had significantly higher hs-CRP levels than control group, hs-CRP levels were 1.40 ± 0.78 mg/dL vs. 0.56 ± 0.47. mg/dL, $p < 0.01$ and significantly higher LDL-C, TG, Apo B and lower HDLC than the control group. TC and Apo E were higher and Apo A-1 was lower in the obesity group than in the control group, but these differences were statistically insignificant.

Table 3 shows Correlation of log hs-CRP with BMI, BP, lipid profile, and apolipoprotein concentrations: Log [hsCRP] showed a positive correlation with BMI ($r = 0.464$, $p < 0.001$), SBP ($r = 0.207$, $p < 0.05$), DBP ($r = 0.225$, $p < 0.05$), Apo E ($r = 0.272$, $p < 0.01$), and TG ($r = 0.298$, $p < 0.05$) by simple regression.

Table 4 shows Unadjusted mean concentrations ± SE, geometric mean concentrations ± SE, and percentage ± SE of CRP > 3.0 mg/L by presence or absence of metabolic syndrome or its five components among patients: Among all patients 50% had no components of metabolic syndrome, 20% had one component, 6% had two components, 20% had three components, and 4% had four components. No participants had all five components. The prevalence of the metabolic syndrome was 24% (no sex difference was found, $P > 0.05$). Furthermore, 40% had a large waist circumference, 32% had

Table 2. Comparison of metabolic parameters between obesity and control groups.

metabolic parameters	Obesity (N = 100)	Control (N = 50)	P
TC (mg/dL)	180.45 ± 25.30	160.23 ± 24.67	NS
HDL-C (mg/dL)	53.40 ± 11.78	61.45 ± 14.08	< 0.05
LDL-C (mg/dL)	105.04 ± 22.70	92.62 ± 22.61	< 0.05
TG (mg/dL)	130.45 ± 68.37	88.23 ± 45.23	< 0.01
Apo A1 (mg/dL)	65.13 ± 21.67	68.65 ± 22.55	NS
Apo B (mg/dL)	27.78 ± 30.0	20.00 ± 8.47	< 0.05
Apo E (mg/dL)	2.89 ± 1.23	2.32 ± 1.35	NS
hs-CRP (mg/L)	1.40 ± 0.78	0.56 ± 0.47	< 0.01

TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B; Apo E, apolipoprotein E; NS,not significant; p, p-value.

Table 3. Correlation of log (hsCRP) with BMI, BP, lipid profile, and apolipoprotein concentrations.

Variables	hs-CRP R	P
BMI	0.464	< 0.001
SBP	0.207	< 0.05
DBP	0.225	< 0.05
TC	0.087	NS
LDL	0.101	NS
HDL	0.113	NS
TG	0.298	< 0.01
ApoA	0.173	NS
ApoB	0.015	NS
ApoE	0.272	< 0.01

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B; Apo E, apolipoprotein E; r, correlation coefficient; p, p-value.

Table 4. Unadjusted mean concentrations ± SE, geometric mean concentrations ± SE, and percentage ± SE of CRP > 3.0 mg/L by presence or absence of metabolic syndrome or its five components among patients.

Metabolic syndrome or components	Sample Yes	Size No	Mean Yes	+_SE No	(mg/l) P	Geometric Yes	mean +_SE (mg/l) No	p	%_SE of Yes	CRP No	> 3 mg/l p
Metabolic syndrome	25	75	3.8 ± 1.2	1.3 ± 0.2	< 0.05	1.9 ± 0.5	0.4 ± 0.04	< 0.001	35 ± 7.2	12.7 ± 0.3	< 0.05
Abdominal obesity	40	60	3.8 ± 0.3	0.9 ± 0.4	< 0.001	1.8 ± 0.4	0.5 ± 0.02	< 0.001	33.0 ± 5.0	5.4 ± 0.8	< 0.001
Hypertriglyceridemia	32	68	1.6 ± 0.4	1.4 ± 0.8	> 0.05	0.6 ± 0.2	0.4 ± 0.04	< 0.05	12.6 ± 2.1	10.8 ± 109	> 0.05
Low HDL cholesterol	28	72	2.1 ± 0.9	1.6 ± 0.6	> 0.05	0.8 ± 0.3	0.4 ± 0.03	< 0.05	12.4 ± 0.3	8.8 ± 0.6	> 0.05
High blood pressure	18	82	5.1 ± 0.3	1.7 ± 0.8	> 0.05	0.7 ± 0.4	0.5 ± 0.07	< 0.05	20.2 ± 6.5	11.3 ± 0.3	< 0.05
0.6 Hyperglycemia _110 mg/dl	6	94	2.4†	1.7 ± 0.6	-	0.9 †	0.5 ± 0.03	-	16.4†	11.2 ± 0.7	-

P value calculated based on log-transformed concentration of CRP. †Unstable estimates.

Table 5. Results of multiple linear regression analysis with concentration of log-transformed hsCRP as the dependent variable among patients.

Independent variables	Regression coefficient	SE	P
Age (years)	0.104	0.129	0.003
Sex (male vs. female) (ref.)	−0.068	0.113	0.524
Abdominal obesity Yes vs. no (ref.)	1.876	0.123	0.001
Hypertriglyceridemia Yes vs. no (ref.)	−0.061	0.132	0.670
HDL < 40 Yes vs. no (ref.)	0.157	0.120	0.117
High blood pressure Yes vs. no (ref.)	0.257	0.264	0.134
Glucose_110 Yes vs. no (ref.)	0.038	0.253	0.139

hypertriglyceridemia, 28% had a low concentration of HDL cholesterol, 18% had high blood pressure and 6% had a concentration of glucose ≥ 110 mg/dL. Mean and geometric mean concentrations of hs CRP were higher among patients who had the metabolic syndrome (mean 3.8 mg/L, geometric mean 1.9 mg/L) than among those who did not (mean 1.3 mg/L, geometric mean 0.4 mg/L). Among patients with the metabolic syndrome, 35% had a concentration of hsCRP > 3.0 mg/L, a concentration considered to place adults at high risk for cardiovascular disease. In comparison, 12.7% of adolescents without the syndrome had such a concentration of hsCRP ($P < 0.05$).

Table 5 shows results of multiple linear regression analysis with concentration of log-transformed CRP as the dependent variable among patients.

Of the five components of the metabolic syndrome, mean concentration of hs CRP was higher only among those with abdominal obesity. However, mean concentrations of log-transformed hsCRP were higher among patients with abdominal obesity, hypertriglyceridemia, low HDL cholesterol, and high blood pressure compared with patients without those conditions. In multiple logistic regression analysis with age, sex, and all five components of the metabolic syndrome added as independent variables, only abdominal obesity was significantly and independently associated with log-transformed concentration of hsCRP. When the same five components were examined as continuous variables in another linear regression model, only waist circumference was significantly associated with concentrations of log-transformed hsCRP. Results from an analogous logistic regression model with dichotomized concentration of hsCRP as the dependent variable and the five components added as continuous independent variables yielded similar conclusions.

5. DISCUSSION

While there have been many previous studies relating CRP and cardiovascular risk factors, the association of CRP with subclinical cardiovascular complication has been examined primarily in adults, with few studies in obese children [13]. Traditional risk factors for cardiovascular disease include aging, hypertension, dyslipidemia, smoking, and diabetes. The contribution of lipid accumulation to atherosclerotic disease is well known, but laboratory and experimental evidence indicates that chronic inflammatory processes also play an important role in the development of atherosclerosis [14]. In order to prevent the incidence of cardiovascular events, it is important to weigh the influence of each risk factor on the cardiovascular system [15]. In this respect, we investigated the relationship of inflammatory markers with other risk factors. In our study, we found that height,

weight and BMI were significantly higher in the obesity group than in the control group. Also, obese group had significantly higher hs-CRP, LDL-C, TG and Apo B and significantly lower HDLC than the control group. These findings were in concordance with Quijada *et al.* (2008) who stated that, Systolic, diastolic, and mean blood pressures (MBP), low-density lipoprotein cholesterol (LDL-C), Tg/HDL-C, total cholesterol/HDL-C, LDL-C/HDL-C ratios, CRP, and leptin were significantly higher in the obese group [16]. Our study showed elevated serum hsCRP levels were positively associated with elevated BMI, confirming previous observations in both children [17] and adults [18]. The mechanisms underlying this association with BMI or obesity might be as follows; the adipose tissue is a source of cytokines such as tumor necrosis factor-a (TNF-a) and interleukin-6 (IL-6), and these cytokines stimulate the production of acute-phase proteins such as C-reactive protein in the liver. Not unexpectedly, then, we found that serum hsCRP levels were positively associated with BMI [19]. Our results are in agreement of Martos *et al.* (2009) who reported that C-reactive protein (CRP) levels were significantly ($P < 0.001$) higher in obese children than in controls. After 9 months of treatment, obese children with lowered BMI SD score (SDS-BMI) displayed a significant decrease CRP ($P = .006$), levels compared with obese children with stable SDS-BMI. In addition to BMI, hsCRP was also found to be closely correlated with SBP, DBP, and ApoE and TG concentrations. Previous studies reported that CRP levels are significantly positively correlated with TG, total ratio of serum cholesterol to serum HDL cholesterol, fibrinogen levels, heart rate, SBP, smoking, and white blood cell count and negatively correlated with HDL-C levels [20]. In this study, the prevalence of the metabolic syndrome was 24%. Mean and geometric mean concentrations of hs CRP were significantly higher among patients who had the metabolic syndrome than among those who did not. Among patients with the metabolic syndrome, 35% had a concentration of hsCRP > 3.0 mg/L, a concentration considered to place adults at high risk for cardiovascular disease. In comparison, 12.5% of adolescents without the syndrome had such a concentration of hsCRP ($P < 0.05$). Soriano-Guillén *et al.* (2008) carried out his study on 115 obese children, 24% showed signs of metabolic syndrome. Those with metabolic syndrome presented higher levels of hs-CRP in comparison with the obese patients who did not show signs of metabolic syndrome After a multivariate analysis, the variables that appeared to influence the changes in (hsCRP)were BMI, triglycerides and HDL-cholesterol levels. From his study he concluded that (hsCRP) is a useful tool for early diagnosis of cardiovascular risk in obese children and teenagers [21]. Current estimates are that approximately 25% of American adults, and an increasing percentage of children and adolescents, can be classified as having Metabolic Syndrome. The wide prevalence of metabolic Syndrome makes this condition a major contributor to cardiovascular risk [6]. Of the five components of the metabolic syndrome, mean concentration of (hsCRP) was higher only among those with abdominal obesity. However, mean concentrations of log-transformed hs CRP were higher among patients with abdominal obesity, hypertriglyceridemia, low HDL cholesterol, and high blood pressure compared with patients without those conditions. Weiss *et al.*, 2004 agreed that log-transformed (hsCRP) levels were affected by abdominal obesity, hypertriglyceridemia, hypertension, HDL cholesterol, and high blood pressure [22]. The metabolic syndrome has generated a great deal of interest in recent years. Among adults and adolescents, components of the metabolic syndrome and the metabolic syndrome itself are associated with measures of inflammation, such as concentrations of (CRP). This low-grade inflammation, which has been associated with an increased risk for cardiovascular disease and diabetes may provide a mechanism for the increased risk of these conditions experienced by individuals who have the metabolic syndrome, however, abdominal obesity was the component that was responsible for much of the difference in concentrations of (hsCRP) [23]. The present study showed association between obesity and elevated concentrations of (hsCRP) in children and adolescents. In univariate analysis, concentrations of (hsCRP) have also been significantly associated with the other four components of the metabolic syndrome. Our results suggest that the presence of the metabolic syndrome and abdominal obesity among children and adolescents may be laying the foundation for the emergence of cardiovascular disease and diabetes later in life through early low-grade inflammation. Unfortunately, the sample size was inadequate to provide results separately for males and females. Similar results were obtained by Cizmecioglu *et al.* (2009) as they found that Waist circumference had the highest sensitivity and specificity for predicting MS in their patients [24]. We appreciate the comments of Dr. Kholeif regarding the utility of (CRP) measurement in stratifying cardiovascular disease (CVD) risk as it relates to our results of patients with the metabolic syndrome categorizing CRP into normal (_1 mg/l), borderline (1-3 mg/L), and high-risk (_3 mg/l) levels are appropriate for stratifying patient risk in combination with other risk factor [25]. While our data must be interpreted cautiously because they are of a cross-sectional nature, our findings are consistent with those of Ridker *et al.* showing metabolic syndrome patients with elevated (hsCRP) levels to

have a less optimistic prognosis than those with normal (hsCRP) levels [26]. Although our study relied on a single measurement as provided by the National Health and Nutrition Examination Survey (NHANES) study and thus did not have duplicate measures over time, Dr. Kholeif reported that a single CRP measurement, given its intraindividual biological variability, is not suitable and that the use of multiple measures would establish the certainty of a given level [25]. Recently, the Centers for Disease Control (CDC)/American Heart Association (AHA) workshop on markers of inflammation and cardiovascular disease did recommend that the mean of only two measures taken 2 weeks apart could be averaged to provide a clinically useful value [27]. The studies above demonstrate that vascular risk prediction and the prediction of type 2 diabetes can be improved by knowledge of (hsCRP) levels, even among those with metabolic syndrome. Recent studies relating (hsCRP) to incident hypertension serve to reinforce the importance of blood pressure in the metabolic syndrome complex [28,29] Investigators have long hypothesized links between the metabolic derangements of insulin resistance syndrome/type 2 diabetes and the development and progression of atherosclerosis. A number of investigators have similarly concluded that IL-6 and CRP are associated with hyperglycemia, insulin resistance, and overt type 2 diabetes, and both are strong predictors of cardiovascular disease in apparently healthy people [30]. Although ultrasonography allows visualization of early subclinical stages of atherosclerosis in obese children, the measurement of the serum (hsCRP) level is simpler and cheaper than ultrasonography, is highly reproducible, and well correlates with carotid intima-media wall thickness (IMT) and brachial flow-mediated dilation in obese children (FMD). Thus, (hsCRP) would be a useful screening marker for evaluating and estimating the degree of atherosclerosis in children [31].

6. CONCLUSIONS

Our results suggest that the presence of the metabolic syndrome and abdominal obesity among children and adolescents may be laying the foundation for the emergence of cardiovascular disease later in life through early low grade inflammation. hsCRP is one of the inflammatory markers that can be easily estimated in these patients.

7. RECOMMENDATION

hsCRP can be used as a useful screening test for prediction of cardiovascular changes in Obese children and adolescents. More work is needed to establish whether intervention targeting “highrisk” metabolic syndrome patients, identified on the basis of elevated (hsCRP), effectively lowers CVD risk. To reduce adverse effects of inflammation that accompanies the metabolic syndrome, children should avoid excessive energy intake, limit sedentary behavior, and increase their energy expenditure. In contrast to other biomarkers that also reflect inflammation, (hsCRP) measurement is inexpensive, standardized, widely available, and has a decade-to-decade variation similar to that of cholesterol. Given the consistency of prognostic data for (hsCRP) and the practicality of its use in outpatient clinical settings, we believe the time has come for a careful consideration of adding (hsCRP) as a clinical criterion for metabolic syndrome and for the creation of an (hsCRP) modified coronary risk score useful for global risk prediction.

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Myocardial infarction in antiphospholipid antibody syndrome

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ABSTRACT

A 52-year-old man was admitted to hospital with chest pain after physical activity. Emergency coronary angiography showed multiple thromboembolic occlusions in the anterior descending coronary artery and in the right coronary artery. Further testing revealed anticardiolipin and β2-glicoprotein antibodies (the patient had been diagnosed for ulcerative colitis and polymyalgia rheumatica). Heparin and nitrate were administered intravenously in addition to oral aspirin and metoprolol. Soon after, the patient referred a withdrawal of chest oppression, and his general clinical condition rapidly stabilised. A follow-up examination was performed 9 months later the discharge: he had resumed most of his activities and sieric concentration of lupus anticoagulant antibodies and anticardiolipin antibodies, IgM isotype, were decreased.

Keywords: Antiphospholipid Antibody Syndrome; Myocardial Infarction; Anticardiolipin Antibodies; β2-Glicoprotein Antibodies

1. INTRODUCTION

Antiphospholipid antibody syndrome is a disorder of coagulation characterized by recurrent vascular thrombosis, pregnancy loss and thrombocytopenia associated with persistently elevated levels of antiphospholipid antibodies. Antiphospholipid antibodies are a heterogenous group of immunoglobulins IgG, IgM or, less frequently IgA.

Antiphospholipid antibody syndrome is present if there are at least one of the clinical criteria (vascular thrombosis, pregnancy morbidity) and one of the laboratory criteria (lupus anticoagulant, anticardiolipin antibody, anti β2-glicoprotein antibodies) [1]. Antiphospholipid anti body syndrome falls into two main classifications: PRIMARY, if there are no other autoimmune disorder such as systemic lupus eythematosus (the cause of primary antiphospholipid syndrome in unknowm, but some factors are associated with developing antiphspholipid antibodies, such as infections, certain drugs or genetic predispositions); SECONDARY, if there are other autoimmune disorders [2], such as systemic lupus erythematosus.

2. CASE REPORT

A 52-year-old man was admitted to our hospital with a 20-hour history of angina pectoris and dyspnoea. He referred chest pain following physical activity for several days.

Family medical history was insignificant.

The patient had been diagnosed for ulcerative colitis (1995) and polymyalgia rheumatica (2000). In 2001 the patient tested positive for lupus anticoagulant antibodies. Further testing revealed elevated titlers of anticardiolipin antibodies (89 U/ml ; NV = < 28 U/ml); β2-glicoprotein antibodies (100 MU/ml; NV = < 17 MU/ml) and rheumatoid factor (190 U/ml ; NV = < 15 U/ml). Both the anticardiolipin and the β2-glicoprotein antibodies were IgM isotypes. No clinical symptoms of venous thrombosis were found. In 2002, the lupus anticoagulant antibodies were absent, and the patient was diagnosed for ischemic dilated (congestive) cardiomyopathy with occlusion of the distal portion of the anterior descending coronary artery. Prior to hospitalization the patient was taking prednisone 5 mg diem, lysin acetylsalicylate 75 mg diem and mesalazin 4 g periodically.

On clinical examination, the patient had a normal body temperature, a blood pressure of 100/60 mmHg, a heart rate of 78 beats/min and a respiratory rate of 22 breath/min. Heart beats were regular without murmurs, bruits or gallops, while lung auscultation revealed fine and coarse crackles. He reported muscle aching and a bit of stiffness in the neck, shoulders and upper arms. The rest of his examination was normal.

The electrocardiogram showed a QS pattern without ST elevation in leads V1-V6, DII and aVF. An emergency

echocardiogram demonstrated hypokinesis of the anterior, lateral and septal walls.

Emergency coronary angiography showed multiple thromboembolic occlusions in the distal circumflex and anterior descending coronary artery and in the marginal branch of the right coronary artery. Haemoglobin was 11 g/dl (NV = 12 g/dl); white blood cell count was 13 × 10^3/μl (NV = 8 – 10 × 10^3/μl) and platelets were 317 × 10^3/μl (NV = 150 – 400 × 10^3/μl). Partial Thromboplastin Time was 27.3 sec (NV = 25 – 39 sec) and the International Normalized Ratio was 1.3 (NV = 0.8 – 1.2). Creati-nine Kinase—MB peaked at 140 U/l (NV = 5 – 130 U/l).

Heparin and nitrate were administered intravenously in addition to oral aspirin and metoprolol. Soon after, the patient referred a withdrawal of chest oppression, and his general clinical condition rapidly stabilised. The patient was discharged from the hospital on the seventh day with only aspirin therapy at 325 mg/die [3].

A follow-up examination was performed 9 months later. The patient was well and had resumed most of his activities. The clinical examination was normal. The C-reactive protein level was 6.76 mg/l, and sieric concentration of lupus anticoagulant antibodies and anticardiolipin antibodies, IgM isotype, were 30.5 U/ml.

3. DISCUSSION

We believe this case report to be of some interest because it underlines some unusual aspects of secondary antiphospholipid antibody syndrome such as the absence of venous thrombosis, multiple thromboembolic occlusions of coronary arteries without interest elsewhere [4,5], the temporary absence of the lupus anticoagulant antibodies and the absence of thrombocytopenia. Another interesting fact is the contemporary presence of antiphospholipid antibody syndrome and polymyalgia rheumatica, independent diseases rarely present in the same patient [6].

The diagnosis of definite antiphospholipid antibody syndrome requires the presence of at least one of the clinical criteria and at least one of the laboratory criteria. However, no limits are placed on the interval between the clinical event and the positive laboratory findings [7]. In this patient the antiphospholipid antibody syndrome was diagnosed by the presence of one clinical (episodes of coronary thrombosis with angina) and two laboratory criteria (anticardiolipin IgM antibodies present in the blood on two occasions at least six weeks apart; lupus anticoagulant antibodies detected in the blood on two occasions at least six weeks apart).

This case-report underlines the presence of antiphospholipid antibody as a risk factor in ischemic cardiopathy.

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How the community pharmacist contributes to the multidisciplinary management of heart failure

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ABSTRACT

Objective: To define how the community pharmacist contributes to the management of heart failure by exploring the type of service he provides to patients and by assessing what patients expect from him. Setting: Pharmacists of the Franche-Comte region (France) and patients of the Franche-Comté Heart Association. Method: Two questionnaires were drawn up and sent to pharmacists and patients. Results: The 118 pharmacists participating in this survey (36.9%) felt that they had a role to play in dispensing drugs (100.0%), educating patients about their treatment (83.1%), informing patients about the importance of observance (81.4%) and over-the-counter drugs (58.5%), distributing heart failure brochures (51.7%) and providing medical equipment (44.9%). On the other hand, only a third of them thought that they should inform patients about their illness and give advice by phone. On the whole, knowledge level is good for disease, drug therapy, contraindicated drugs, medical supervision and hygieno-dietetic management, but intermediate or poor for alert signs of decompensation, essential vaccinations and patient associations. University training in this area during formal pharmacy studies is considered either "insufficient" or "very insufficient" in 56.9% of cases. Although more than 99% of the pharmacists think that additional training is needed, only 33.1% had actually benefited from such training. Of the 96 patients (48.0%) who completed the questionnaire, 92.6% are faithful to their pharmacist. They contact him more about drug therapy than about their disease, or information related to treatments. Roles attributed to their pharmacist are mainly related to drug therapy explanation and information concerning over-the-counter drugs. Therapeutic education is known to 40.6% of interrogated patients. Among these patients, two-thirds depend on their pharmacist and feel that he is capable of providing the necessary education. Moreover, 46.2% of patients had received some form of therapeutic education from their pharmacist. Pharmacists believe that they are able to assume this role in 67.8% of cases. Conclusion: In spite of biases, this study allowed us to assess the expectations of heart failure patients with regard to the pharmaceutical management of their disease, thus clarifying the indispensable contribution that pharmacists make in the management of this disease.

Keywords: Heart Failure; Management; Community Pharmacist

1. BACKGROUND

Heart failure remains a common diagnosis and is an important public health problem [1]. The prevalence of heart failure exponentially increases with advanced age [2]. Depending on the severity of symptoms, heart dysfunction, age and other factors, heart failure can be associated with an annual morbidity and mortality of 5% to 50% [2]. Although many causes of heart failure exacerbations requiring hospitalisation can be identified, medication and dietary noncompliance have been reported as contributing factors in up to 33% of hospitalised patients [3]. However major advances in both diagnosis and management have occurred and will continue to improve symptoms and patient outcomes [4-6].

A multidisciplinary approach to managing patients suffering from heart failure has been shown to improve outcome [7]. Yet the place and the role of the community pharmacist in the multidisciplinary management of heart

failure have not been defined. Pharmacists may play a role in drug dispensation, patient follow-up and monitoring of drug therapy. However, the role of the pharmacist could be extended.

Therefore, the aim of this study was to define how the community pharmacist contributes to the management of heart failure by exploring the type of service he provides to patients and by assessing what patients expect from him.

2. METHOD

2.1. Study Design

To achieve the aim of our study, we developed and sent two anonymous questionnaires, first to pharmacists and then to heart failure patients.

The first questionnaire, for pharmacists, was subdivided into five parts: 1/pharmacist characteristics: permanent (yes or no), how long he has had his qualifications (< 5, (5-9), (10-14), > 15 years), pharmacy location (country, city center, urban district, shopping center), number of follow-up heart failure patients (0, (1-5), (6-10), (11-15), > 15), faithful patients (yes or no); 2/ knowledge of heart failure (disease, alert signs of decompensation, drug therapy, contraindicated drugs, hygieno-dietetic management, medical supervision, essential vaccinations, patient association) and role to play (in dispensing drugs, educating patients about their disease and treatment, informing patients about over-the-counter drugs and the importance of observance, referring patients to other health professionals or patient/support associations, providing medical equipment, giving advice by phone, distributing brochures about heart failure); 3/asked questions by patient: frequently (yes or no), ability to answer (frequently, sometimes, rarely, never), adopted behaviour if no answer (searching for answer in documents, on internet, contacting the patient's physician); 4/initial university training (very satisfactory, satisfactory, insufficient, very insufficient) and continuing education (necessary, yes or no); 5/therapeutic education: knowledge (yes or no), investment and motivation to provide it (yes or no), privacy space (yes or no).

This questionnaire was distributed to the pharmacists of the Franche-Comté region, via three wholesale distributors. One pharmacist per pharmacy was allowed to answer the questionnaire.

The second questionnaire, for patients, was subdivided into three parts: 1/patient characteristics: age (years), sex (female or male), duration of disease (years), residence (city or country), faithfulness to community pharmacist (yes or no) and grounds (good drug therapy knowledge or good advice provided by pharmacist; pharmacist listens attentively to patients and when necessary refers them to other health professionals; proximity of pharmacy, other); 2/roles and expectations regarding the way the pharmacist manages their disease: a) how often patients ask questions about disease or drug therapy (frequently, sometimes, rarely, never), pharmacists responses (very satisfactory, satisfactory, insufficient, very insufficient); b) good contact for any request related to disease or drug therapy, information on over-the-counter drugs, medical follow-up, medical supervision, hygienodietetic management, referral to other health professionals or support/patient associations (yes or no); 3/therapeutic education: knowledge (yes or no), trust pharmacist to provide it (yes or no), in pharmacy (yes or no).

This questionnaire with stamped envelope for return was distributed to patients of Association de Cardiologie de Franche-Comté (id est. Franche-Comté Heart Association).

Both questionnaires were accompanied by a letter explaining the aim of the study and instructions on how to return the completed questionnaire. Questionnaires were collected, centralized and analyzed.

2.2. Statistical Analysis

SAS 9.1® software was used for questionnaire analysis. Continuous variables were described by mean ± standard deviation and median with ranges [minimum value – maximum value] and qualitative variables by the number and percentage. Quantitative and qualitative variables were compared respectively by the Wilcoxon Mann-Whitney and the Fisher exact test or the chi square test. The tests were significant at an alpha threshold of 5% (p).

3. RESULTS

3.1. Pharmacist Point of View

3.1.1. Pharmacist Characteristics

Out of the 320 distributed questionnaires, 118 (36.9%) were analysed. Results revealed that pharmacists are mainly permanent (75.4%) and have been qualified for more than 15 years (57.7%). Permanent pharmacists have been qualified longer than assistant pharmacists ($p < 10^{-4}$). More than half of the pharmacists work in rural areas (53.8%) and others in urban districts, city centers and shopping centers respectively in 23.1%, 19.7% and 3.4% of cases. On the whole, they provide follow-up to more than ten heart failure patients (70.1%). Heart failure patients tend to be very faithful (96.6%).

3.1.2. Knowledge of Heart Failure and Role to Play

Pharmacists' assessment of their own knowledge of heart failure is summarized in **Table 1**.

Their knowledge level with regard to drug therapy, hygieno-dietetics, and essential vaccinations was significantly related to the number of patient follow-ups in the pharmacy (respectively, $p = 0.04$, $p = 0.01$ and $p = 0.02$). Pharmacists' knowledge level increased with the number of patients. Disease knowledge was significantly positively related to drug therapy knowledge ($p < 10^{-3}$), contraindicated drugs and alert signs of decompensation knowledge ($p = 0.01$).

Without taking into account drug dispensation (100.0%), the pharmacist plays different roles in: educating patients about their treatment (83.1%), informing them about the importance of observance (81.4%) and over-the-counter drugs (58.5%), distributing brochures about heart failure (51.7%) and providing medical equipment (44.9%).

One-third of the pharmacists in our study also play a role in educating patients about their disease (35.6%) and providing advice by phone (33.0%). Referral to other health professionals and support/patient associations was only found for respectively 22.0% and 11.0% of pharmacists.

3.1.3. Questions Asked by Patients

Pharmacists estimated that more than a third of all patients (33.9%) often ask them questions. However, pharmacists were unable to answer these questions in 69.1% of cases. There is no significant difference between the frequency of questions and the ability to answer ($p = 0.69$). If the pharmacist cannot answer, immediately, he tries to find the answer in documents (82.8%), on internet (43.1%) or by contacting the patient's physician directly (68.1%).

Table 1. Pharmacist self-evaluation: Knowledge of heart failure.

n = 118	Knowledge Level, number (%)			
	Very Good	Good	Average	Poor
Disease	4 (3.4)	68 (57.6)	43 (36.4)	3 (2.5)
Alert signs of decompensation	6 (5.1)	36 (30.5)	55 (46.6)	21 (17.8)
Drug therapy	11 (9.3)	88 (74.6)	18 (15.3)	1 (0.9)
Contraindicated drugs	11 (9.3)	59 (50.0)	42 (35.6)	6 (5.1)
Medical supervision	5 (4.3)	51 (43.2)	51 (43.2)	11 (9.3)
Essential vaccinations	3 (2.5)	34 (28.8)	48 (40.7)	33 (28.0)
Hygieno-dietetic management	15 (12.7)	70 (59.3)	31 (26.3)	2 (1.7)
Patient association	0 (0.0)	3 (2.5)	29 (24.6)	86 (72.9)

3.1.4. Initial University Training and Continuing Education

Initial university training about heart failure was judged satisfactory to very satisfactory by 43.1% of pharmacists. More than 99% of them consider it necessary to have a additional training. However, only 33.1% of pharmacists ever actually had continuing education. The older the qualifications, the more dissatisfied the pharmacist was with his initial university training ($p = 0.02$) and the more interested he was in additional training ($p < 10^{-3}$). Continuing education was also significantly positively related to the number of heart failure patient follow-ups in the pharmacy ($p = 0.04$), permanent pharmacist status ($p = 0.04$), and how long the pharmacists has been qualified ($p < 10^{-3}$).

3.1.5. Therapeutic Education

77.1% of pharmacists participating in our study known about therapeutic education and they think that they are able to play this role in 67.8% of cases. More than two-thirds (70.3%) have privacy space.

3.2. Heart Failure Patient Point of View

3.2.1. Patient Characteristics

Of the 200 questionnaires distributed to patients, 96 (48.0%) were collected and analysed. Patient characteristics are summarized in **Table 2**. The mean age of disease was estimated at 8.3 ± 0.9 years, with a median of 6 years (1-51).

Patients are faithful to their pharmacist in 92.6% of cases for different grounds (**Table 3**). Patients living in the city are significantly more faithful than patients living in the country ($p < 10^{-2}$). Listening and referral to other health professionals are significantly related to the sex of patients ($p = 0.02$): these roles are important for

Table 2. Heart failure patient characteristics.

n = 96	number (%)
Sex	
Female	31 (32.3)
Male	65 (67.7)
Age classes (years)	
< 60	12 (12.5)
(60–75)	64 (66.7)
> 75	20 (20.8)
Residence*	
City	60 (63.2)
Country	35 (36.8)
Faithfulness to the pharmacy*	
Yes	88 (92.6)
No	7 (7.4)

32.0% of male patients as opposed to only 9.8% of female patients.

3.2.2. Pharmacist Roles and Expectations Regarding the Management of their Disease

Patients contact their pharmacist mainly to ask questions about drug therapy rather than about their disease (respectively, 58.0% and 31.0% of cases). Reponses are satisfactory or very satisfactory in 77.2% of cases. But more than 15% of patients do not have an opinion.

Patients state that contact with their pharmacist is good for any request of information related to treatments (explanation, information on over-the-counter drugs) (**Table 4**).

However, for disease explanation or medical follow-up/supervision, attitudes differ. Men think that the pharmacist has a role in medical follow-up and medical supervision, whereas most women do not (respectively, $p = 0.04$ and $p = 0.02$).

3.2.3. Therapeutic Education

40.6% of patients indicated that they were familiar with therapeutic education and this was not significantly related to the sex ($p = 0.65$) or age ($p = 0.15$) of patients or to their place of residence (city or country, $p = 0.35$). Among patients familiar with therapeutic education, two-thirds (69.2%) depend on their pharmacist and think that he can. Moreover, 46.2% of patients had received some form of therapeutic education from their pharmacist.

4. DISCUSSION

Heart failure management is a public health priority. The multidisciplinary approach to managing it has been shown to improve outcome, in particular in terms of hospitalisation [4-6,8]. However, the role of the community pharmacist has not been evaluated. Since patients always have to visit their pharmacy to collect their drug therapy, it seems coherent to include community pharmacists in multidisciplinary management. We therefore felt that, by using two questionnaires, we could assess: 1/pharmacists: their knowledge of heart failure and their roles especially concerning their ability and willingness to provide therapeutic education and 2/patients: roles and expectations regarding pharmacist management of their disease, and also whether or not they trust pharmacists to provide therapeutic education. Pharmacists and patients included in this study constitute a specific sample. Thus, 320 of 437 community pharmacists of the Franche-Comté region (73%) received a questionnaire and 118 of them (37%) responded. Among patients of the Franche-Comté Heart Association, 96 responded. They were not representative of the total number of heart failure patients (selection bias) because they have already accepted the disease and are willing to share their experience with other patients. On the whole, we can consider the patient response rate satisfactory, especially since our study did not include a reminder or anonymous

Table 3. Grounds of faithfulness.

Grounds of faithfulness, n = 88	number (%)
Pharmacist's good drug therapy knowledge	57 (64.8)
Pharmacist's good advices	33 (37.5)
Pharmacist's ability to listen to and refer patients to others health professionals	14 (15.5)
Proximity of pharmacy	69 (78.4)
Other	7 (8.0)

Table 4. Roles attributed to the pharmacist by heart failure patients.

n = 96	Is the pharmacist qualified and capable of providing necessary information? number (%)
Disease explanation	
Yes	20 (20.8)
No	76 (79.2)
Drug therapy explanation	
Yes	68 (70.8)
No	28 (29.2)
Information on over-the -counter drugs	
Yes	55 (57.3)
No	41 (42.7)
Medical follow-up	
Yes	36 (37.5)
No	60 (62.5)
Medical supervision	
Yes	13 (13.5)
No	83 (86.5)
Hygieno-dietetic management	
Yes	29 (30.2)
No	67 (69.8)
Referral to support associations	
Yes	9 (9.4)
No	87 (90.6)
Referral to others health professionals	
Yes	18 (18.8)
No	78 (81.2)

follow-up mail to patients.

Heart failure patients, over sixty years old in 88% of cases (over seventy-five years old in 21% of cases) are satisfied with their community pharmacist and are faithful to him. They express some expectations related to their questions. To our knowledge, patient expectations have never been studied. For most patients, the community pharmacist is a drug therapy specialist (explanation and information on over-the-counter drugs). However, few patients (between 9% and 38%) think that the pharmacist must play other roles such as: disease explanation, medical follow-up, medical supervision, hygieno-dietetics management, referral to support associations or to other health professionals. A better integration and involvement of the community pharmacist in the managing heart failure patients could improve this image. Thus, for example, in terms of public heath, collaboration between support association/health professionals and community pharmacists could be envisaged.

Efforts to promote adherence should be included in programs involving a multidisciplinary team with community pharmacist participation designed to improve heart failure therapy and outcomes. Pharmacists have an important role to play in educating patients. Patient therapeutic education is a vital component of heart failure management and reinforces the importance of medication adherence. Thus, in spite of the fact that 59% of patients polled did not know about therapeutic education, among those familiar with it, two-thirds depend on their pharmacist and think that he is capable of providing it. Moreover, 46.2% of patients had received some form of therapeutic education from their pharmacist. 77% of pharmacists polled know about therapeutic education and they feel that they are able to play this role in 68% of cases. Therapeutic education ordinarily takes place in a hospital setting. It would be interesting to consider and promote the community pharmacy as an additional setting.

Community pharmacist follow-up more than ten heart failure patients and they tended to return regularly. Indeed, community pharmacists are in a good position to provide a local service. Their work should not be limited to drug dispensation, but must include educating patients about their treatment, informing about the importance of observance and therapeutic education, and providing medical equipment. However, community pharmacists are not comfortable in all fields. Their initial university training about heart failure was considered insufficient or very insufficient by 57% of community pharmacists. The older the qualifications, the more dissatisfied the pharmacist was with his initial university training. This could explain why pharmacists were unable to answer in 69% of cases when patients inquired about heart failure. Almost all feel that it is necessary to have continuing education, but only one-third have ever had it. An additional refresher course is essential to remedy this situation and to ensure an effective and competent participation in the multidisciplinary management of heart failure patients.

The design of our study is original, exploring both the pharmacist and the patient's point of view. It is debatable whether or not the present study results may be compared with those obtained in the literature. Some studies have assessed the role of the community pharmacist in the management of heart failure [9-13]. Thus, Gattis et al. show that heart failure outcomes can be improved with a clinical pharmacist as an important component of the multidisciplinary heart failure team [14]. Pharmacists contribute to the overall care of these patients, but should be appropriately trained. Murray *et al.* show that pharmacist intervention for outpatients with heart failure can improve adherence to cardiovascular medications and decrease health care use and costs, but the benefit probably requires constant involvement because the effect dissipates when the intervention ceases [13]. Pharmacist received training for their intervention. The same is true of the Bouvy *et al.* study [9].

In the United Kingdom, the government has been encouraging an extension to the role of community pharmacists, including independent prescribing, medicine use review and a health promotion role to provide advice about, diet and nicotine addiction, among other issues [15]. In the United Arab Emirates, the introduction of a clinical pharmacy programme involving optimization of drug treatment and intensive education and self-monitoring of patients [11]. In Canada, the involvement of pharmacists in problems of patient compliance goes back many years and has been studied through the PRECEDE pharmacist education program which espouses a thorough structured approach to patient education incorporating patient's beliefs [16]. Community-based pharmacists are embedded in an infrastructure where they are essential for patients to receive medication.

Pharmacist's involvement in a disease management program will improve the care given to patients with heart failure.

5. CONCLUSIONS

In spite of biases, this study allows us to assess the expectations of heart failure patients with regard to the pharmaceutical management of their disease, thus, clarifying the indispensable contribution that pharmacists make in managing this disease.

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7. CONFLICTS OF INTEREST

There is no potential conflict of interest related to the content of this manuscript.

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Health

A new device for the identification of lymph nodes removed during different types of neck dissection

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ABSTRACT

Meticulous mapping of the lymph node status is a general principle in present-day head and neck surgery. The removal of a certain number of lymphatic levels during neck dissection may well be therapeutic in intent, but it is also mandatory for correct tumour staging. We present a precise lymph node mapping during different types of neck dissection in the course of major head and neck surgery by a sterile plastic tray moulded in the shape of the neck. This device makes lymph node mapping simpler, safer, quicker and methodically more structured than any of the present methods. It facilitates the work of the pathologist and the flow of reliable information along the surgeon ─ pathologist-oncologist chain. With this device, a more structured, methodical means of lymph node removal has become possible.

Keywords: Head and Neck Surgery; Lymph Node Mapping; Neck Dissection

1. INTRODUCTION

In 1906, George Crile published a report on what is now considered the first surgical procedure for the en bloc resection of cervical nodes [1]. Until the 1950s, his radical neck dissection technique underwent only modest technical improvements and there was little clarification of the indications for the procedure. During the 1960s, the ideas of Suarez and Ballantyne led to advances in the technique of conservative neck dissection [2]. For the first time the non-lymphatic structures (the spinal accessory nerve, the internal jugular vein and the sternocleidomastoid muscle) were preserved, and only the lymph nodes between the aponeurotic compartments of the neck were removed [3]. Today, a variety of different types of neck dissection are available that are considered on- cologically, functionally and cosmetically effective in the therapeutic or prophylactic treatment of the neck in patients with head and neck cancers. These less radical surgical procedures are often performed bilaterally, and may be followed by postoperative radiotherapy with a very similar recurrence ratio as observed after radical/modified radical neck dissections [4,5].

Meticulous mapping of the lymph node status is a general principle in present-day head and neck surgery. The removal of a certain number of lymphatic levels during neck dissection may well be therapeutic in intent, but it is also mandatory for correct tumour staging [6]. Te decisions concerning the prognosis, postoperative adjuvant therapy at the individual level and the audit at the departmental level are impossible without proper TNM staging [7].

As in other manual specialities, the identification, handling, collecting and appropriate labelling of the individual lymph nodes according to their origin during different types of neck dissection is a time-consuming procedure all participants concerned. This seemingly minor problem is customarily solved by the usage of labelled individual vials or small bottles.

The routine of individual ENT surgeons practising neck dissections is determined by a long list of factors, but it is clear that lymph node mapping is not ideally standardized and integrated into the daily routine [6]. Deficiencies and substandard attitudes towards the importance of the mapping can not be dealt with here, but it is perfectly obvious that after a neck dissection, careful attention and an extra workload are needed on the part of all the theatre staff. Their number, quality and level of enthusiasm are frequently underestimated potential sources of the misplacing and mishandling of specimens. Over noisy verbal instructions given in the course of

sampling with reference to the designation of the individual specimen in question can also be distractive. Even the necessary communication between the surgeon/scrub nurse and the circulating staff during identification is another possible source of misunderstanding. The routine of the postoperative filling of the pathological request forms by the junior staff and their re-checking of the designation of the vials are likewise not the strongest elements in the information chain. In spite of being a seemingly unrelated area of trouble-shooting, the interdepartmental transfer can be another source of problems.

Lymph node mapping is time and energy consuming activity in every phase starting with removal of one or more lymphatic levels from their anatomical surroundings until the specimens land on the microscope plate of the pathologist. We have modified a tool invented and originally introduced for mediastinal lymphnode mapping during lung cancer surgery and applied it for the systematic collection of different types of neck dissection specimen [6].

2. MATERIAL AND METHOD

A plastic tray shaped in accordance with the outline of the anatomy of the neck with the designated lymph node position represents the anatomical field of the origin of the individual specimens. The small built-in containers it holds, also made of plastic, contain a fluid (water, alcohol or formaline) and are fitted with airtight rubber caps **(Figure 1)**. The invidual lymph node stations are denoted in accordance with the standard [6]. The whole complex can be handled by the surgeon, the assistant or the scrub nurse at the time of the surgical removal of the lymphatic levels as it is sterile. Sterilization is achieved with formaldehid steam**,** no disturbing interference with any other intraoperative activity.

The method was evaluated by personal interviews with the personnel concerned (4 ENT surgeons, 6 theatre nurses, 6 theatre assistants and 2 pathologists) during a 2-months test period. Application of the tool was approved in advance by the Ethical Committee of Pécs University, Medical School.

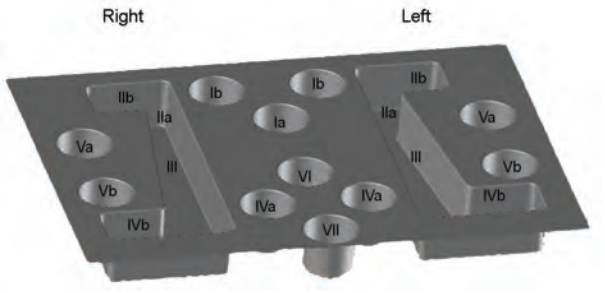

Figure 1. The original plastic tray containing built-in vials closed by rubber caps. The device measured 34 cm in diameter and can easily be handled by the assistant or the scrub nurse.

3. RESULTS

The prototype of the tool was applied between December 1st 2006 and Janury 31st 2007 in 14 consecutive cases, without any adverse event. The type of neck dissections was as follows: radical neck dissections: 2 cases, modified radical neck dissections: 2 cases and selective neck dissections: 10 cases. The theatre staff did not consider the extra workload caused by the usage of the tool to be excessive. Their most important observation related to the ease of following the Health and Safety Regulations as their exposure to biohazard was obviously reduced. The positive comments from the pathologists emphasized the simplicity and reliability of processing the lymph nodes.

4. DISCUSSION

In 1991, the Committee for Head and Neck Surgery and Oncology created by the American Academy of Otolaryngology Head and Neck Surgery, in conjunction with the Education Committee of the American Society for Head and Neck Surgery [8], developed a classification system based on the following concepts: 1) radical neck dissection is the fundamental procedure with which all neck dissections has to be compared; 2) modified radical neck dissection denotes the preservation of one or more non-lymphatic structures; 3) selective neck dissection denotes the sparing of one or more lymph node levels; and 4) extended neck dissection denotes the removal of more lymphatic and/or non-lymphatic structures. The terminology for the current classification of neck dissections is detailed in **Table 1** [9].

For the categorization of neck dissections, we must first adopt a common nomenclature for the lymph node groups of the neck. The classification recently proposed by Som *et al.* [10] is simple and clear. It includes seven levels and proposes precise imaging-based anatomical landmarks for use in classifying metastatic cervical adenopathy. The lymph node groups that correspond to the neck levels and subgroups are outlined in **Table 2**. This classification defines in a more precise manner the anatomical zones or levels of the neck previously classified by Shah *et al.* [11] and by Robbins *et al.* [8]. We took this classification into account while planning our device in order to simplify neck node identification during neck dissections.

Meticulous mapping of the lymph node status of the neck demands the systematic use of neck dissection classification. Our preliminary experience indicates that

Table 1. Terminology of the current classifiation of neck dissections.

Type of neck dissection	Lymph node levels removed	Structures preserved
Comprehensive		
Radical	I, II, III, IV, V	None
Modified radical		
Type 1	I, II, III, IV, V	SAN
Type 2	I, II, III, IV, V	SAN, IJV,
Type 3	I, II, III, IV, V	SAN, IJV, SCM
Selective		
Suprahyoid	I, II	SAN, IJV, SCM
Supraomohyoid	I, II, III	SAN, IJV, SCM
Extended supraomohyoid	I, II, III, IV	SAN, IJV, SCM
	I, II, III, IV, V	SAN, IJV, SCM
Posterolateral	suboccipital and retroauricular nodes	
Lateral	II, III, IV	SAN, IJV, SCM
Anterior	VI	SAN, IJV, SCM
Anterolateral	II, III,IV,VI	SAN, IJV, SCM
	I, II, III, IV, V	None
Extended neck dissection	and one or more additional lymph node groups (such as the paratracheal nodes or anterior compartment lymph nodes)	and structures that are not routinely removed by radical neck dissection (such as the carotid artery, the hypoglossal nerve, the vagus nerve) are removed

SAN: spinal acessory nerve; IJV: internal jugular vein; SCM: sternocleidomastoid muscle

the new device has already demonstrated obvious advantages in five different areas: 1) it reduces operating theatre movement as there is no need for the separate step of passing the individual lymph nodes to the circulating staff for further handling; 2) unnecessary verbal communication is avoided as the identification of individual lymph node levels is self-explanatory; 3) the quality of the lymph nodes reaching the pathologist is improved as tissue trauma during grasping and transfer is minimized; 4) the risk of exposure of the handling staff to dangerous materials (specimens and formalin) is lower than on the use of individual vials/small bottles; and 5) from an educational point of view, it is important that the device as it makes the cancer surgeon and trainees more aware of lymph node staging. With this device,

Table 2. Lymph node groups corresponding to levels I-VII and the various subzones.

Level	Lymph node group
Ia	Submental nodes
Ib	Submandibular nodes
IIa	Upper jugular, anterior to n. IX
IIb	Upper jugular, posterior to n. IX (submuscular recess)
III	Middle jugular nodes
IVa	Lower jugular nodes (behind sternal head of sternocleidomastoid muscle
IVb	Lower jugular nodes (behind clavicular head of sternocleidomastoid muscle)
Va	Posterior triangle nodes (spinal accessory group)
Vb	Posterior triangle group (transverse cervical artery group, supraclavicular group)
VI	Anterior (central) compartment lymph nodes (paratracheal, perithyroideal, Delphian)
VII	Superior mediastinal nodes

a more structured, methodical means of lymph node removal has become possible, and the importance of lymph node mapping gets the emphasis it deserves.

5. CONCLUSIONS

The reported device makes lymph node collection and identification simpler, safer and quicker. Industrial production is planned, with the whole complex made as one integrated plastic tray. The removable vials will be replaced by designated capped bays.

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Quantitative assessment of heavy metals in some tea marketed in Nigeria

——Bioaccumulation of heavy metals in tea

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ABSTRACT

Bioaccumulation of heavy metals in tea marketed in Nigeria was investigated. Four major and most consumed brand of tea were selected for the present study. Both aqueous and dry methods were used. Total contents of metal were determined by digesting 1g of each brand using a mixture (3:1) concentrated nitric acid (NNO_3) and hypochlorous acid ($HCLO_4$). The second method involved hot water extract of tea samples. After boiling and filtration, the residue was evaporated to near dryness and digested with concentrated HNO_3 and $HCLO_4$ as described above. Results indicate that Zn, Cd, Cu, and Pb were present in lowest concentrations in ascending order for which there were two significant differences between the four sources of samples. The general characteristics of heavy metal concentrations in aqueous extract showed high level of Fe and Mg in a descending order. Going by the correlation study of our result indicates that there is no significant relationship between the two elements of Fe and Mg, though, the numerical values of the two elements varied widely among the samples. These differences may have major impact on human health. However, the beneficial effects of tea are in a fairly narrow concentration range between the essential and the toxic level. In conclusion, the variations in heavy metals content of tea brands may be due to geographical, seasonal changes and the chemical characteristics of the growing regions.

Keywords: Bioaccumulation; Tea; Heavy Metals; Human Health; Toxic Level

1. INTRODUCTION

Exposure to various metal containing components of tea varied widely and may have varying health implications [1]. Depending on the origin of tea leaves, heavy metals accumulation can be derived naturally by soil contamination, use of pesticides and fertilizers [2]. Some trace metals Cr, Fe, Co, Ni, and Zn are essential for growth of organisms, while other heavy metals Pb, Cd, Hg and As are not only biologically non essential, but toxic [3]. A very important biological property of metals is their tendency to bioaccumulations. Bioaccumulation is therefore essential in hazard evaluation strategies. For example, calculation of percent available of Aluminum (Al) and Zinc (Zn) in tea consumed by human showed that tea can provide 37.2% of the daily dietary intake of Al, the percent available for absorption in the intestine is only 1.78% for overall mean concentration [4]. Similarly, daily dietary intake of Zn was 2.13% while percentage available for absorption in the intestine was 0.72% [5]. Thus chronic metal toxicity may often characterized by tissue/organ damage resulting in mortalities which are related to secondary physiological disturbances [5,6]. The extent of physiological disturbances depends upon uptake and bioaccumulation of metals [3,7].

Considering that an estimated amount of 18 billion teacups are consumed daily in the world [1,8,9] its economic and social importance are unprecedented. In Nigeria, people drink tea by the bowl because of its therapeutic value. It is valuable in the treatment and prevention of many diseases [8].

The presence of heavy metals in tea has become world-wide study. For example, the concentrations of Fe and Cu in Poland [10], Cu in India and US [9], Se in Pakistan [11], As in Iran [2,12], Al in China [13] and in Lithuania [14] have recently become the subject of wide spread concern, since beyond the tolerable limits they become toxic [15,16]. Determination of harmful and toxic heavy metals in different tea marketed in Nigeria

gives direct information on the significance of these elements in tea beverages. Lack of basal data on the contents of heavy metals in tea and the regulated on the maximum allowable and safe concentration of metal in tea are needed. This predicated the present study to determine the quantitative assessment of heavy metal contaminants in some popular tea marketed in Nigeria.

2. MATERIALS AND METHODS

Several samples of tea leaves which are commonly consumed in Nigeria were procured from provisional stores. Accordingly each sample tea was coded to conceal the original source. The code is tagged by a letter designating the type of tea, that is black tea-Lip; green tea-Gin; white tea-Tia and Top. Three replicate samples of each tea were quantified using two different standard methods of [11,17].

Total contents of metals were determined by having a portion of one g of sample tea digested in 12 mL of a mixture (3:1 v/v) concentrated HNO_3 and $HCLO_4$. The mixture was heated until the solution turned white. The digested sample was filtered and transferred to a 100ml flask and the volume was adjusted to the mark with 5% HNO_3 acid. This digestion procedure was validated by using the reference certified material of National Agency for Food and Drug Administration and Control (NAFDAC) [18].

Hot water extract of metals were determined by having a portion of one g of each brand boiled in 50 ml of distilled water for 10min in a porcelain cup and filtered. The residue was evaporated to near dryness and digested with concentrated HNO_3 as described earlier. The final volume of the solution was made up to 100 ml. Following digestion, ten drops of H_2O_2 were added and centrifuged. The acid digested sediments were filtered and capped.

A Perkin Elmer Analyst 300 flame atomic absorption spectrometer (AAS) (Central Science laboratory, Obafemi Awolowo University, Ile-Ife) was used to quantify the heavy metal concentrations [19]. Calibration standard curves provided the basis for quantifying metal contents for both sediments and plant tissues after the initial ashing digestion. Correlation coefficients for metals in plant tissues and sediments were found to be 99.59% and 99.54% respectively. Calibration curves, developed using standards, provided the basis for quantifying metals concentrations for analysis of plants tissues and sediments using both the dry and wet analysis techniques.

The data were statistically analyzed and the least significant differences (SD) at the 5% level used to separate means. The relationship between the different variables was elevated by a simple correlation and regression analysis [20].

3. RESULTS AND DISCUSSION

Results of the present study show the actual concentration of heavy metals in tea samples after digestion (**Table 1**). The metals present in lowest concentration (< 1.00 mg/kg) were Zn, Cd, Cu, and Pb in ascending order respectively, for which there were two significant differences between the four sources of samples. The sources differ with regard to Se and Fe contents, but similar with respect to Cu, Pb, and Zn and more substantially with respect to Fe and Mg with total metal content in Gin samples about double the other samples.

Table 2 shows the general characteristics of metal concentrations transferred to hot water extracts from brand of teas. The metals present in greatest concentration were Fe (442-1344 mg/kg), followed closely by Mg (123-239 mg/kg) and highly toxic element Cu (2-7 mg/kg). Except for Pb, all these metals are release very slowly from tea leaves because they are complexed by porphyrins [21]. The mean SD values of all determined heavy metals for the group of tea are indicated in the Tables. Going by the performed t-test the concentrations of heavy metals were not significantly different ($P > 0.05$). The numerical values of heavy metals concentrations of Fe and Mg contents varied widely among the samples. From the regression plot, it can be inferred that since the significant value is 0.414 which is far higher than 0.05 and the R and R square values are 0.586 and 0.344 respectively, that there is no significant relationship between the two elements (**Figure 1**). According to correlation study between heavy metals Fe-Mg, and Cd-Cu showed no significant relationship in all the tea samples, otherwise, the differences can have major impact on staying healthy. The excessive heat in tea boiling for example can alter the natural chemical nature of these metals.

The result of total contents of the studied heavy metals (As, Se, Zn, Fe, Mg, Cd,, Cu, Pb) in these teas compared to tea grown in other countries showed accumulation of different heavy metals, for example, studies have shown that Cu in Iranian, Lithuanian and Chinese tea [12-14] respectively, K in Pakistanis [1] and Pb in Tunisian tea [22] bioaccumulations. It goes to show the ability of these plants in accumulating metals. Other studies showed accumulation of Al [23] and Fe [10] in tea leaves. However, in the present study, Fe and Mg complexes are higher than other metals in tea marketed in Nigeria and may be due to high metal levels in the geographical locations and more to preferential absorption of these metals. The percent solubility of Fe and Mg revealed these are in form of least water soluble complexes.

From all indications, differentiation of metal contents

Table 1. Total (mean ± SD) contents of heavy metals in tea samples.

Samples	metal ions							
	As	Cd	Cu	Fe	Mg	Pb	Se	Zn
Lip	1.48 ± 0.01	0.01 ± 0.00	0.12 ± 0.02	2.39 ± 0.22	5.22 ± 2.54	0.03 ± 0.00	0.52 ± 0.01	0.02 ± 0.01
Tia	2.73 ± 0.01	0.31 ± 0.01	0.31 ± 0.04	0.99 ± 0.10	4.06 ± 2.16	0.13 ± 0.01	1.53 ± 0.01	0.01 ± 0.01
Top	1.48 ± 0.01	0.02 ± 0.02	0.12 ± 0.02	2.23 ± 0.21	2.31 ± 1.47	0.33 ± 0.02	3.21 ± 0.01	0.01 ± 0.00
Gin	3.17 ± 0.01	0.39 ± 0.01	0.34 ± 0.05	0.99 ± 0.10	2.96 ± 1.75	0.27 ± 0.02	3.26 ± 0.09	0.02 ± 0.01
Mean ± SD	2.22 ± 0.01	0.12 ± 0.01	0.22 ± 0.03	1.65 ± 0.16	3.64 ± 1.98	0.19 ± 0.02	2.13 ± 0.03	0.02 ± 0.01

All the values are in mg/L

Table 2. Mean (± SD) heavy metals contents in tea aqueous extract.

Samples	As	Cd	Cu	Fe	Mg	Pb	Se	Zn
Lip	1.65 ± 1.60	0.37 ± 0.01	4.15 ± 0.09	1716.6 ± 186.15*	244.56 ± 10.07	00 ± 0.07	0.00 ± 0.32	0.80 ± 0.4
Tia	2.3 ± 0.20	7.92 ± 0.73	5.69 ± 0.09	442.95 ± 8.25	123.54 ± 10.92	0.06 ± 0.08	0.00 ± 0.95	0.03 ± 0.04
Top	1.20 ± 0.00	0.31 ± 0.01	2.33 ± 0.71	1551.20 ± 159.15*	221.59 ± 10.36	0.11 ± 0	10.39 ± 0.16	0.12 ± 0.01
Gin	0.30 ± 0	2.41 ± 0.02	3.95 ± 0.54	591.95 ± 27.15	41.10 ± 0.72	00 ± 0.02	11.09 ± 0.29	0.12 ± 0.01
Mean ± SD	1.36 ± 0.83	2.75 ± 0.19	4.03 ± 0.36	975.68 ± 95.16	157.77 ± 8.10	0.09 ± 0.57	10.74 ± 0.43	0.28 ± 0.23

*Significantly different from the rest tea $p < 0.001$; All the values are in mg/L

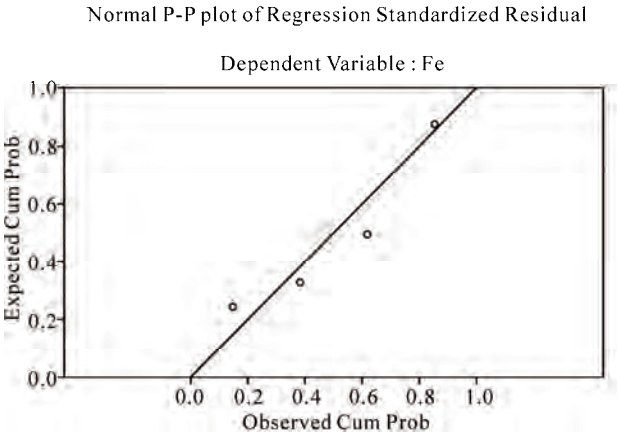

Figure 1. Showing regression table of Mg as constant and Fe as dependent variable.

in variety of tea brand may be due to their geographical origin [21], due in part to leachate characteristic of soil. Long-term plantation of tea can cause soil acidification and elevated concentrations of bioavailable heavy metals in the soil, therefore, enhance the risk of heavy metals accumulation in tea leaves. The variations in the present study might be due to different agro-climatic origins of the imported tea. Although toxic effects of heavy metals have sufficiently being described by WHO 1998a [24], however, the beneficial effects of tea is in a fairly narrow concentration range between the essential and the toxic level [12]. The tea aqueous extracts have considerable amounts of metals ions that could contribute towards daily intake, but these values are lower than the daily requirements of human being (WHO 1998b) [25]. The determination of these elements in beverages, water, food, plant and soil is thus of outermost important tasks. One of the major food sources of these metals is green leafy vegetable [26]. It is equally recommended that aqueous extracts be routinely consumed for the essential nutrients. Routine check and frequent analysis of tea in Nigeria and elsewhere is required to avoid the risk of exceeding the daily in-take beyond the tolerance limits standards.

In conclusion, the geographical variations in heavy metal concentrations among the tea samples were evident in all the brands. At the same time, we found significant differences in the percent solubility of Fe and Mg in all water soluble complexes. Some authors have noted some differences between the metal concentrations in other tea brands. The wide variations of metal concentrations observed could be due to seasonal changes and to the chemical-physical characteristics of the growing regions.

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Temperament and character as predictor of health related quality of life after metacarpophalangeal joint arthroplasty

——Personality and MCP joint arthroplasty outcome

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ABSTRACT

Purpose: To evaluate personality characteristics' impact upon outcome after silicone-based MP arthroplasty in RA patients. Methods: 40 RA patients who had undergone operations on their MP joints were investigated in a one-year follow-up. Objective measurement to assess grip strength and active range of motion—Paper-pencil-tests to assess pain during activity and at rest performance, QoL, and personality. Results: Significant improvement was observed in function and pain related scores except for the pain related VAS and in several QoL facets and domains. Patients who experienced improvement reported higher scores on the activities of daily living facet of the WHO QoL questionnaire. Those with lower pain showed more independence. The variance of the QoL domain scores, other than social and physical domains, could substantially and meaningfully be explained by variance of objective measures combined with personality scores. Conclusions: Most RA patients' QoL can be improved by MP arthroplasty despite remaining substantial level of pain. NS and HA seem to play an important role in the adaptation process during the long term, chronic illness; whereas SD represents a tool of coping with the burden of pain and disability. Personality characteristics are highly predictive for QoL suggesting their important mediating role between experienced pain and disability and HR QoL.

Keywords: Rheumatoid Arthritis; Health Related Quality of Life; MP Joint Arthroplasty; Temperament; Character

1. INTRODUCTION

Rheumatoid arthritis (RA) patients usually suffer from pain, joint or muscle stiffness, and fatigue, causing disability complicated by unpredictable exacerbations [1,2] which impacts upon social and psychological functioning such as employability, independence, self-concept, mood and subsequently upon general wellbeing in terms of health related quality of life (HRQoL). However, there are patients with objective indications of RA who do not report pain and others who report severe pain without positive immunological parameters [3]. This observed variability in pain experience suggests that other factors might be of importance as mediators between objective measures, such as joint dysfunction or clinical findings in the serum, and pain experience or HRQoL as indicators of well-being. There has been much discussion as to whether patients suffering from RA have a particular personality type. The evidence is not convincing and the role of stress in the aetiology of rheumatoid arthritis is not fully understood [4]. However, coping responses to stress (in the case of RA patients in terms of pain, physical and social disabilities) are determined by several psychological processes including personality characteristics. Therefore, personality is considered to be a major phenomenon impacting upon pain-perception and quality of life. Indeed, Chou and Brauer [1] found negative affect in the sense of Watson's and Clark's theory [5] determining subjective health independent of age, marriage, or length of disease. This might

be explained by the fact that negative affect is often combined with self-focused attention and with a negative memory bias. Self-esteem and adjustment to RA has to be regarded as mediators between RA on the one hand, and subjective experience of pain and psychological well-being on the other hand [2]. Furthermore relating to emotional processing in older women with RA, the variability of pain reactivity was found dependent on both emotional intensity and the ability to regulate emotion in a longitudinal investigation by Hamilton, Zautra and Reich [6].

There is general agreement that psychological factors such as personality may influence patients' adjustment and outcome of surgical interventions [7,8]. For example, dissatisfied patients were characterized by significantly higher aggressiveness, lower extraversion, and more health worries compared to satisfied patients without differences in hallux valgus angle or intermetatarsal angle (I-II) three month after operative hallux valgus correction [9]. In another study, Hyphantis *et al.* [10] reported that arthritis-related pain and hostility were negatively related to both physical as well as psychological health in patients suffering from systemic sclerosis.

Silicone-based metacarpophalangeal (MP) arthroplasty still remains the primary treatment for patients with severe degenerative destruction of joints. Joint pain and deformity affecting hand function are the two primary indications for performing metacarpophalangeal joint arthroplasties. Most rheumatoid patients with significant pain and destroyed MP joints are also noted to have palmar subluxation of the proximal phalanx with ulnar subluxation of the extensor tendon, resulting in a fixed flexed posture of the MP joints, which impairs overall function and reduces power. However, the main disadvantage is the inability to obtain a postoperative range of motion close to that of the normal hand [11].

Substantial improvement in range of motion, pain, ulnar deviation and patient satisfaction have been reported as the outcomes of MP arthroplasty with "reported postoperative arcs of motion vary from 38 to 60 degrees" and "extension lags also vary from 9 to 22 degrees" [12]. Rittmeister *et al.* [13] evaluated the outcome of silicon MP arthroplasty as "good" in 40 joints, as "fair" in 10 joints, and as "poor" in none. In a "formal systematic review of all available world literature" Squitieri and Chung [14] found silicone MP joint arthroplasty to be superior to vascularised toe joints or PyroCarbon joints with a mean active range of motion of 47 +/- 16 degrees and a complication rate of 18%. However, maintenance or recurrence even years later after surgery were reported in some patients [12].

The aim of the study was to evaluate if personality characteristics impact upon the outcome of silicone-based MP arthroplasty in RA patients.

The following questions were to be answered; 1) Are there differences between recovered and non-recovered patients pre- and post silicone-based MP arthroplasty according to pain severity, movement capacity, HRQoL, and personality? 2) Is the objectively measureable change associated with perceived improvement? 3) Are there differences in personality characteristics between severe RA patients who received silicone-based MP arthroplasty and healthy individuals? 4) Do psychological or sociodemographic variables predict QoL after silicone-based MP arthroplasty or can related differences between recovered and non-recovered patients be explained by personality characteristics?

2. METHODS

2.1. Sample

40 RA consecutive patients (7 male; 33 female) with an average age of 61.54 ± 9.93 years (range: 32-78) were recruited for this prospective follow-up study. One patient died before follow-up investigation and was therefore excluded from the analysis. All patients had operations on four MP joints, totalling 156 joints. Indications for surgery were pain and severe deformity of MP joints, which had resulted in severe impairment of hand functions in daily life. The MP joints were either volarly subluxated or ulnarly dislocated. No reoperation was performed during the study period.

In order to control for personality characteristics, data was used from a previous standardisation investigation in northern Sweden [15] investigating the Temperament and Character Inventory [16]. From this data a group of individuals from the general population were matched to the patients, with two individuals matched by age (60.5/60.6 ± 10.3 years; range: 30-80) and gender to each patient.

Informed consent was obtained from all participants prior to the investigation; and the study was approved by the Ethics Committee at Umeå University (§127/99 dnr 99-017).

2.2. Measurements

2.2.1. Objective Measurements

Preoperatively and postoperatively, all patients were examined by an independent physiotherapist and an occupational therapist. Maximum and mean grip strength was measured with Grippit (AB Detektor, Göteborg, Sweden) [17].

The active range of motion was measured with goniometry for each phalanx in all joints (MP-metacarpophalangeal joint, PIP-proximal interphalangeal joint, and

DIP-distal interphalangeal joint).

The active arc of motion was determined by subtracting active extension from active flexion.

2.2.2. Rating of Others

The Canadian Occupational Performance Measure (COPM) [18] was used to evaluate patient participation in the goal formulation process. It is a semi-structured interview in which the patients identify their problems in occupational performance and rank them. The patients rate their occupational performance related to identified problems and satisfaction with their performance in areas of self-care, productivity, and leisure. Ten-point scales are used ranging from 1—"not able to do it" or "not satisfied at all" to 10—"able to do it extremely well" or "extremely satisfied".

2.2.3. Self-Report Measurements

A visual analogue scale (VAS) relating to pain during activities and another concerning pain at rest was applied.

The Grade Chronic Pain Status (GCPS—[19]) was developed as "a simple method of grading the severity of chronic pain" (p. 133). Based on the patient's responses to 7 questions, pain severity is classified into 4 hierarchical groups: "Grade I, low disability-low intensity; Grade II, low disability-high intensity; Grade III, high disability-moderately limiting; and Grade IV, high disability-severely limiting" (p. 133). Internationally this scale is often used to classify chronic pain combined with pain related disability [10,20,21].

The Quality of Life questionnaire (WHOQoL-100 – [22]) consists of 100 items covering six domains (physical health, psychological health, level of independence, social relationships, environment, spirituality, and one general domain - overall quality of life). Each item is measured from 1 to 5 according to four underlying Likert scales referring to intensity, capacity, frequency and evaluation. All scores are standardized as 0 = worst quality of life to 100 = best quality of life scale. The internal consistency was reported to be 0.97; the test-retest reliability was 0.70; and an intensive validation study of the Danish version supports the satisfactory external and discriminant validity [23].

A special feature of this WHOQoL questionnaire is its focus on satisfaction with various aspects of life. It covers relevant factors of health related QoL such as pain, physical function, and capacity for work.

The Temperament and Character Inventory (TCI-9 version) is a 238 item true-false self-administered paper-and-pencil test based on Cloninger's biosocial personality theory [16]) and measures the four largely genetically determined and independently inherited temperament dimensions Novelty Seeking (NS—4 subscales); Harm Avoidance (HA—4 subscales); Reward Dependence (RD—3 subscales); and Persistence (PS—single scale) and the three character dimensions Self-Directedness (SD—5 subscales); Cooperativeness (CO—5 subscales); and Self-Transcendence (ST—3 subscales). Temperament refers to individual differences in conditioned emotional responses, such as anger, fear, and disgust; and character refers to individual differences in goals, values and self-conscious emotions like shame, guilt and empathy [16].

2.3. Design

The RA patients were investigated twice—1) preoperative assessment and 2) postoperative assessment after 12 months with all the above described preoperative investtigations conducted repeated.

Physiotherapeutic treatment and training sessions took place between the two assessments. In postoperative week 6 gradually increased motion was initiated without pressure in radial and ulnar directions. In week 8 daily activities were allowed without weight loading in the ulnar direction. From the postoperative third month no restrictions were set limiting daily activities or work except pain; and finally at 6 months, a clinical evaluation was made and an additional training program, some technical advice or equipment was added or optimised.

2.4. Statistical Analysis

Various dichotomous groupings were established relating to the RA patients based on the difference between pre- and postsurgical assessment on several outcome criteria including GCPS, the COPM scores and the visual analogue scales. One group consisted of patients with lower or equal scores (not recovered/not improved) and the other group had higher postoperative scores (recovered, improved). The T-test for independent and paired samples was applied in order to test for various group differences for continuous variables on univariate level and MANOVA was calculated on multivariate level. Hierarchical multiple regression analyses were calculated in order to test for predictive value of personality scores for QoL post-surgery controlling for age and gender in the first level followed by the measured motion and grip-strength as an control-indicator for objecttive surgery results.

3. RESULTS

We found a significant difference between pre- and postsurgical assessment concerning all the parameters measured except for pain. The functional arc of motion in the metacarpal joint changed from 30.8° preoperative to 48.6° one year postoperative ($p < 0.001$). The active flexion arc changed from 69.4° preoperative to 82.5° (p

< 0.001). Extension lag improved significantly in all fingers between 51.7º to 20.8º ($p < 0.001$). The range of movement of each of the operated fingers was postoperatively significantly wider than preoperatively (t between 3.15; $p = 0.002$ for the little finger and 4.16; $p < 0.001$ for the ring finger). Patients improved signifycantly in joint mobility and strength ($t = 2.57$; $p = 0.010$).

The COPM score of performance and satisfaction with occupational performance was significantly higher after surgery. The disability score, number of disability days and the disability points as well as the characteristic pain intensity measured by the GCPS were significantly lower after surgery, whereas the difference in self-evaluation of pain intensity in rest and while active assessed by means of the visual analogue scales did not reach any meaningful level of statistical significance (**Table 1**).

On average the scores of the QoL facets pain and discomfort, energy and fatigue, sleep and rest, activities of daily living, and work capacity were significantly higher one year after surgery (t between –4.60; $p < 0.001$ for work capacity and –2.02; 0.050 for energy and fatigue), but the score for home environment and transport decreased ($t = 2.2.5$; $p = 0.031$, $t = 5.09$; $p < 0.001$ respectively). At the domain level, QoL relating to physical ($t = –3.68$; $p < 0.001$) and independence ($t = –3.25$; $p = 0.002$) were found to be improved (**Table 2**).

When referring to the dichotomised outcome indicators GCPS, COPM performance and satisfaction, as well as the visual analogue scale for pain in rest and in activity with constant or lower scores at postsurgical assessment were considered to be not recovered and higher scores considered recovered 54%, 78%, 81%, 47%, and 47% respectively were characterised as recovered (question A).

Multivariate analyses of variance were separately calculated with groups (recovered versus not recovered) based on every outcome indicator as fixed factor and with the active range of motion of the operated fingers and the strength of the hand grip at the follow-up assessment as well as with the change of these parameters as dependent variables. None of these models proved to be statistically significant (question B). Nevertheless, the model with COPM performance group as a fixed factor demonstrated a significant tendency (Wilk's Lambda = 0.68; $F(5/27) = 2.53$; $p = 0.053$; $\eta^2 = 0.319$; power = 0.695); and the change in grip strength yielded a significant result in the test of between-subject-effects relating to the COPM performance grouping ($F = 6.92$; $p = 0.013$) and in the GCPS grouping MANOVA ($F = 4.56$; $p = 0.040$), even though the latter overall model was not significant.

When referring to the same groups in MANOVA with QoL facets or domains at 12 months after the operation as dependent variables, none of the models showed a significant main effect of the group variable except for 1) the groups based on the COPM performance scale (Wilk's Lambda = 0.16; $F(24/11) = 2.47$; $p = 0.060$; $\eta^2 = 0.840$; power = 0.762) with the activities of daily living facet showing a significant between-subject-effect test result

Table 1. Mean scores (SD) of outcome indicators at baseline and one year follow-up (paired sample t-test) and follow-up scores dependent on recovery classification (based on CPGS classification change—no significant differences).

	n	Baseline prior to surgery	One year after surgery	T	p	Recovered N = 21	Non-recovered N = 18
COPM performance	36	4.1 (1.8)	6.9 (2.3)	–6.05	0.001	7.0 (2.3)	6.7 (2.2)
COPM satisfaction	36	3.5 (2.0)	6.6 (2.5)	–6.05	0.001	6.7 (2.7)	6.5 (2.3)
VAS Pain at rest	33	3.7 (3.0)	3.3 (2.6)	0.72	0.478	3.2 (2.5)	3.2 (2.9)
VAS Pain in activity	33	4.1 (2.9)	3.4 (2.7)	1.30	0.203	3.3 (2.6)	3.2 (2.9)
GCPS Classification	39	2.6 (1.2)	1.8 (2.0)	3.52	0.001	1.7 (1.0)	2.0 (1.3)
GCPS Disability points	39	3.1 (2.1)	1.8 (2.0)	3.11	0.001	1.5 (1.6)	2.2 (2.3)
GCPS Disability Days	39	1.4 (1.2)	0.7 (1.1)	2.89	0.006	0.6 (1.0)	0.9 (1.2)
GCPS Characteristic Pain Intensity	39	47.9 (19.0)	39.0 (22.0)	2.14	0.039	34.6 (18.3)	44.1 (25.2)
Range of motion forefinger	37	134.9 (47.4)	154.1 (55.4)	–3.90	< 0.001	154.7 (55.0)	153.3 (57.4)
Range of motion middle finger	37	144.6 (45.8)	167.8 (46.0)	–5.30	< .001	173.2 (47.2)	162.2 (45.5)
Range of motion ring finger	35	135.0 (50.0	161.9 (46.7)	–5.43	< 0.001	163.9 (50.6)	159.7 (43.6)
Range of motion little finger	35	126.7 (50.1)	152.6 (54.5)	–3.57	0.001	149.2 (57.8)	156.2 (52.4)
Hand-grip strength	35	70.9 (46.4)	87.0 (48.9)	–2.17	0.036	93.8 (61.8)	79.5 (28.6)

Table 2. WHOQoL questionnaire facets and domains pre- and post-surgical（× (SD)—paired sample t-test).

	Prior to surgery	One year follow-up	T	p
Pain and discomfort	45,8 (15,2)	59,6 (17,1)	–3.97	< 0.001
Energy and fatigue	49,0 (15,4)	54,8 (14,2)	–2.02	0.050
Sleep and rest	59,8 (21,9)	68,4 (19,1)	–2.94	0.006
Positive feelings	60,6 (11,8)	59,6 (10,1)	0.50	0.623
Thinking, concentration	64,9 (12,2)	64,6 (12,0)	0.18	0.857
Self-esteem	58,2 (11,1)	57,7 (13,4)	0.28	0.784
Body image	64,7 (15,1)	65,4 (14,5)	–0.24	0.810
Negative feelings	64,7 (18,1)	64,9 (16,5)	–0.06	0.950
Mobility	56,3 (16,7)	60,4 (19,1)	–1.77	0.085
Activities of daily living	59,8 (16,4)	65,9 (18,0)	–2.15	0.028
Medication	48,6 (17.0)	49,8 (18,8)	–0.38	0.707
Work capacity	43,6 (21,7)	62,0 (22,6)	–4.60	< 0.001
Personal relationships	73,4 (12,7)	72,6 (15,2)	0.43	0.673
Social support	72,1 (16,2)	69,9 (18,5)	1.06	0.297
Sexual activity	54,2 (20,9)	55,5 (17,7)	–0.64	0.529
Physical safety	64,1 (12,9)	63,3 (12,8)	0.39	0.697
Home environment	70,2 (16,6)	66,7 (14.0)	2.25	0.031
Financial resources	65,5 (21.0)	63,9 (21,6)	0.96	0.342
Health and social care	61,2 (10,6)	61,1 (12,6)	0.10	0.924
New information	65,2 (13,7)	65,1 (14,1)	0.07	0.945
Recreation/leisure	57,2 (14,7)	59,0 (14,8)	–0.88	0.384
Environment	65,2 (13,3)	62,5 (11,8)	–0.96	0.344
Transport	71,6 (19,1)	68,4 (19,9)	5.09	< 001
Spirituality	47,9 (21,7)	50,2 (21,2)	–0.95	0.350
Physical	51,6 (13,2)	61,0 (13,5)	–3.68	< 0.001
Psychological	62,6 (8,9)	62,5 (10,3)	0.14	0.888
Independence	52,0 (13,6)	59,5 (14,9)	–3.25	0.002
Social	66,6 (13,2)	65,9 (14,2)	0.42	0.676
Environment	65,0 (10,6)	63,7 (10,6)	1.20	0.239
Spiritual	47,9 (21,7)	50,2 (21,2)	–0.95	0.350

(F = 6.65; p = 0.014) and 2) groups based on COPM satisfaction scale (Wilk's Lambda = 0.09; F(24/11) = 4.43; p = 0.007; η^2 = 0.906; power = 0.967).

When using the change score of the WHOQoL questionnaire as dependent variables the MANOVA models for all the various groups failed to reach significant main effects. However, several significant between-subjects-effects appeared. Therefore, we decided to add analyses on the univariate level. We could not find any difference relating to finger-motion or hand-grip strength between recovered and non-recovered patients on any of the outcome indicator groupings. However, recovered patients, as defined by the

1) GCPS change score, reported higher scores on the

WHOQoL facets thinking and concentration (z = –2.32; p = 0.020), activities of daily living (z = –2.18; p = 0.029), work capacity (z = –2.53; p = 0.011), and financial resources (z = –2.97; p = 0.003), as well as on the domains independence (z = –2.14; p = 0.032) and environment (z = –2.30; p = 0.022);

2) COPM performance scored higher than non-recovered patients on the thinking and concentration facets of the QoL measurement (z = –2.10; p = 0.040);

3) "pain at rest visual analogue scale", reported a lower chronic pain intensity in the CPGS (z = –2.31; p = 0.021), as well as higher scores in QoL facets energy and fatigue (z = –2.10; p = 0.036), negative feelings (z = –2.13; p = 0.034), and financial resources (z = –2.09; p = 0.036);

4) "pain in activity visual analogue scale" reported a lower chronic pain intensity in the CPGS (z = –2.92; p = 0.003), as well as higher scores in QoL facets negative feelings (z = –2.38; p = 0.019), work capacity (z = –2.03; p = 0.045), and financial resources (z = –2.09; p = 0.041).

None of the MANOVA models with the outcome groups as fixed factors and the personality domains of the TCI as dependent variables yielded a significant result; and only ST appeared as significantly differentiating between satisfied and dissatisfied patients after surgery (F = 4.95; p = 0.009) in the between-subjects-effect tests. However, when using the TCI subscales as dependent variables MANOVA gave significant results for the outcome group-differentiation except for the groups relating to GCPS scores (**Table 3**). The subscales 'disorderliness versus regimentation' (NS 4), 'anticipatory worry versus uninhibited optimism' (HA 1) and 'fatigability versus asthenia and vigour' (HA 4), and 'self-forgetfulness versus self-conscious experience' (ST 1) as well as 'transpersonal identification versus self-isolation' (ST 2) were most often of differentiating effect between improved and not improved patients.

The comparisons of personality domain scores between RA patients and the two groups of general population controls matched for age and gender did not render any significant result by means of t-test for dependent samples (question C). Based on the subscales of the TCI, the controls of both matched samples had significantly higher scores for 'disorderliness' (NS 4–t = –2.66/–2.29; p = 0.011/0.028) and HA 1 (t = –2.30/–2.75; p = 0.027/0.009); as well as lower scores for 'fatigability' (HA 4–t = 3.08/3.02; p = 0.004/0.005). Partly contradictory results between the two comparisons occurred for 'sentimentality' (RD 1–t = 2.14/.257; p = 0.039/0.798), 'compassion' (CO 4–t = 2.54/1.74; p = 0.015/0.090), 'pure-hearted conscience' (CO 5–t = –0.49/–2.14; p = 0.628/0.039), 'self-forgetfulness versus self-conscious experience' (ST 1–t = 1.73/3.74, p = 0.090/0.001) and 'transpersonal identification versus self-isolation' (ST 2–t = 1.87/2.02; p = 0.069/0.050).

In order to test for prediction of QoL hierarchical multiple regression analyses were calculated entering gender and age at the first step, either the functional range of motion and hand-grip strength from follow-up assessment or the change in these scores at the second step and TCI dimensional scores at the third step as independent variables together with either the six WHOQoL domains or the change in these domains as dependent variables (question D). We could not find any significant regression model relating to the change scores between pre- and postoperative assessments except for domain 'environment' with the difference in hand-grip-strength as a single substantial indicator in the equation (standardised Beta = –0.54; F = –3.25; p = 0.004); whereas, based on the scores from follow-up assessment, the variance in this set of variables could substantially explain variance in the QoL domains other than social and physical (**Table 4**).

Table 3. Results of MANAOVA with outcome scores as fixed factors and TCI subscales as dependent factors.

	Wilk's λ	F	Df/df	P	η^2	Power	Significant-between-subjects-effects
CPGS	0.55	1.26	50/180	0.138	0.260	0.987	NS4: F = 3.92; p = 0.023; HA1: 3.49; p = 0.034; HA4: F = 6.42; p = 0.002; ST1: F = 4.56; p = 0.012
COPM performance	0.44	1.80	50/174	0.003	0.340	1.000	NS4: F = 4.14; p = 0.019; HA1: 4.36; p = 0.015; HA4: F = 5.23; p = 0.007; ST1: F = 6.17; p = 0.003; ST2: F = 3.53; p = 0.033
COPM satisfaction	0.46	1.67	50/174	0.008	0.324	0.999	HA1: 3.63; p = 0.030; HA4: F = 5.37; p = 0.006; ST1: F = 7.06; p = 0.001; ST2: F = 3.63; p = 0.030
VAS pain rest	0.42	1.83	50/168	0.002	0.352	1.000	NS4: F = 3.28; p = 0.041; HA1: 3.85; p = 0.024; HA4: F = 3.72; p = 0.027; ST1: F = 5.83; p = 0.004; ST2: F = 3.46; p = 0.035
VAS pain activity	0.47	1.54	50/168	0.023	0.314	0.997	HA1: 3.10; p = 0.049; HA3: F = 4.34; p = 0.015; HA4: F = 3.72; p = 0.027; CO4: f 3.28; P = 0.041; ST1: F = 5.67; p = 0.005

HA1 'anticipatory worry versus uninhibited optimism'; HA3 'shyness with strangers versus confidence'; HA4 'fatigability versus asthenia and vig- our'; NS4 'disorderliness versus regimentation'; CO 'compassion versus revengefulness'; ST1 'self-forgetfulness versus self-conscious experience'; ST2 'transpersonal identification versus self-isolation'; GCPS Grade Chronic Pain Status; COPM Canadian Occupational Performance Measure; VAS visual analogue scale.

Table 4. Hierarchical multiple regression on QoL domains at one year follow-up.

WHOQoL domain	Standardised r^2	Model F	P	r^2	F	P	Variables in final equation with significant standardised Beta
Psychological	–0.06	0.22	0.941				HA (B = –0.48; t = –2.22; p = 0.038)
	–0.14	0.40	0.891				
	0.33	2.21	0.050	0.51	3.72	0.010	
Independence	–0.03	0.21	0.434				Motion range middle finger (B = –1.17; t = –2.74; p = 0.013)
	0.26	2.68	0.030				
	0.17	1.51	0.196	0.43	1.09	0.422	
Environment	–0.05	0.24	0.786				Age (B = 0.27; t = 2.66; p = 0.015); motion range middle finger (B = 0.87; t = 3.06; p = 0.006); little finger (B = –0.60; t = –3.08; p = 0.006); NS (B = –0.37; t = –3.61; p = 0.002); RD (B = 0.33; t = 2.70; p = 0.014); PS (B = –0.57; t = –4.51; p < 0.001); CO (B = 0.44; t = 3.95; p = 0.001); ST (B = –0.29; t = –2.92; p = 0.008)
	–0.07	0.69	0.681				
	0.74	7.92	< 0.001	0.70	13.02	< 0.001	
Spiritual	0.04	1.72	0.195				Gender (B = 0.48; t = 2.68; p = 0.014); motion range ring finger (B = –0.81; t = –2.28; p = 0.034); hand-grip strength (B = 0.68; t = 2.96; p = 0.008); PS (B = –0.45; t = –2.39; p = 0.027); ST (B = 0.35; t = 2.38; p = 0.027)
	0.22	2.39	0.049				
	0.42	2.77	0.019	0.28	2.33	0.065	

4. DISCUSSION

For this follow-up investigation, 40 RA patients could be recruited representing a reasonable sample compared with other studies in this field (e.g., 33 patients [24]; 45 patients [25]; 68 patients from three sites [26]).

Our results showed an average improved range of motion in all MP-joints resulting in improved grip strength after surgery. We assume that our good results in the whole group are a consequence of early controlled active motion; intense physiotherapy with special focus on functional grip; and the use of the metacarpal joint instead of the distal interphalangeal joints combined with the functional plasters individually manufactured by the occupational therapist. This assumption is supported by a review of effective post-operative therapy for MP arthroplasty that reported passive motion as ineffective in increasing motion or strength under this condition [27].

Moreover, we found average substantial improvement in all applied functional related and pain related scores except in the pain related VAS and, consequently, in several QoL facets and domains at the one year follow-up after surgery. This might be caused by the fact that surgery in RA patients only addresses one of several problems in the hand and the effects of surgery may be overshadowed by the more general nature of the disease. Colville *et al.* [28] reported improvements in active daily living and reduced hand pain but could not report any improvement in arthritis activity, mood or QoL after surgery in correspondence with our findings. Furthermore the difference found between objectively measureable changes and the subjective experience, particular in pain, represents further evidence of their relative association mediated by several other conditions. The objective change in motion and strength is obviously not directly and linearly correlated with changes in pain intensity or QoL.

Nevertheless, there are about 50% of the RA patients who reported less pain compared to pre-surgery (based on the dichotomized sample on pain related visual analogue scales and the GCPS score) and about 80% reported improved function and satisfaction one year after the surgery. Seemingly, the strength of the hand-grip is an important indicator of a perceived improved functional status of the hand causing an increased independence in activities of daily living and overall QoL. This might in turn imply an increase of QoL based on an improved functional status despite still suffering from severe pain.

Our RA patients were characterised by some deviations in personality compared to the general population subjects, implying that RA patients are more organised, preferring activities with strict roles and that they lose their temper more slowly (NS 4). Furthermore, they are pessimistic, anticipating harm or failure and ruminating about embarrassing experiences for long time (HA 1); and they are asthenic, lacking energy and recovering slowly from minor illness or stress (HA 4). These differences compared to people from the general population can probably be explained to a substantial amount by adaptation to the pain, impairment and disability caused by RA.

However, it partly confirms findings of Chou and Brauer [1] of high negative affect in RA patients as well of difficulties in regulating emotions in pain patients by Hamilton *et al.* [6]. The many anticipatory worries identified, combined with the limited openness and flexibility in their behaviour might in turn cause more self-focussed attention, including attention to pain signals, leading to increased passivity and avoidance of activity in order to prevent increasing pain which in turn would negatively affect QoL. This explanation would argue against an interpretation in favour of a RA personality and only supports the assumption that the identified deviations in personality characteristics of RA patients compared to healthy people are primarily personality changes due to RA.

Interestingly, improved and not improved patients after surgery differ on the same personality characteristics in the same way as the RA patients differ from healthy subjects – namely, a high self-transcendence in the sense of abilities to transcend their boundaries when deeply involved in something or concentrating on the present activity (ST 1) and highly intensive perception and experience of connectedness to the world (ST 2). These self-transcendent skills might enable the patients to be more accepting, better able to cope and to be more satisfied with their lives than those lacking these abilities.

HA as a personality trait reflecting the type or colour of focus and orientation on the world, the ability to deal with uncertainties, strange and unfamiliar situations as well as the overall level of energy was found to be the only substantially predicting variable for 'Psychological health' as one important domain of QoL after controlling for age, gender and range of motion. This finding was expected because there are many reports in the literature of close relationships between HA and various psychopathological manifestations, suggesting a non-specific vulnerable role of HA in relation to psychopathology in general [16]. Interestingly, the QoL domain 'Independence` is substantially predicted by one of the objective indicators implying that the improved movement abilities after surgery cause an improved independency in self-care and other general daily living activities. Particular personality characteristics do not increase the prediction of 'Independence.

It appears somewhat curious that the QoL 'Environment' domain is predicted by personality characteristics to the greatest degree; 74% of the variance could be explained and 70% only by personality characteristics in terms of TCI temperament and character domains. This QoL domain integrates several areas of life including the availability of health-care services, possibilities of information and knowledge acquisition, as well as the possibilities of active participation at recreational and leisure activities. Even though age and movement abilities are of significant predictive value, a wide range of personality characteristics consisting of NS, RD, PS, CO, and ST is of predictive power; with only HA as less meaningful.

The interpretation of the study results is limited by the consecutive nature and the small size of the sample. This might have caused an under-evaluation of findings because of the level of statistical significance. However, well established measurements were applied and the use of two matched general population sample concerning personality measurement can be considered as strength as it allowed a cross-validation of the differences between RA patients and controls to be performed. The combined consideration of objective and subjective indicators of surgery outcome and HRQoL can be seen as an additional strength.

In summary, RA patients' QoL can be significantly improved by MP arthroplasty in most cases by improveing their movement abilities, despite substantial levels of pain remaining. NS and HA temperament systems of personality seem to play an important role in the adaptation process during the long term, chronic illness causing measureable differences compared to general population subjects. However, the character dimension ST represents a tool for coping with the burden of the pain and disability leading to a better experienced QoL after hand-surgery. Finally, personality characteristics are highly predictive of QoL, particularly relating to 'Environment' and 'Psychological Health' suggesting their important mediating role between experienced pain and disability and HRQoL.

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List of Abbreviations

CO Cooperativeness
COPM Canadian Occupational Performance Measure
GCPS Grade Chronic Pain Status
HA Harm Avoidance
HRQoL health Related Quality of Life
MP Metacarpophalangeal
NS Novelty Seeking
PS Persistence
QoL Quality of Life
RA Rheumatoid Arthritis
RD Reward Dependence
SD Self-Directedness
ST Self-Transcendence
TCI Temperament and Character Inventory
VAS Visual Analogue Scale

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Effect of user fee on patient's welfare and efficiency in a two tier health care market

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ABSTRACT

This is a theoretical paper examining the effect of user fee on patients' welfare and social welfare under three forms of provider reimbursements: full cost, prospective payment and cost sharing. The paper extends Rickman and McGuire (1999) by introducing user fee to the public sector and maintaining the assumption that providers can work in both the private and public health sectors. Contrary to previous studies, this study shows that efficiency is possible under the full cost reimbursement. The paper also shows the conditions under which efficiency is possible under each reimbursement scheme. Patient's welfare can improve with the introduction of user fee when services in the public and private sector are complementary.

Keywords: User Fee; Two Tier Healthcare; Mixed Financing; Prospective Payment; Cost Sharing

1. INTRODUCTION

Many countries have the two tier health care system: the coexistence of public and private health care sectors. And over the years, public health care reform in many countries has involved a switch from fully financed system to mixed ones [1,2]. Such a switch is observed in both industrialized and developing countries. The mixed financing system involves making the patient bear at least part of the cost of care provided in the public sector. The intention is to partly relieve the government of the burden of funding public health care and at the same time reduce excessive use of care that might exist under the fully funded system. Various payment schemes are also used in the public health sector to pay health care providers. The purpose of this paper is to find the impact of patient direct payment at the point of purchase, in the public health sector, on the efficiency of three payment schemes: full cost reimbursement, prospective payment and cost sharing in a two tier health care sector.

Example of mixed financing systems in industrialised countries consists of a combination of compulsory social security system covering a package of essential services and private insurance policy to cover the rest. The patient then has to pay premium and co-payment [3]. Some of the industrialised countries that have adopted this include Australia, Italy, and the United States (in the Medicare plan) [3]. In developing countries the mixed financing system in general involves introduction of user fee. Many Sub-Saharan countries such as Cote d'Ivoire, Kenya, and Nigeria have adopted this system.

There is considerable theoretical literature on the effect of private insurance (with co-payment) on quality of care and the efficient provision of services (e.g., [4-8]. While these analyses centred more on the effect of insurance on quality and efficiency of services than on the reimbursement schemes, others examined the role of different reimbursement schemes in the efficient provision of services [9-17]. The question then is does the effect of mixed financing system on efficiency depend on the type of reimbursement scheme to providers.

To answer the question, the current paper extended a model on a two tier health care system in [16] that examined the effect of the private health care sector on the efficiency of provider reimbursement schemes. The extension involves the introduction of a user fee to the public sector. Reference [16] in turn, was an extension of [18], which examined the effect of reimbursement scheme on the supply of services in the public sector. In their study, the public health care was a fully funded system (e.g., the National Health Services in the Britain) and so the patient did not pay for the services received. Reference [16] extended [18] by including a private sector while maintaining the full funding system in the public sector. The specific interest in the paper then is to examine how their results of the two previous papers would be affected when a user fee is included in the public sector.

Earlier studies that examined health production in private and public sector with user fee in the public sector include [18-20]. Reference [18] presented an empirical model in which effort was not observable and patients had to choose medical contracts for health care provision from government and mission hospitals in the Cameroun. Reference [20] also presented an empirical study, which examined how the introduction of user fee to the public sector affects quality and accessibility of services. In a theoretical model in which public sector services are covered partly by compulsory social insurance, partly by private insurance and partly by out-of-pocket, [21] examined the conditions for optimal rates of social insurance and private coinsurance. Following [16] the current paper focuses on three forms of provider-reimbursement schemes: full-cost reimbursement, prospective payment and cost sharing.

2. METHOD

This is a model of mixed health care market, public and private, that allows physicians to work in both sectors. Let q represent publicly provided health care received by a representative patient and s represent privately provided health care. The patient receives benefit, $B(q,s)$, from treatment and pays for it according to the marginal benefit it provides: $B_s(.)s$, for treatment in the private sector and $(1-\alpha)B_q(.)q$ for treatment in the public sector, where $0 < \alpha < 1$ is the fraction of the fee paid by the government. These fees do not have to equal the full cost of treatment. The patient could have partial insurance and bear only part of the cost of treatment. What is important is that the patient bears some cost for treatment in both sectors. $B(.)$ has positive marginal products, with unique maxima in q and s, $B_{qq} < 0$, $B_{ss} < 0$. Treatments, q and s, can be complements, $B_{sq}(.) > 0$, substitutes, $B_{sq}(.) < 0$ or unrelated $B_{sq}(.) = 0$. Treatments are complements if, for example, the physician uses the public sector to request the patient to do some tests in the private sector to help with diagnoses in the public sector. When the physician uses, for example, the private sector to treat an illness that can be treated in the public sector then q and s are substitutes. The patient's net benefit is:

$$N(q,s) = B(q,s) - B_s(q,s)s - (1-\alpha)B_q(q,s)q \quad (1)$$

With the exception of the last term on the RHS, which represents the fee in the public sector, (1) is identical to the patient's net benefit in [16]. The marginal net benefits are:

$$N_q(q,s) = \alpha B_q(q,s) - q(1-\alpha)B_{qq}(q,s) - sB_{sq}(q,s) > 0 \quad (2)$$

[1]

$$N_s(q,s) = -sB_{ss}(q,s) - (1-\alpha)B_{qs}(q,s)q > 0 \quad (3)$$

Physicians are regulated and are often expected to follow a code of ethics with the purpose of taking the patient's interest into account when choosing treatment. Following [22] it is assumed that the physician cares about the ethics of treatment[2]. Thus, the physician cares about the well-being of his patient, $N(q,s)$, as well as the profit of his public hospital, π^h and private sector profit, π^p:

$$\pi^h = R(q) + (1-\alpha)B_q(q,s)q - c(q) \quad (4)$$

where $R(q)$ is the revenue that the public hospital receives from the government. Again with the exception of $(1-\alpha)B_q(q,s)q$ (4) is identical to the public hospital profit in [16]. The private profit, however, is the same as that in [16]:

$$\pi^p = B_s(q,s)s - c(s) \quad (5)$$

where $c'(i) > 0$, $c''(i) > 0$ $(i = q, s)$,[3] and π^h and π^p have unique maximum in q and s respectively, the marginal profits are:

$$\pi_s^h = (1-\alpha)B_{sq}(q,s)q \gtrless \text{ or } < 0 \quad (6)$$

$$\pi_q^h = R'(q) + (1-\alpha)B_q(q,s) + qB_{qq}(q,s) - c'(q) \lessgtr \text{ or } > 0 \quad (7)$$

where $R'(q) > 0$.

$$\pi_q^p = B_{sq}(q,s)s \gtrless 0 \quad (8)$$

$$\pi_s^p = B_s(q,s) + sB_{ss}(q,s) - c'(s) \gtrless 0 \quad (9)$$

The physician's utility function is $U(\pi^h, \pi^p, N)$, with $U_N > 0$, $U_{\pi^h} > 0$, $U_{\pi^p} > 0$, $U_{NN} < 0$, $U_{\pi^h\pi^h} < 0$, and $U_{\pi^P\pi^P} < 0$. The physician chooses q and s to maximize his utility. The first order conditions are:

$$U_{\pi^h}\pi_q^h + U_{\pi^p}\pi_q^p + U_N N_q = 0 \quad (10)$$

$$U_{\pi^h}\pi_s^h + U_{\pi^p}\pi_s^p + U_N N_s = 0 \quad (11)$$

[1]Note that N_q can be negative when services are complements and s is very large. This case is not examined.

[2]I do not use the assumption in [16] that patients do not search among alternative physicians but because physicians care about the welfare of patients they do not charge a monopolist price. In the current model, the physician's care for the patient represents his care for ethics of treatment .This prevents the physician from charging monopolist price. As shown in **(11)** the physician would charge a monopolist price if $U_N = 0$. This is consistent with [23], where the physician is constrained by patient information. In [23] even though, patients cannot evaluate the marginal benefit from treatment from a given physician they can evaluate the absolute utility upon treatment. Patients can observe the utility of other patients after treatment. If patients of one physician end up with a lower utility on average than others then the physician looses patients.

[3]In order to avoid the implied requirement that the marginal cost in the two sectors be equal, I do not assume constant marginal costs for the provision of treatment as in [16].

$$\Rightarrow U_{\pi^p} = -\frac{U_{\pi^h}\pi_s^h + U_N N_s}{\pi_s^p} \quad (12)$$

By substituting (12) into (10) and rearranging produces:

$$\pi_q^h = MRS_{\pi^h N} N_s \left(\frac{\pi_q^p}{\pi_s^p} - \frac{N_q}{N_s}\right) + \frac{\pi_s^h \pi_q^p}{\pi_s^p} \quad (13)$$

where $MRS_{N\pi}{}^h = U_N / U\pi^h > 0$. **Eq.13** defines a locus $\{q,s\}$ that maximize the physician's utility. With the exception of the second term on the RHS, (13) is identical to what [16] obtained. This second term appears here because the inclusion of a fee in the public sector makes π_s^h, which is zero in [16], positive, negative or zero, in the current model, depending on whether q and s are complements, substitutes, or unrelated respectively. The terms in brackets are the magnitudes of the slopes of the private sector iso-profit (π_q^p/π_s^p) and the patient's indifference curve (N_q/N_s).

The welfare function, $W(q, s) = B(q, s) - c(q) - c(s)$, is used to find efficiency under the various reimbursement schemes. It is assumed that the welfare function is concave and has unique maxima in q and s. Efficiency requires that the following first order conditions for the maximization of the welfare function are satisfied:

$$B_q(q,s) - c^h = 0 \quad (14)$$

$$B_s(q,s) - c^p = 0 \quad (15)^4$$

The three physician reimbursement rules in the public sector and their effect on equilibrium q and s provided by the physician are now examined. The reimbursement rules are full-cost reimbursement, prospective payment, and cost sharing. The patients in [16] did not have to pay fees in the public sector and so the full-cost reimbursement involved the government providing enough revenue to cover the cost of production. In the current model, however, the full-cost reimbursement, involves the public hospital receiving $R(q)$ from the government to cover part of the cost not covered by the user fee. Prospective payment involves the government giving a fixed amount of revenue, G, to the hospital regardless of the total cost of production and of the total fees collected. The cost-sharing rule is a combination of prospective payment and cost reimbursement. The government gives fixed revenue, G, and then pays for a fraction of the cost of production. Under each rule, (13) is used to examine how the q and s chosen by the physician affect optimality from the points of view of the patient and society.

The patient's indifference curves are downward sloping for both complements and substitutes[5]. This is because the slope of the indifference curve is $-N_q/N_s$ and with $N_q > 0$, $N_s > 0$ regardless of the relationship between q and s. **Figures 1(a)** and **1(b)** shows the iso-profits for the physician's private profit. The slope of the iso-profit $(-\pi_q^p/\pi_s^p)$ depends on whether treatments are substitutes or complements. As shown in (8) and (9), π_q^p is positive when treatments are complements and negative when they are substitutes; π_s^p is positive when s is very small and becomes negative as it is increases. Thus, when treatments are substitutes, the iso-profit slopes upwards when s is small and downwards when s increases with profit increasing as q falls. The opposite occurs when treatments are complements. When treatments are complements the iso-profit slopes downwards when s is small and upwards as s increases with profit increasing in q. These are shown in **Figure 1**.

3. RESULTS AND DISCUSSION

3.1. Full Cost Reimbursement

Under this rule, the government provides the revenue required to cover part of the cost of production that the user fee could not cover. Hence $\pi^h = 0$ and so $\pi_q^h = 0$; i.e., the physician chooses q to maximize public hospital profit. Equation (13), then, becomes:

$$0 = MRS_{\pi^h N} N_s \left(\frac{\pi_q^p}{\pi_s^p} - \frac{N_q}{N_s}\right) + \frac{\pi_s^h \pi_q^p}{\pi_s^p} \quad (16)$$

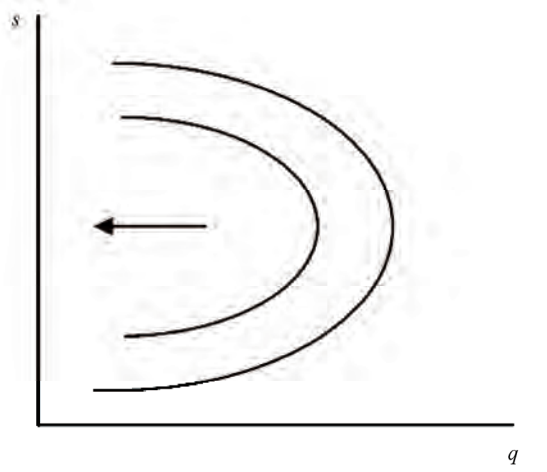
Rearranging (16) produces:

$$\frac{MRS_{N\pi^h} N_s + \pi_s^h}{MRS_{N\pi^h} N_s} \frac{\pi_q^p}{\pi_s^p} = \frac{N_q}{N_s} \quad (17)$$

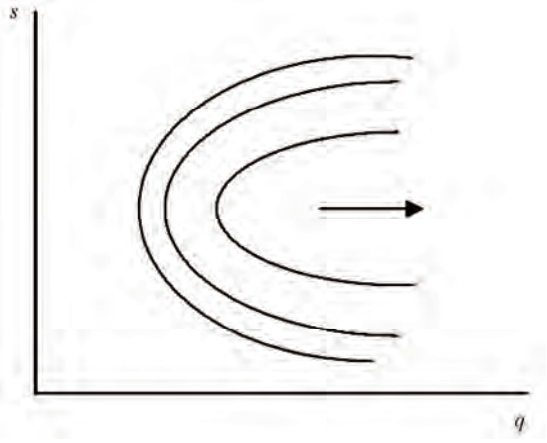
Notice that when $\pi_s^h = 0$, the two slopes in brackets in (16) are equal, and the results are identical to those in [16], *i.e.*, the physician chooses q and s to equate the slopes of the iso-profit and the patient's indifference curves. Such result is possible in the current model if q and s are unrelated, *i.e.*, if $B_{qs}(q, s) = 0$ implying that $\pi_s^h = 0$. It is of interest to examine the optimality of equilibrium q and s from the patient's and society's perspective when services are complements, $\pi_s^h > 0$, and when they are substitutes, $\pi_s^h < 0$. **Eq.17** shows that in equilibrium the difference between the slopes of the iso-profit and indifference curves depends on the sign of π_s^h. When treatments are substitutes, $\pi_s^h < 0$, the iso-profit curve is steeper than the patient's indifference curve. The resulting q lowers the patient's welfare compared to [15] as well as [16]. Reference [15] showed that in the ab-

[4]where c^h and c^p represent the marginal cost of q and s respectively.

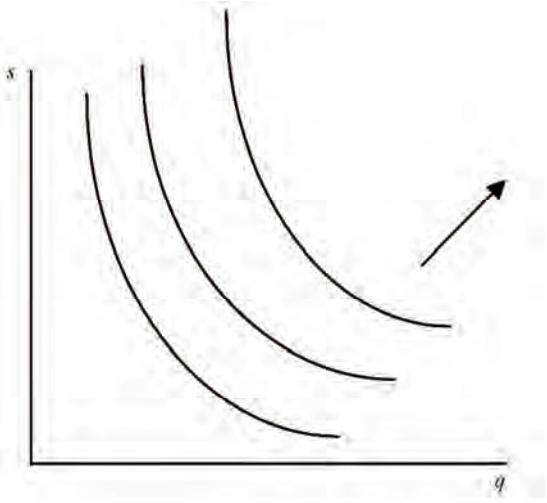
[5]Because the marginal net benefits in this model differ from those in Rickman and McGuire the patient's indifference curves in this model are also different. The indifference curves in Rickman and McGuire slope downward when q and s are substitutes and upward when they are complements (U-shaped indifference curves). In Rickman and McGuire $N_s > 0$, whether q and s are substitutes or complements; $N_q > 0$ when they are substitutes and $N_q < 0$ when they are complements.



(a)



(b)



(c)

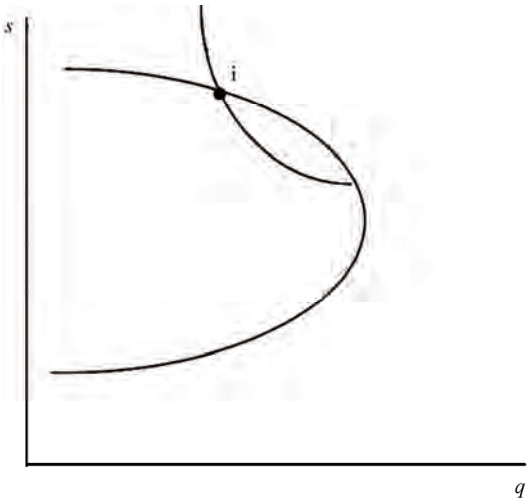
Figure 1. (a) Private iso-profit curves for substitutes; (b) private iso-profit curves for complements; (c) patient's indifference curves for substitutes and complements.

sence of a private sector and a user fee, the optimal q chosen by the physician coincides with what the patient would choose under full information, *i.e.*, at the point where net marginal benefit is zero. However, by including the private sector, [16] showed that the physician reallocates treatments between the private and public sector such that the patient's marginal net benefit in equilibrium is positive; hence, from the patient's perspective, the q chosen by the physician is sub-optimal. In the current model, the inclusion of a user fee further increases the patient's marginal net benefits. The patient, then, gets less q and lower welfare than in [16]. **Figure 2(a)** shows the equilibrium treatments from each sector when treatments are substitutes.

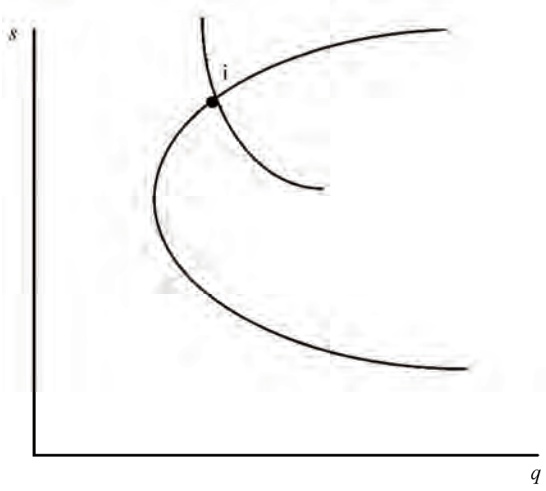
For complements $N_s > 0$, $N_q > 0$ and $\pi_s^h > 0$ and so in equilibrium the patient's indifference curve is steeper than the iso-profit. The patient, then, receives less supply of both services than he would have chosen himself. This is contrary to [16] where the patient received oversupply of q and undersupply of s. It is thus not clear if the patient is worse off or better off in this model than in [16] when services are complements. The equilibrium is shown in **Figure 2(b)**.

The difference between the sign of π_s^h in [16] and the current paper makes an important point. With $\pi_s^h = 0$ in [16], the quantity of s chosen by the physician does not affect the profit of the public hospital. The introduction of user fee in the current paper, however, makes the public hospital profit dependent on the quantity of services supplied in the private sector with public profit decreasing in s when services are substitutes and increasing s when services are complements. When services are substitutes, the two sectors compete for services and so every unit of private treatment supplied represents a loss of fee to the public sector. With complementary services, however, the two sectors become partners and so an increase in supply of private treatment is accompanied by an increase in supply of public treatment and thus increases the amount of fees received by the public hospital. Since the physician is agent to the public as well as the private hospitals, a change in the physician's behaviour that depends on the relationship between the services represents the conflict of interest that exist in the agency relationship. Such conflict of interest can adversely affect the public hospital if the physician puts more weight on private profit than public profit when services are substitutes. When services are complements the two hospitals becomes partners and so any conflict of interest is eliminated. Thus, the standard notion that physician has incentive to create artificial shortages in the public sector in order to increase services in the private sector is relevant when services are substitutes.

The efficiency of the equilibrium is now considered by using (16):



(a)



(b)

Figure 2. (a) Full cost equilibrium for substitutes; (b) full cost equilibrium for complements.

$$0 = MRS_{N_{\pi^h}} N_s \left(\frac{\pi_q^p}{\pi_s^p} - \frac{N_q}{N_s} \right) + \frac{\pi_s^h \pi_q^p}{\pi_s^p} \tag{16}$$

By substituting (9) into (16) and rearranging (16) becomes:

$$v = \frac{MRS_{N_{\pi^h}} N_s + \pi_s^h}{MRS_{N_{\pi^h}} N_q} \pi_q^p - sB_{ss} \tag{18}$$

where $v = B_s - c^p$. Assuming efficiency in the private sector, $v = 0$, it is of interest to find out if efficiency in the public sector is also achievable. This is done by setting $v = 0$ and substituting (2), (3), (6) and (8) into (18) and solving for B_q:

$$B_q = \frac{(1-\alpha)qB_{qq} + \alpha sB_{sq} - 1}{\alpha} + \frac{(1-\alpha)qB_{sq}(1 - MRS_{N_{\pi^h}})}{\alpha sB_{ss} MRS_{N_{\pi^h}}} \tag{19}$$

The sign of B_q depends on whether services are complements or substitutes as well as on the size of $MRS_{N\pi}^{\ h}$. For substitutes, $B_q < 0 < c^h$ when $MRS_{N\pi}^{\ h} \geq 1$ and for complements, $B_q < 0 < c^h$ is possible when $MRS_{N\pi}^{\ h} < 1$. However, $B_q > 0 \lessgtr c^h$ is possible when services are substitutes and $MRS_{N\pi}^{\ h} < 1$ or when services are complements and $MRS_{N\pi}^{\ h} > 1$. For complements when $MRS_{N\pi}^{\ h} = 1$, $B_q < 0 < c^h$ when $q > s$ and demand for q is inelastic[6]. Similarly, $B_q > 0 \lessgtr c^h$ when $q < s$ and demand for q is elastic. $MRS_{N\pi}^{\ h} = 1$, means that the physician puts equal weight on the public hospital's surplus and patient's utility, when choosing treatment. Reference [15], call this behaviour perfect agency. Recall that $MRS_{N\pi}^{\ h} = U_N / U_\pi^{\ h}$, and so $MRS_{N\pi}^{\ h} > 1$ when the physician puts more weight on the patients net benefit than public hospital surplus. Similarly, $MRS_{N\pi}^{\ h} < 1$ when the physician puts more weight on the public hospital surplus than the patient's net benefit. As described in [15], such imperfect agency is likely, because the hospital often has a stronger bargaining power on the physician than the patient. Hence the analysis will focus on the case where $MRS_{N\pi}^{\ h} \leq 1$. Perfect agent is used to refer to the case in which $MRS_{N\pi}^{\ h} = 1$ while imperfect agent refers to the case in which $MRS_{N\pi}^{\ h} < 1$.

For substitutes, (19) shows that when the supply of s is efficient the physician oversupplies q if he is a perfect agent. In the same way the physician can oversupply q when for complementary services he is an imperfect agent or is a perfect agent, $q > s$, and demand for q is inelastic. For both substitutes and complements efficiency in both sectors can be ruled out under these circumstances. However, efficiency is possible when providing services that are substitutes and the physician is an imperfect agent. When services are complements perfect agency combined with elastic demand for q and a less supply of q than s is required for efficiency.

These results are important in several respects. First, contrary to [16], where efficiency in both sectors is not possible under the full-cost reimbursement rule, efficiency in both sectors is possible in the current model under the full cost. This gives credibility to the argument that cost control policy on the demand-side in the form of user fee, coinsurance, and deductibles is essential to reducing the excessive use of care that exist under the full cost reimbursement scheme with full insurance in the public sector. However, even though efficiency is

[6]Note that price elasticity of demand for q is: $\varepsilon_q = \frac{B_q}{qB_{qq}} \Rightarrow B_{qq} = \frac{B_q}{q\varepsilon_q}$

possible it is achieved at the expense of the patient's welfare. This is not surprising because the user fee forces the patient rather than the provider to internalize any externality that existed in the market. As already explained, the user fee reduces the equilibrium supply of services and this makes the patient worse off. Secondly, imperfect agency is required for efficiency. Unlike [16] as well as [15] where the agency role is not able to achieve efficiency under full cost reimbursement, imperfect agency is crucial for efficiency in the current model. In [16], efficiency in the private sector is only achievable at the expense of oversupply of services in the public sector. The possibility of efficiency in the current model then implies that when services are substitutes, the introduction of user fee in the public sector constraints the physician from oversupplying services to the extent of supplying the amount that maximizes social surplus as long as the physician is an imperfect agent. The user fee then transfers any cost caused by the imperfection of the agency unto the patient. Thirdly, the elasticity of demand for public service is important for efficiency when services are complements. For complementary services, the user fee maximizes social surplus by reducing oversupply as long as the physician is a perfect agent when demand for q is elastic and the equilibrium supply of q is less than s. When services are complements and there are no close substitutes the resulting inelastic demand for q makes even perfect agency unable to achieve efficiency in the public sector. This weakens the ability of the user fee to achieve the efficiency and so weakens the argument for cost control on the demand side when services are complements.

The results are also contrary to what [24] and [25] found in comparing full cost reimbursement with prospective payment. They show that cost-reimbursement like fee-for-service is characterized by oversupply because it does not provide the provider incentive to economize on the quantity of services. Like [15] they did not have user fee in their models.

3.2. Prospective Payment

Under this rule, the government gives a fixed amount of revenue, G, to the public hospital regardless of cost of the production and revenue collected from user fee. Thus, (4) becomes:

$$\pi^h = G + (1-\alpha)B_q(q,s)q - c(q) \tag{20}$$

The resulting marginal profit with respect to q is negative:

$$\pi_q^h = (1-\alpha)B_q(q,s) + q(1-\alpha)B_{qq}(q,s) - c'(q) < 0 \tag{21}$$

Eq.13, then, becomes:

$$\begin{aligned}&(1-\alpha)B_q(q,s) + q(1-\alpha)B_{qq}(q,s) - c'(q)\\ &= MRS_{N\pi^h} N_s \left(\frac{\pi_q^p}{\pi_s^p} - \frac{N_q}{N_s}\right) + \frac{\pi_s^h}{\pi_s^p}\frac{\pi_q^p}{}\end{aligned} \tag{22}$$

Rearrange to get:

$$\begin{aligned}&\frac{c'(q) - q(1-\alpha)B_{qq}(q,s) - (1-\alpha)B_q(q,s)}{MRS_{N\pi^h} N_s}\\ &+ \frac{MRS_{N\pi^h} N_s + \pi_s^h}{MRS_{N\pi^h} N_s}\frac{\pi_q^p}{\pi_s^p} = \frac{N_q}{N_s}\end{aligned} \tag{23}$$

With the exception of the first term on the left hand side (LHS), (23) is identical to (13). This (positive) term determines the difference between the equilibrium q and s under the full-cost payment scheme and the prospective payment scheme. Again, the relationship between the slopes of the indifference curve and the iso-profit depends on whether services are substitutes or complements. For substitutes, the coefficient of the iso-profit's slope is less than one. Thus, (23) shows that, under the prospective payment scheme, the equilibrium q and s occurs at a point where the iso-profit is steeper than the indifference curve but not as steep as under the full-cost scheme. This is shown in **Figure 3(a)** as (ii) which has less q and more s than the full cost equilibrium[7]. This result is equivalent to [16]. When services are complements, the coefficient of the iso-profit's slope in (23) is greater than one implying that in equilibrium the iso-profit is flatter than the indifference curve but not as flat as under the full cost. As shown in **Figure 3(b)** as ii, the equilibrium q and s are both less than those of the full cost and for a given level of private profit, the patient ends up on a lower indifference curve than the full cost equilibrium. This again is consistent with the results of [16].

To determine the efficiency of this equilibrium (2), (3), (6), (8), and (9) are substituted into (23) set $B_s = c^p$ (*i.e.*, efficiency in the private sector), and rearranged to produce:

$$k = (1 - MRS_{N\pi^h})\left[\alpha B_q + q(1-\alpha)\frac{B_{sq}^2 - B_{qq}B_{ss}}{B_{ss}}\right] \tag{24}$$

where $k = B_q - c^h = 0$ is required for efficiency in the public sector. **Eq.24** shows that $k = 0$ when $MRS_{N\pi}{}^h = 1$. However, $k > 0$ when $MRS_{N\pi}{}^h < 1$ and $k < 0$ when $MRS_{N\pi}{}^h > 1$[8]. Efficiency in the public sector depends

[7]Note Note that a fall in q causes an increase in private profit when services are substitutes. By using the positive sloped portion of the iso-profit it is possible to obtain similar results if the private iso-profit curve is allowed to shift to the left and indifference curve remains unchanged.

[8]The underlying assumption is that $\frac{B_{sq}^2 - B_{ss}B_{qq}}{B_{ss}} > 0$, *i.e.*, the q and s maximize patient benefit if cost were zero, making the term in the square bracket positive.

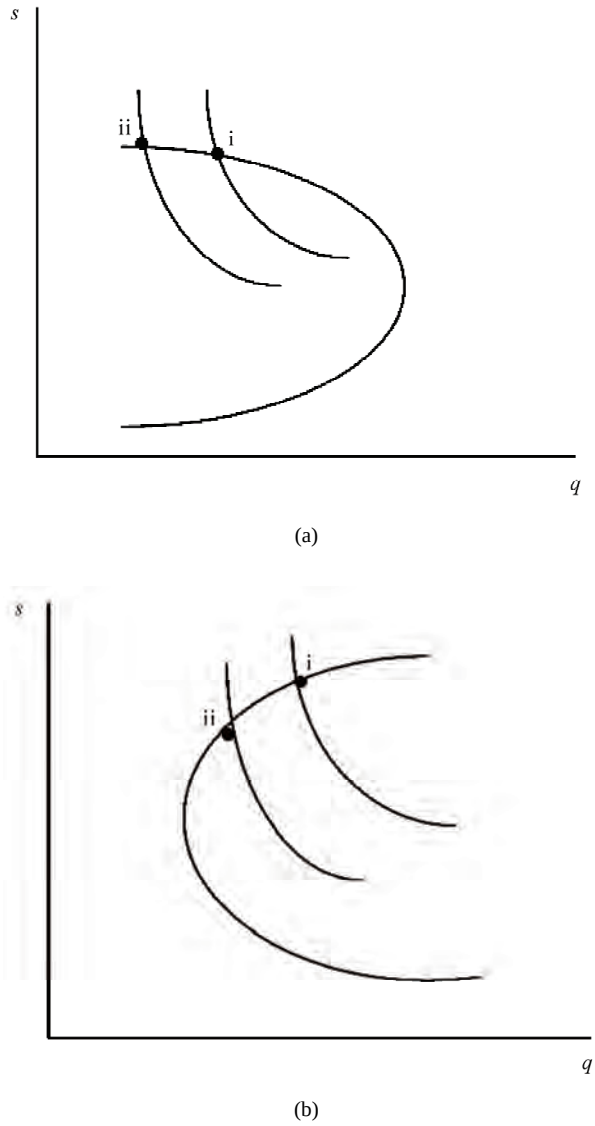


Figure 3. (a) Prospective payment equilibrium for substitutes relative to that of full cost; (b) Prospective payment equilibrium for complements relative to that of full cost.

on the agency behaviour of the physician and not on the relationship between the services. Perfect agency is required for efficiency in the public sector while imperfect agency produces too little q in equilibrium. Given that the physician is an imperfect agent, (24) shows that term in the square bracket increases when the price elasticity of demand for s increases[9]. As the demand for services in the private sector becomes more elastic the imperfect agent supplies an optimal amount of private services at the expense of too little supply of public services regardless of the relationship between the services.

These results are also important. First, compared with the cost reimbursement case, the disincentive that accompanies the provision of q under the prospective payment causes the imperfect agent to cut down the oversupply of q that exist under the cost reimbursement when services are complements as well as the optimal supply of q when services are substitutes such that too little q is produced whether services are complements or substitutes. Second, the results here are contrary to [16] where efficiency in both sectors is possible under the prospective payment when services are substitutes regardless of the agency type of the physician. Third, the results are similar to [15] in that perfect agency is required for efficiency. Reference [15] argues that the need for perfect agency weakens the argument in favour of prospective payment scheme. The influence of hospitals on physicians' behaviour is manifested in the change in services provided by physicians in accordance with changes in the payment scheme to hospitals. Thus, physicians are more likely to be imperfect agents than perfect agents implying that efficiency cannot be actualized under the prospective payment. The results also provide interesting comparison with [10], [16] and [12]. Reference [10] presents a model in which all firms (hospitals) produce homogeneous product but differ by the cost of production. He concludes that prospective payment, because it makes payment independent of the hospital's cost, is optimal. Reference [16] considers a model with heterogeneous patients, in terms of costliness, that can be treated with varying efforts with demand responding to the variation. Managerial effort is required for the enhancement of quality and reduction of cost. His results show that prospective payment can elicit the efficient effort if the provider has to treat all patients. Selden's results, however, showed that prospective payment is not optimal even under full insurance.

The question then is what size of α can lead to efficiency in the public sector, under the prospective payment, given efficiency in the private sector. This is found from (24) by setting $k = 0$ and solving for α:

$$\alpha^{*} = \frac{q(B_{sq}^{2} - B_{ss}B_{qq})}{q(B_{sq}^{2} - B_{ss}B_{qq}) - B_{q}B_{ss}} \tag{25}$$

Thus, there is an optimal α at which efficiency in both sectors is possible under the prospective payment regardless of the type of agency role played by the physician as well as the relationship between services. Obviously, $\alpha^* < 1$. Note, however, that α^* increases as the elasticity of demand for private service falls regardless of the relationship between the private and public ser-

[9]Note that $\varepsilon_s = \frac{B_s}{sB_{ss}} \Rightarrow B_{ss} = \frac{B_s}{s\varepsilon_s}$, substituting this into **(24)** and differentiating with respect to ε_s yields $sq(1-\alpha)\frac{B_{sq}^{2}}{B_s} > 0$.

vices[10]. This is interesting because (24) shows that given that the physician is imperfect, a fall in the elasticity of demand for private services causes the physician to increase services in the public sector given an optimal supply of services in the private sector. Thus, (25) implies that as the services in the private and/or the public sector become necessities (and so less elastic) efficiency demands that the government reduces the user fee for public services. This is consistent with [16] that when α = 1 efficiency is possible under prospective payment regardless of the type of agency.

3.3. Cost Sharing

Under this rule, the government makes a fixed payment, G, and covers a fraction, γ, of the cost of production:

$$R(q) = G + \gamma c(q) \tag{26}$$

The public hospital profit becomes:

$$\pi^h = G + (1-\alpha)B_q(q,s)q + (\gamma - 1)c(q) \tag{27}$$

where $0 < \gamma < 1$. The government can increase, G, and reduce γ such that the total payment remains constant. Note that $\gamma = 0$ implies prospective payment while $G = 0$ and $\gamma = 1$ represents full cost reimbursement. The marginal profit is:

$$\pi_q^h = (1-\alpha)B_q(q,s) + q(1-\alpha)B_{qq}(q,s) - (1-\gamma)c'(q) < 0 \tag{28}$$

Eq.13 becomes:

$$\frac{(1-\gamma)c'(q) - q(1-\alpha)B_{qq}(q,s) - (1-\alpha)B_q(q,s)}{MRS_{N_{\pi^h}} N_s} + \frac{MRS_{N_{\pi^h}} N_s + \pi_s^h}{MRS_{N_{\pi^h}} N_s} \frac{\pi_q^p}{\pi_s^p} = \frac{N_q}{N_s} \tag{29}$$

With the exception of - $\gamma c'(q)$, (29) is identical to (23). **Eq.29** shows that for substitutes the iso-profit is steeper than the patient's indifference curve but not as steep as under the prospective payment. When services are complements, however, the iso-profit is flatter than the patient's indifference curves but by lower degree than under the prospective payment. These are shown as iii in **Figures 4(a)** and **4(b)**:

Thus, patients are worse off under cost sharing than under full cost but are not as worse off as under prospective payment.

Following [16], it is important to consider the size of γ at which optimality can be achieved in the public sector given that the private sector is optimal. This is obtained by substituting (2), (3), (6), (8) and (9) into (29) and setting $k = 0$:

$$\gamma^* = (1 - MRS_{N_{\pi^h}}) \left(\alpha + \frac{q(1-\alpha)(B_{sq}^2 - B_{ss}B_{qq})}{c'(q)B_{ss}} \right) \tag{30}$$

Clearly γ^* is zero when the physician is a perfect agent in which case prospective payment is optimal.

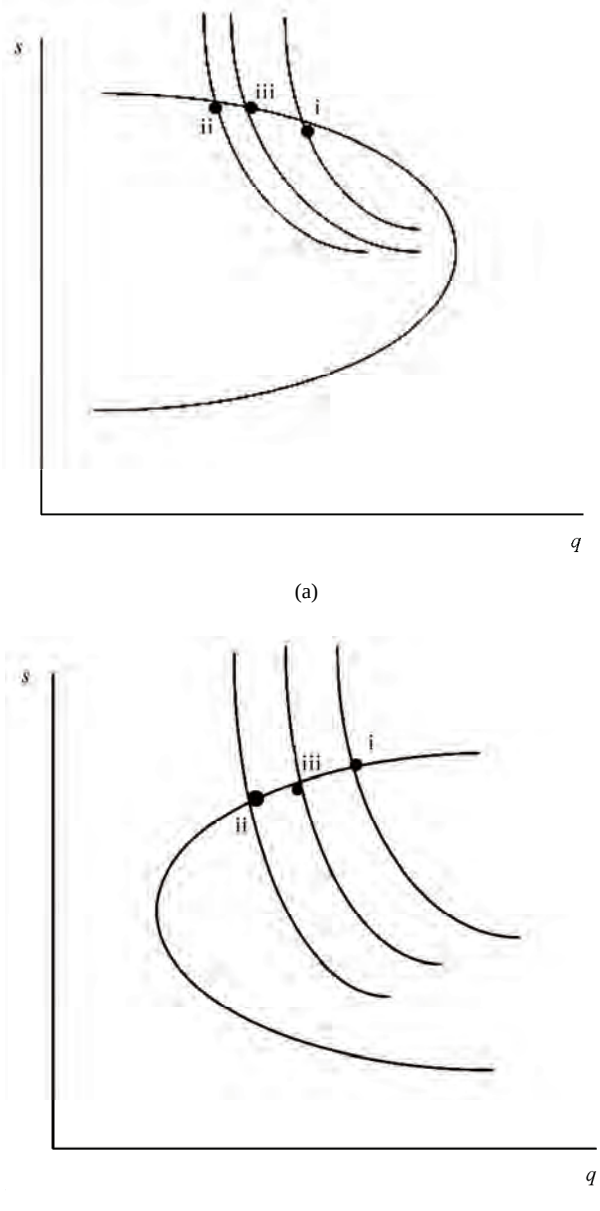


Figure 4. (a) Cost sharing equilibrium for substitutes, relative to those of full cost and prospective payment; (b) Cost sharing equilibrium for complements relative to those of full cost and prospective payment.

[10]The elasticity of demand for s is: $\varepsilon_s = \frac{B_s}{sB_{ss}} \Rightarrow B_{ss} = \frac{B_s}{s\varepsilon_s}$;

$\frac{\partial \alpha^*}{\partial B_{ss}} = \frac{qB_q B_{sq}^2}{[q(B_{sq}^2 - B_{ss}B_{qq}) - B_q B_{ss}]^2} > 0$.

However, γ^* cannot be one[11], *i.e.*, cost sharing can be used to ensure optimal supply of services. It is optimal for the government to use a combination of fixed fee and incentive payment to reward the hospital because this forces providers to internalize the externalities that leads to undersupply under prospective payment (Selden 1990; [15] 1986) and oversupply under full cost reimbursement. Equation (30) shows that the size of γ^* does not depend on the relationship between services *i.e.*, it is optimal for the government to pass on the same fraction of cost for substitutes and complements. In [16] efficiency in both sectors requires that $\gamma^* = (1 - MRS_{N\pi}^h)$. This is identical to what [15] recommend for efficiency in the public sector when there is no private sector. Thus, in Rickman & McGuire, the optimal fraction of cost that is passed on to providers does not depend on the relationship between the services.

The term in the square bracket in (30) represents the effect of the user fee in the public sector on the size of γ^*. Note that when $\alpha = 1$ (30) becomes $\gamma^* = (1 - MRS_{N\pi}^h)$, which is the same as in [16] as well as in [15]. With the term in the square bracket less than one, γ^* in the current model is less than that in [16]. Thus, when there is a user fee in the public sector, efficiency requires that less revenue be retained to induce optimal provision of public services for both substitutes and complements than when there is no user fee. Note that the second term in the square bracket increases in the elasticity of services in both sectors[12]. The γ^* here is subject to the same setback as in [15] as well as [16] in that information on an unobservable variable, the physician's utility, is required. However, the effect of elasticity on γ^* in the current model reduces the dependence on information on the physician's utility. The presence of the positive term in the bracket which depends on elasticity reduces the range within which γ^* falls. Thus, under the user fee system in the public sector in a two-tier system, cost sharing can ensure efficiency in both the public and private sector regardless of the relationship between services in the two sectors. This is also intuitive. Compared to the full cost reimbursement system (where $G = 0$ and $\gamma = 1$), the fall in marginal revenue resulting from letting $\gamma^* < 1$ gives the imperfect agent the incentive to reduce the oversupply of complementary services while maintaining supply of substitutes at the efficient level. For comparison with prospective payment, (where $G > 0$ and $\gamma = 0$) the increase in marginal revenue from setting $\gamma^* < 1$ induces the imperfect agent to increase services.

[11]This is based on the assumption that $\frac{q(B_{sq}^2 - B_{ss}B_{qq})}{c'(q)B_{ss}} < 1$

[12] $\frac{\partial(\ )}{\partial B_{ss}} = -\frac{q(1-\alpha)B_{sq}^2}{B_{ss}^2} < 0$ and $\frac{\partial(\ )}{\partial B_{qq}} = -\frac{q(1-\alpha)}{c'(q)} < 0$

4. CONCLUSIONS

Many recent health care reforms introduce user fees to the public sector. This paper examined patient's welfare and efficiency under different provider reimbursement schemes in the public sector in a mixed health care system where the patient bears cost for treatment in both the public and private sectors. The paper extended previous studies by introducing user fee in the public sector. The provider reimbursement schemes examined are full-cost reimbursement, prospective payment and cost sharing.

The results show that efficiency is possible under the full cost reimbursement scheme if the physician trades off public hospital surplus for patient net benefit when services are substitutes and trading off patient's net benefit for public hospital surplus or being a perfect agent when services are complements. This is contrary to the results in previous studies where there is oversupply of services in equilibrium in the public sector under the full cost reimbursement. Under the prospective payment efficiency is only possible when the fraction of cost not covered by the user fee is at its optimal level. Similarly, under the cost-sharing scheme, efficiency occurs only if the fraction of cost that the government passes on to providers is at its optimal level. This is similar to the results in previous studies; however, the optimal fraction in this paper is less than that in previous studies. Of the three reimbursements schemes, the patient is worst off under the prospective payment and has the highest utility under the full-cost reimbursement. In general the introduction of user fee in the public sector makes the patient worse off when services are substitutes. However, it is not clear whether the user fee makes patients worse off or better off when services are complements.

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The need for a new framework for the economic evaluation of health services in a national health scheme

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ABSTRACT

The normative theory of economic evaluation and its welfare theoretic basis are deeply problematical and result in recommendations which are potentially unfair. The root cause of the problem is the set of assumptions behind the theory which posit behaviours and motivations that are not universal, and which exclude other behaviours and motivations that are potentially important. As falsification of assumptions may be evaded indefinitely this paper presents an alternative critique. We commence with six anomalies with the theory which are attributable to the assumptions. The first three—the net present value criterion, the willingness to pay criterion and moral hazard—arise from welfare theory. The remaining three are associated with the present definition of cost, the concept of efficiency and the omission of sharing, which are common to most economic evaluation. We argue that these anomalies are indicative of a defective core theory and that they are equivalent to observations that conflict with a positive theory. In the final section we outline and illustrate a more general framework for decision making that is capable of overcoming the anomalies we discuss.

Keywords: Economic Evaluation; Economic Costs; Welfare Theory; Empirical Ethics; Cost Benefit Analysis

1. INTRODUCTION

Between 1990 and 2003 public expenditures on health per capita in the OECD doubled. Unsurprisingly this has resulted in closer scrutiny of the services provided. Commencing with Australia in January, 1993, an increasing number of countries have passed legislation to ensure the economic evaluation of pharmaceuticals before receiving subsidy. Economic evaluation is also increasingly adopted for other public health services, a trend which is likely to be of increasing importance in rationing health services, even in the USA where current legislation prevents its full use in Medicare.

Given this scenario, the relatively low level of self-criticism of fundamental elements of the theory of economic evaluation is of concern. In the financial sector the recent world-wide crash signalled the existence of defects in both practice and theory. The doubtful activities of many financial managers are now well known. Less publicised is that in the 1960s Mandelbrot demonstrated that a fundamental tenet of financial theory was wrong [1]: movements in the stock exchange follow a power function and deviations are not normally distributed. Sometime earlier, Keynes had argued that the return on assets was often subject to uncertainty, not risk (*i.e.*, there is not enough information to justify assuming a particular distribution of expected returns) [2]. Both observations strike at the basis of the theory which underpinned the financial structures of recent times, but both have been largely ignored. The recent crash will, hopefully, provoke introspection and error learning.

In contrast, there are no external events that could encourage examination of the theory and practice of economic evaluation. However, there are anomalies in its use in the health sector and elsewhere which are at least as important as and much more obvious than those identified by Mandelbrot and Keynes. Some of these are outlined in Section 2 below. Those relating to the health sector are largely associated with the neglect of fairness and the drive to find technical answers to questions relating to social values.

The proximate reasons for this outcome are discussed in Section 3. To increase analytical rigour, assumptions have been made in economic theory that oversimplify or seriously distort reality (cf. risk versus uncertainty).

Equally damaging is the application of the standard "*ceteris paribus*"—all else equal—caveat. Especially in the health sector, conclusions are often context-specific and neglect of some elements may be as damaging as the misrepresentation of others. Finally, in Section 4 we outline and illustrate some principles for the construction of a better framework for economic evaluation.

The existing framework is broadly concerned with the comparison of benefits and opportunity costs where the latter are the benefits foregone by using resources in one way rather than another. A key criterion in evaluation theory is that the present value of benefits exceeds cost ($B > C$), or that net present value is positive ($B - C > 0$). A necessary condition for this is that the benefit-to-cost ratio exceeds unity ($B/C > 1$), or the cost per unit of benefit is less than unity ($C/B < 1$). This criterion defines efficiency. If there is a constraint, such as a fixed budget, then 'unity' is replaced by a threshold reflecting the opportunity cost of capital or use of the constrained resource.

In the health sector, however, there is tension about the application of this broad principle. In more orthodox 'welfare theory' (WT) benefits are equated with individual 'utility' where this, in turn, is equated with the strength of a person's preferences. While it is argued (following Robbins [3]) that these subjective preferences cannot be directly compared, they can be revealed by a person's willingness to pay [4], and social welfare—the welfare of society—is a function only of individual utilities: the doctrine of "welfarism". The more orthodox arm of health economics attempts to implement these principles. For example, the gold standard for benefit measurement is revealed preferences, but in its absence the second best approach is to use stated preferences and/or stated willingness to pay. This includes the willingness to pay for life itself, or at least a 'statistical life', which is often inferred from an individual's willingness to pay for a reduction in the risk of death or the willingness to accept compensation for assuming a risk of death [5,6].

In contrast, in Cost-Effectiveness Analysis (CEA) benefits are "external" and measured in physical terms, either as lives saved, life years or "quality-adjusted life years" (QALYs). The term "extra welfarism" has been used to describe the theory that the only—or only relevant—benefit which needs inclusion in CEA in the health sector is health outcome [7]. While this 'external benefit' or 'material welfare' tradition is the earlier historically, welfare theory and welfarism are widely regarded as a gold standard with various attempts being made to either reconcile CEA with them or to show the special circumstances when CEA provides a consistent 'theoretically correct' solution to economic problems [8].

Some of the anomalies with the theory of economic evaluation are unique to welfare theory. However, there are anomalies which it shares with extra-welfarism based CEA.

2. SOME ANOMALIES

2.1. Net Present Value: The Wrong Criterion

Welfare theory evolved from the context of the private sector in which individuals chose whether or not to purchase a commodity for their own benefit. Unsurprisingly, it is assumed in WT that well-informed and rational individuals will apply the criterion that (personal) benefits should equal or exceed (personal) costs—the positive net present value criterion. If costs equal benefits plus $1 there will be, or should be, no transaction. This theoretical point has been transferred to the health sector: if total costs as described by WT exceed total benefits as described by WT no service should be provided even if the patient dies. Welfare theory allows for compassionate externalities—others may obtain utility if the patient lives, but if not, then the patient should die as greater utility can be gained if the resources are used elsewhere.

However, changing the context from a private market to a national health service (NHS) may have profound consequences for almost all aspects of evaluation including the comparison of costs and benefits. At present, the bulk of health costs are borne by 'society' (in the form of tax or premium payments) and benefits are obtained by an individual who will not bear (at least the full) cost. Different principles almost certainly apply to the willingness to provide benefits, or else the NHS would have been unnecessary and if the scheme is successful then the demands upon the national health system will differ from the demands upon the private system. With full knowledge of the magnitude of the utilities involved those making decisions for society may elect to save life, or improve life, when it is 'uneconomic' according to WT. There are well known ethical and rational reasons for this, including those appealing to 'sympathy', 'reciprocal altruism', and 'process utility' (fairness). More significantly, there may be 'counter-preferential' reasons, based on 'duty' or 'commitment'.

A well-known argument against duty and commitment, to which we return below, is that they may simply be another source of utility: they are only superficially 'counter-preferential'. More fully, since utility is revealed by decisions, the decision to carry out one's duty indicates that the decision maker must have received utility. However, while the revealed preference criterion is potentially useful in a context where an individual is well-informed and known to be only self interested, this interpretation of the revealed preference definition of

utility converts the argument into a tautology: all action, by definition, reflects utility. Some people may, indeed, gain utility from fulfilling their duties, but as Sen has pointed out [9], other motivations are clearly possible.

2.2. Willingness to Pay: An Unwanted Element

Private willingness to pay (WTP) is still widely used and vigorously promoted in the literature as a method for evaluating improvements in quality of life (QoL), and the value of life itself [6,10]. However, the wedge between recipients and funders of health services invalidates the logical connection between social goals and private WTP and makes the interpretation of WTP data problematical. As noted, if there was no difference between an individual's WTP for a service for themself and the WTP of the society, then it is unlikely that there would be significant support for an NHS. Private, risk-based insurance would probably satisfy the need for ensuring health. But the great appeal of an NHS is that it promotes fair access to health services. That is, the appeal relates to the use, or potential use, of services specifically by those who would not otherwise have access through lack of financial resources. Restated, the purpose of an NHS is to ensure that services are available to people when their private WTP is less than the cost. That is, *the purpose of an NHS is to ensure that the 'optimal outcome' described by welfare theory does not occur*.

Adopting the WTP criterion of benefit results in a further paradox for the orthodox economist attempting to achieve economic efficiency. For every additional dollar that an individual is willing to spend on themself, the NHS should also be willing to spend an additional dollar on this person. With WTP benefits determined by income, taxpayers would have to subsidise the wealthy, who are willing to pay more for their own health care, and spend less on the poor, who are the usual target group.

A final defence of the WTP criterion is more ad hoc. Since economists have not devised a better method for determining the dollar value of health services, the WTP may indicate the order of magnitude of appropriate spending in the health sector. Some have adjusted the WTP to take account of an individual's wealth, but this remains an ad hoc approach as it does not overcome the theoretical problems outlined above [11,12]. The ad hoc solution may, indeed, be the best available, but it has the theoretical status of an opinion poll and should not claim greater authority.

2.3. Moral Hazard: Replacing Normative with Technical Objectives

Disagreement exists about the benefits of health insurance and at least part of this has arisen because of the different lenses through which the protagonists have viewed the issue. Following Pauly [13], economists have almost universally accepted that health insurance is subject to 'moral hazard'. That is, it causes an 'excess burden'—an inefficient use of resources, where the benefit (determined by willingness to pay, or the area under the demand curve) is less than cost. This arises because the price to the patient has been reduced (by the insurance rebate) to be less than the cost (possibly zero in a 'free' scheme). Patients are therefore willing to pay the reduced (or zero) cost of additional care for which society pays the full cost. It is usually concluded that insurance therefore induces the use of resources where costs exceed benefits and that this inefficiency should be limited by the use of patient co-payments to reduce the "excess burden".

The argument appears, *prima facie*, a good example of value free social science. However, social welfare groups and supporters of universal health insurance tend to have a different focus. The demand curve used in the economist's argument is a simplification. There are, in fact, many demand curves determined primarily by levels of illness and ability to pay. The demand of the wealthiest will be relatively unresponsive to prices, with responsiveness, or 'elasticity', increasing as family income falls and price becomes a larger real burden. As the patient co-payment rises (to reduce the excess burden) the reduction in health care will be determined very largely by income. The savings to a tax-financed NHS will primarily benefit wealthy taxpayers. In the extreme, the 'excess burden' may be interpreted not as a measure of pure inefficiency, but as a measure of the extent of the redistribution of income from the wealthy and healthy to the poor and unhealthy, and it is to the former group that there is an "excess burden". In Australia, at least, co-payments have been supported for reasons of sectional interest by doctors (to give some control over incomes), by government (to shift costs to patients) and by social welfare groups (to protect the poor). Economists alone have perceived co-payments to be primarily related to efficiency.

The literature on moral hazard is an example of the consequences of the previous error of treating the net present value (NPV) as a gold standard criterion. Because behaviour changes as a result of an NHS the deviation from the (NPV) criterion is considered to be a source of inefficiency, and to be avoided, even if from a social perspective this deviation was the purpose of the scheme.

Of less relevance here, the moral hazard anomaly also applies in a private market with an additional error caused by an omission from the theory, namely utility obtained in

the "pre outcome" period before a person knows whether or not they will be sick [14]. The disutility of risk induces the purchase of insurance in the knowledge and with the purpose of altering realised spending in the case of illness. The moral hazard argument defines this deviation as inefficiency reflecting the analytical weakness of orthodox economics' treatment of time.

2.4. Economic Costs: The Wrong Comparator

The problems outlined above arise in cost-benefit analysis (CBA) where both benefits and costs are reduced to dollars. In practice, the predominant form of economic evaluation in the health sector is cost-effectiveness analysis, or cost minimisation analysis, which seeks to rank services according to their cost-per-unit of benefit (lives, life years, or quality adjusted life years in the case of cost-utility analysis). Cost-effectiveness analysis can be justified independently of welfare theory as it indicates how benefits, somehow measured, may be maximised given resources or from a given budget. Benefits need not be measured by individual utility. Nevertheless it encounters problems which also apply to CBA.

A simple extension of the earlier arguments indicates that the 'costs' that are relevant in evaluation studies may not be the resource costs of economic theory, i.e. the value of the resources expended (labour, time, capital, stock depleted, etc). The 'social willingness to pay' - the amount an individual is willing to contribute to an NHS (or the willingness of the individual's "agents" on their behalf)—depends upon their "social generosity". This may or may not be closely related to an individual's perception of their personal benefits from the NHS. Of greater relevance here, it will not depend upon net use of resources but upon the amount of money lost personally through the taxes and premiums that finance the NHS. These are not the same. The net resource cost of a medical service is equal to the direct cost minus indirect benefits, where the latter is the value of employment gained (or not lost) by a patient's return to the workforce. But the chief beneficiary of this is the employer or patient not the taxpayer. Consequently, an increase in taxes or premiums which resulted in increased employment and in 'negative real costs' would still impose a personal cost on the taxpayer. If willingness to pay and cost are to be equated to achieve the usual notion of efficiency, it is this personal cost to the taxpayer, not the net resource cost, which should be compared with the social willingness to pay.

The argument is even clearer if the scope of government services is broadened to include pensions. In principle every person with a chronic illness could be offered a compensatory pension in exchange for their access to the NHS. As normally understood, pensions are a "transfer" (from the taxpayer to the recipient) and omitted from the calculation of "costs" as no real resources are consumed. Consequently, the compensatory pension would generate similar satisfaction but no real cost. It would always be the better strategy. But the strategy would impose a (possibly much higher) personal cost on taxpayers and the optimal strategy could not avoid taking this into account.

2.5. Equity and Efficiency: A Misleading Distinction

To a greater or lesser extent every country endorses equity of access to needed services (somehow defined). But the cost of providing needed services varies. Patients in inaccessible locations are more costly to treat than those close to major hospitals and medical facilities. Even a partial concession to equity therefore implies the provision of services with higher costs per unit of benefit than might be provided to patients in large cities. Minimising cost or cost-per-unit of benefit will not, therefore, achieve social goals efficiently. Stated simply, when there are two objectives—cost and equity—social goals cannot be achieved by focusing only upon one of these.

In principle this is acknowledged and formalised in textbooks by recognising the existence of an equity-efficiency trade-off. In practice the evidence reveals little concern with equity, with most studies providing no evidence relevant for its assessment.

By contrast, attention has been given to what is easily measured, while the various elements of fairness and its inclusion in empirical studies have been neglected [15]. The somewhat emasculated concept of fairness—"equity"—has been largely consigned to the realm of theory. But even the concept of an equity-efficiency trade-off is unsatisfactory. This may be seen by considering a well-defined category of patients, viz, those with a 'difficult disease', where costs are high and the effectiveness of treatment is low because of the type of disease and current state of medical technology. There is no obvious logical or ethical reason why the principle of equal access for equal need should allow an increased cost threshold because of, say, a person's geographical location, but no similar increase because of a person's medical problems. To the contrary, those who live in remote locations do so voluntarily. Those contracting difficult diseases generally do so through bad luck. From behind a veil of ignorance we would therefore expect to give higher priority to the latter, not former group. Of course, not all treatments can be provided irrespective of cost. But just as those who have been historically singled out

as beneficiaries in the equity-efficiency trade-off (e.g. rural patients) receive some (incomplete) compensation, so it might be expected that those with difficult diseases would similarly receive some consideration possibly at the expense of those with "lucky" illnesses. In practice this commonly occurs. Few are left to suffer in a severe condition because all of the options are cost-ineffective: those with only days to live are accommodated in a high-cost hospital minimally receiving palliative care which is highly "cost-ineffective" as judged by the criterion of cost-per-life year gained or cost-per-quality adjusted life year gained. However economic theory has not caught up with practice.

The efficiency goal of minimising the cost of achieving objectives becomes problematical, as one of the objectives is—all else equal—the (part) provision of services to those with inefficient-to-treat diseases. The concept of a trade-off between cost/QALY (efficiency) and cost/QALY (fairness) therefore becomes, at best, ambiguous.

2.6. Sharing: Ignored Social Objectives

Perhaps the greatest anomaly with economic evaluation theory is the theoretical framework itself. The framework is based upon an analysis of the individual and the way each individual maximises their utility or wellbeing. *Prima facie*, however, this is almost the opposite of the perspective embodied in the rhetoric of an NHS. The latter social perspective relies heavily upon the concepts of "community", "solidarity", "sharing" and emphasises the need to off-set the barriers to uneven access caused by price and income inequalities—which are precisely the variables that drive the allocation of resources and define benefits in the orthodox economic model. Indeed, while in theory the vocabulary of the entire English language is available to economists, in practice orthodox welfare economists rarely speak about "community", "sharing", "duty" etc. This can have damaging consequences, as described by George Orwell [16] in an appendix to his classic novel on tyranny, "Nineteen Eighty-Four". He observes that in the absence of appropriate vocabulary the conceptualisation of ideas becomes very difficult[1]. It is arguable that this has happened in orthodox welfare economics in which these important concepts play no role. (For exceptions see the advocacy of communitarianism, in particular, by Mooney [17,18], sympathy and justice by Sen (2009), also discussions of "sharing", "fairness", "altruism" and "trust" in the experimental economics literature [19-22].

This truncation of the available language is, in fact, facilitated by the core definitions of welfare theory. Using Samuelson's revealed preference criterion, it might be argued that if any action, including voting, was observed because of any possible motive, then this would indicate that individuals were obtaining utility from that motive, otherwise they would not have done it. Therefore, the reason for self-sacrifice is that it maximises utility. In the extreme case of one person giving their life for another, this must be construed as utility maximising. However, the logic is vacuous. As illustrated in **Figure 1**, the answer to the question "why does an individual take a particular action", is that "this action maximises utility". But the answer to the question "how do we know that this action maximises utility", is that 'they have taken the action'.

The more defensible argument offered by orthodox economics is that its framework includes "externalities"—the utility which people obtain from something external to the market. There is a long and detailed literature demonstrating how, in theory, "fairness", "reciprocity", "participation", and so on, understood as externalities, can be employed to "shoehorn" the elements of a communitarian framework into an orthodox framework (see particularly the works of Culyer [7,23]. In practice this theory is never operationalised. Rarely are measurements of the benefit of this type of externality included in economic evaluations. Rather, it has been used to justify the retention of the narrow focus of current practice.

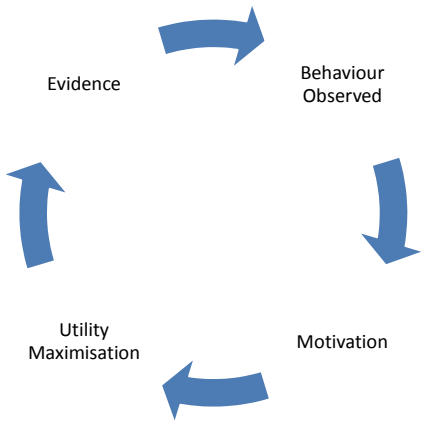


Figure 1. Revealed preferences and the resurrected primacy of utility.

[1]In '1984' the control of thought was achieved by the truncation of language 'It was intended that when Newspeak had been adopted once and for all ... heretical thought ... should be literally unthinkable, at least so far as it is dependent upon words. This was done ... chiefly by eliminating undesirable words ... countless other words such as honour, justice, morality, internationalism, democracy, science and religion had simply ceased to exist. A few blanket words covered them, and in covering them, abolished them. What was required in a party member was an outlook similar to that of the ancient Hebrew who knew, without knowing much else, that all nations other than his own worshiped "false gods" ... he knew Jehovah and the commandments of Jehovah; he knew, therefore, that all gods, with other names or other attributes were "false gods".' [16] pp. 317-319.

The defensive logic of the argument is effectively that other motivations may (possibly) be attributable to (selfish) externalities and other, non-selfish motives may (possibly) not exist. Alternatively, if they exist, that they are of no interest to economics. Therefore we may treat them as if they do not exist or are irrelevant. Externalities are a satisfactory explanation for altruism and commitment; orthodox theory and therefore current practices are justified.

However, there is strong evidence that people have additional motivations to selfish utility maximisation and that these can be behaviourally significant. Beinhocker [24] for example, summarises a large body of evidence obtained from experiments and anthropology which indicates that human beings are *conditional cooperators and altruistic punishers*, behaviour sometimes described as "*strong reciprocity*". This results in a predisposition to cooperate with others and punish (even at personal cost if necessary) those who violate the norms of cooperation, even when it is implausible to expect these costs to be recovered at a later date [25].

One of the most widely replicated experiments demonstrating strong reciprocity is the so-called ultimatum game. Using real money, one individual divides the money between themselves and a second person who has the ability to either accept the money or reject the offer. In the latter case neither person receives any money. The utility maximising strategy for the second person is to accept what they are offered, however small it is. However, the universal observation is that the second person will reject the offer if it is too small, in order to punish the first person. In countries as varied as the USA, Mongolia and Zimbabwe it has been found that most individuals divide the money more or less equally and that the second person will reject the offer when it is less than about 30 per cent of the total [24]. One group of respondents has been found to behave in the way predicted by economic theory, namely those who have a disorder related to autism in which they have little capacity to empathise with others [26]. Alternatively, people could derive from such behaviour benefits that go beyond the monetary payoff - e.g. the 'warm glow' that derives from sharing, defending justice, etc. [27,28].

The prevalence and importance of motivations apart from utility maximisation are apparent in the results from two research projects undertaken at the Centre for Health Economics at Monash University. In the first, 501 Australians were asked to indicate their agreement or disagreement with a series of statements relating, inter alia, to their motivation. Results reported in **Table 1** indicate that the majority of respondents care about relative and not just absolute income levels (Question 1) and an even larger number rejected the view that the maximisation of happiness is the most important ethical principle (Question 2). An overwhelming majority rejected the view that they, personally, fulfilled their duty to achieve happiness (Question 3,4) and less than 1 in 5 believe that other people help one another only if they gain something personally (Question 5). This is not conclusive however. For example, 84.2 per cent of respondents also agreed with the statement: "I fulfil my duties to individuals and organisations (to family, country etc) because doing so will make me happier in the long run". This suggests that motivations may not be clearly separated in people's minds. People may fulfil their duties out of genuine self-sacrifice (which conflicts with utility maximisation as a psychological hypothesis) or it may be because it will enhance their own well-being, or both.

The second study was web based. Individuals were asked to allocate a block of money to one of four patients, each of whom would die without treatment. Patients were identical except for the cost of treating their disease, which led to a different outcome. One block of money could produce 12, 8, 6 or 4 additional years of life. After allocating the first block of money respondents were asked to allocate a second, third and fourth block, etc, to any of the patients. This left respondents with a choice of allocating resources where they pro-

Table 1. Results from the monash ethics survey.

Questions/Statements	% (Strongly) agree	% Neutral	% (Strongly) disagree
1. Australia is better off if the wealthy receive even higher incomes so long as the income of the poor does not fall.	22.8	22.8	57.4
2. Maximising happiness is more important than any other ethical principle.	14.3	19.8	65.9
3. I have some duties that I must fulfil even if doing so makes me a little less happy.	91.5	4.7	3.9
3. I fulfil my duties to individuals and organisations (to family, country etc) not primarily because it will make me or others happy, but because it is my role (e.g. as a mother, father, employee etc).	77.8	5.6	16.7
4. People help others only because they gain something personally.	18.2	21.2	60.7

Source: [44]

duce most life (minimum cost per life year), sharing resources or some combination of the two strategies.

Figure 2 is similar to the visual representation given to respondents. Initially only the blocks in bold type were shown. When the respondent clicked on 'patient 1' the second block of 12 years (in broken bold type) appeared which could be selected in the second choice. The exercise ended when there was no longer a choice. The order in which resources were allocated was recorded and analysed.

If respondents behaved as economic theory predicts they do (or should) the life years gained with each new block of money would be maximised - that is, in the order: 12, 12, 12, 12, 8, 8, 8, 8, 8, 8, 6...6, 4...4. In contrast, the 532 respondents allocated resources so that an average of only 62.5 per cent of the maximum life years were obtained. 37.5 per cent were sacrificed in order to share resources between the four patients. The statistical analysis of choice indicated that this was dominated by the life expectancy of different patients which accumulated as more resources were given to them. The cost per life year was statistically significant but relatively unimportant despite the fact that the 'opportunity cost' of allocating resources to a 'high cost' patient was (visually) obvious in the form of a greater number of years of life which might have been obtained by allocating resources to another patient.

3. THEORY

Economic theory consists of a set of assumptions and logical deductions. As the latter are generally correct, criticism necessarily focuses upon the assumptions and there have been numerous critiques. In the context of health economics perhaps the most comprehensive is Rice [29] but the present authors have also made a contribution [15,30]. In the present context, we focus upon the subset of assumptions of orthodox economics which

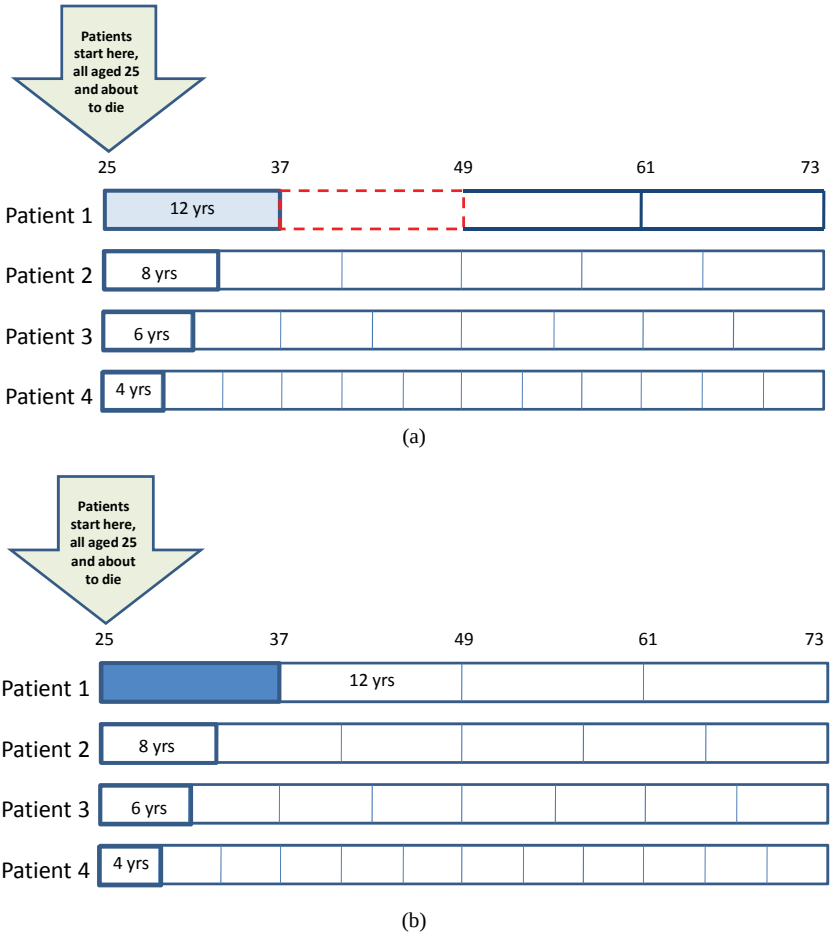


Figure 2. Web-based allocation exercise. (a) The diagram represents 4 patients, all aged 25 facing immediate death. Whichever block is selected will extend that patient's life for the number of years indicated in that block; (b) When a block is clicked, it fills with colour and the next block becomes available. The patient will now live until the end of the filled block.

facilitate the normative conclusions of economic evaluation theory, namely that in an NHS services should be offered ideally when their net present value is positive and, when budgets are constrained, according to the ratio of costs to benefits as these terms are defined.

Using Sen's terminology, the most fundamental assumptions behind welfarism economics are these:

A.1 Individuals are motivated only by utility which they seek to maximise;

A.2 Utility is only derived from the consequences of actions (consequentialism); and;

A.3 Social welfare—the wellbeing of society—is a function of (only) individual utility;

However, these assumptions are insufficient to allow applied evaluation studies and, in practice, some ancillary assumptions are also made. These are that;

A.4 If one person's utility is increased without a reduction in the utility of anyone else—a 'Pareto improvement' - then society may be presumed to be better off;

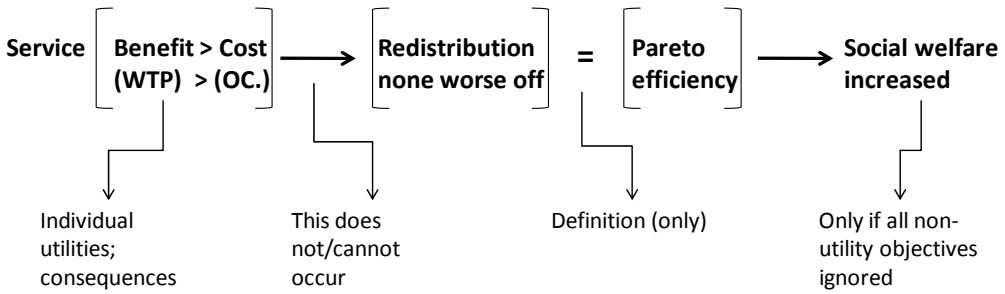
A.5 People are 'rational' and well-informed implying that their choices reveal their utility maximising strategies;

A.6 A net increase in utility increases social welfare, even if there are winners and losers, provided it is theoretically possible for the former to fully compensate the latter;

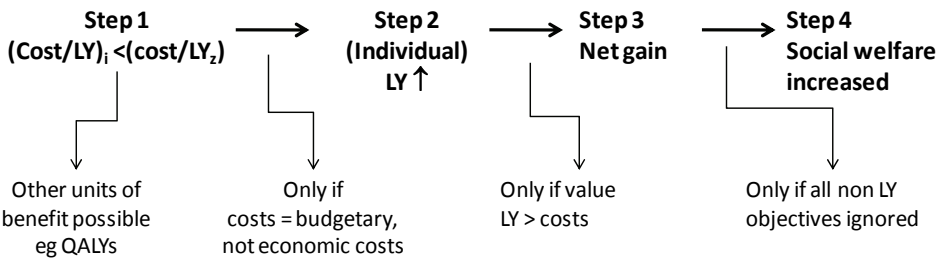
The final assumption—the potential compensation or Kaldor-Hicks criterion—permits each dollar to be treated as of equal value since it may be redistributed and dollars may therefore be added to obtain potential benefits and costs;

To a greater or lesser extent each of these assumptions is necessary to justify the practice of cost benefit analysis (CBA) as shown in Box 1. If a willingness to pay (WTP) methodology is used to reduce benefits to dollars (the defining characteristic of CBA) a positive net present value (NPV) assures a surplus, as measured in dollars, which might, in principle, be redistributed (step 2 Box 1). In principle no one need have fewer dollars while leaving the beneficiary better off. In the case of health economics, the WTP is for a final health state (consequentialism) and from the revealed (stated) preference criterion the surplus indicates an increase in the person's utility. Consequently the positive NPV is 'Pareto efficient' (step 3) and social welfare will be increased (step 4). Note that only rarely are *relative* costs and benefits taken into account, although they can significantly affect social welfare via envy.

With CEA the role of utility in this argument is largely replaced by an explicit target, commonly the life year (LY) or quality adjusted life year (QALY). Consequentialism is more explicit and social welfare is implicitly a linear function of the number and quality of life years deflated by a rate of time preference. The causal sequence is represented in Box 2. As benefits cannot be reduced to dollars, the cost/LY of different



Box 1. The logic of cost benefits analysis.



Box 2. The logic of cost effectiveness analysis.

services are compared (step 1). Selecting the service with the minimum cost/LY allows more life years to be produced (step 2) which is considered to be a net gain (step 3) which increases social welfare (step 4).

As with CBA, social welfare is only affected by total life years or QALYs (consequentialism), not by relative gains and losses. The explicit view in CBA that a dollar should be treated as a dollar due to potential compensation is replaced by the view that all QALYs should be treated equally [31]. As this cannot be judged by the potential to compensate those who do not receive QALYs (and are therefore dead) the judgement is an ethical assumption.

Box 1 and Box 2 highlight the reliance of the two forms of economic evaluation upon the various assumptions. In Box 1 benefits are reduced to dollars (normally) by assuming that revealed preferences are the result of rational well-informed choice (step 1). But only the utility of the final health states, and utility-based indirect benefits and externalities, are included in the evaluation. Non-utility consequences and processes are excluded. The redistribution of benefits to ensure that no one is worse off in step 2 never occurs in practice in the health sector and often cannot occur - e.g. when people die [30,32]. Pareto efficiency need not result in an increase in social welfare if any of the objectives assumed irrelevant by welfarism (e.g. those relating to equity which are independent of utility effects) are in fact quantitatively important.

The logic of CEA side-steps the initial problems of CBA typically by assuming that some spending will occur or that there is a fixed budget and that the problem is how best to spend the resources. But this justification alters the logical sequence. Life years (or QALYs) (step 2) are only maximised if the 'cost' in step 1 is the constrained (budgetary) cost not resource cost. To illustrate this consider a project with small benefits but with no resource cost because the exceedingly high cost-to-budget is off-set by indirect benefits—cost savings through an early return to work by patients. With costs/LY = 0 the project would be prioritised, exhausting the budget and crowding out projects with a lower budget cost-to-benefit ratio which would achieve greater net benefits.

In the UK the National Institute for Clinical Excellence (NICE) has recognised this problem and its guidelines specify that the relevant costs are those that relate to the NHS; that is, economic evaluation for the NHS longer employs economic costs. Similarly, NICE has sanctioned a (particular type of) QALY (based upon the EQ-5D QoL instrument) for cost utility analysis [33]; that is, it no longer employs the usual concept of economic benefits. The latter change has commonly been justified by an appeal to extra-welfarism. Coast *et al.* [34] note that 'the apparent success of the extra-welfarist approach ... in the UK ... might lead to the view that there is general satisfaction with the theoretical approach in health economics' (p 1994). (The authors then express concern with this view.)

However, the chief characteristic of extra-welfarism, on one interpretation [7], is its minimalism. (Other interpretations of extra-welfarism, which allow a place for health, utility, capabilities, and other factors, are also possible (Hurley 2000). It amounts to the assumption that the only purpose of the health sector, or at least an NHS, is the maximisation of health. The NICE directive to employ NHS costs may be considered a consequence of this theory/assumption.

The unique feature of extra-welfarism - on its minimalist interpretation - is its assertion that *only* health is of concern for economic evaluation, and the number of issues excluded by this form of extra-welfarism makes it highly contentious and, indeed, far more restrictive than welfare theory in which, in principle, no objectives are excluded as long as they are consequences which affect utility. The importance of health may indeed be implied by Sen's capabilities approach (and by other social philosophies [35]) but this does not establish it as being the exclusive objective for an NHS.

The assumptions that are necessary for the validity of the theory of CBA and CEA and the causal pathways shown in Boxes 1 and 2 are responsible for the anomalies that started this paper. The relationships between the anomalies, the (chief) assumptions from which they arise, and the (chief) problems with the assumptions are summarised in **Table 2**. A problem which emerges in the context of each anomaly is that the assumptions do not permit a satisfactory treatment of the distribution of costs and benefits. In effect, they extinguish the need for considerations of fairness and, more generally, any normative judgement with the sole exception of the assumption that social welfare as a function of utilities (in CBA) and that health is the only benefit of interest (in CEA). With the doctrine of revealed/stated preferences or the assumption of a single objective, economic evaluation becomes a purely technical matter of observing preferences or the assumed maximand and following through the implications for costs and benefits. Other social judgements are unnecessary.

Two assumptions are of particular importance in this process of excluding matters of fairness. First is the key assumption of welfarism stated above that only utility matters for social welfare. Apart from the circular argument summarised in **Figure 1** there is no satisfactory argument for believing that welfarism is true. The existence of other ethical theories - including the deon-

Table 2. Anomalies and justifying assumptions.

Are of anomaly	Justifying assumption	Problem*
NPV	• Potential Pareto efficiency; potential compensation; validity of WTP	• Different persons receive benefits, costs; and no compensation occurs • Benefits are redistributed • Procedural and deontological goals are excluded
WTP	• Benefits = (selfish) utility (only) • Individualism, rationality	• Benefits (objectives) not restricted to selfish utility • Communitarianism • Non-rational behaviour • Unwanted distribution of benefits
Moral hazard	• Consequentialism • Potential compensation • Individualism	• Ex-ante (pre-outcome) benefits/costs • Non transferable benefits • Communitarianism • Inequitable distribution of benefits
Economic costs	• Sacrifice by a person = resource loss	• Health budgets not resources are the constraints in an NHS • Tax burden to an individual = transfer, not a resource cost
Equity and efficiency	• Separability of equity, efficiency • Consequentialism	• Unfair to high CB patients • Sharing, duty, procedural justice • Distribution of benefits excludes high CB patients
Sharing	• Individualism	• Communitarianism • Non welfarist goals motivation • Distribution of benefits unfair

*Some of these are practical problems rather than theoretical - e.g. no compensation occurs in practice, procedural goals ('process utility') are not included in practice, etc.

tology and religiously-based views, which are nominally endorsed by the vast majority of the population - at least suggests the need for more than a bold assertion of welfarism. The compatibility of observations with welfarism is of lesser significance for its universality than the incompatibility of other observations (*i.e.* anomalies).

The second key assumption is the Kaldor-Hicks potential compensation principle. It permits abstraction from the distribution of costs and benefits. Once NPV is positive it is as if it were 'disembodied' - unattached to any particular person - and can be shifted at will from person to person. But the principle is misleading. Firstly, 'potential' is not the same as 'actual'. No amount of persuasion is likely to convince a person who has suffered a financial catastrophe that this outcome is desirable because another person has obtained a windfall from the same event that is more than sufficient to compensate the first person for their loss even though they will not do so. They may agree that the new situation is "efficient" according to the Kaldor-Hicks criterion. They could not disagree since the criterion is treated as a definition. But they might certainly object that the outcome is unfair.

Secondly when patients are left in a state of extreme disability or allowed to die, compensation is impossible even in principle and the Kaldor-Hicks criterion is irrelevant. Finally, in the context of an NHS the argument for potential compensation conflicts with the purpose of the NHS. This is to redistribute the cost of illness from those who are sick to the taxpayer: the taxpayer is the final loser (if only money and health are taken into account). Compensation would involve a reversal of this redistribution. The unhealthy who benefit from the NHS would be taxed in order to compensate the healthy. But this conflicts with the purpose of the scheme implying the irrelevance of the Kaldor-Hicks principle (again, unless "process utility", "participatory utility", "duty utility", etc, are taken into account, which does not happen in practice).

In CEA the assumption of welfarism is replaced with the adoption of a stated objective (LY, QALYs). There is no explicit equivalent to the Kaldor-Hicks criterion as the theory has been less developed than welfare theory. As noted, theoretical concerns have focused upon its consistency with the assumptions of orthodox economics rather than the development of an independent rationale for its methods. However, like CBA it has no satisfactory method for resolving issues associated with the distribution of benefits, and generally there is an implicit assumption that social welfare rises directly with the number of life years or QALYs gained. The assumption plays the same role as the Kaldor-Hicks principle in abstracting evaluation from issues of distributive justice.

3.1. Empirical Ethics and a General Framework

In both its practice and underlying theory economic evaluation based upon welfarism or extra welfarism

may be described as "efficiency focused". As discussed above, assumptions largely purge the theory of the need for ethical decisions and, in practice, measurement focuses upon efficiency. However, the evidence overwhelmingly suggests that the *raison d'être* for an NHS is not the maximisation of individual utility as defined in orthodox welfare economics—*i.e.* the maximisation of self-interested preferences. Nor does it suggest that maximising health is the only goal. Rather, the empirical evidence, based on community surveys, suggest that the purpose of an NHS is to ensure fair treatment in health related matters, and this should be an important focus of the framework.

The difficulty encountered in progressing beyond this point and developing the framework is that there is no objective basis for demonstrating the truth or superiority of a normative rule. There is a sufficient consensus about the importance of health, social justice and costs to conclude that the framework must include these, especially when they remain as abstract notions. But it is not possible to determine what other goals should be included or excluded and, when included, how they should be measured and weighted. With respect to this problem the literature is unhelpful. Welfarism might be considered an exception only because of its longevity and the elegance of the model woven from its troubled assumptions.

$$Cost / LY = 1/b_1\left[a + b_2 Budget + b_3 LE + b_i X_i\right]$$

We cannot offer a final solution to these problems. As argued elsewhere, there can be no objectively correct metric - that is, no metric that everyone will agree upon [36]. Further, drawing normative conclusions directly from objective evidence is a well-known error (the 'naturalistic fallacy').

Nevertheless, for the solution of social problems it is important to know what the public thinks. This has been described elsewhere as empirical ethics [37,38]. It is little more than the suggestion that population values should be taken into account in the final decision algorithm or at least understood through empirical inquiry. Prima facie the proposition is trivial. Nevertheless, the suggestion has not been adopted and the volume of literature investigating population values and the problems with basing normative conclusions on them remains negligible.

Another literature, however, is helpful in this context. The broad principles of decision theory provide a flexible and 'commonsense' approach to decision making. [39]. In this, objectives or dimensions of the choice set are independently obtained and weighted to reflect their relative importance. CBA can be viewed as an example of its simplest application. The two objectives, maximising benefits and minimising costs are each given a unitary weight. However, weights need not be unitary and the objective function need not be additive. In the context of multi-attribute utility instruments for combining dimensions of a health state - the independently determined objectives—both the Health Utility Index (HUI) and Assessment of Quality of Life (AQoL) instruments employ multiplicative models [40,41]. Econometric methods may also be used including the new advanced methods of discrete choice modelling [42].

A major challenge to modelling is the inclusion of sharing as an objective. In the sharing study described in Section 2 Richardson *et al*. employed the econometric approach (a logit model) to predict the probability that a particular life-extending service would be selected from the four options when each option favoured a different person. The model took the form:

$$Ln\left(p/(1-p)\right) = a - b_1\left[Cost / LY\right] + b_2 Budget + b_3 \cdot LE + b_i X_i$$

Where b is the probability of a person/disease receiving resources; LE is the person's life expectancy (severity of condition) and X_i included variables for the share of the budget already received and life expectancy relative to that of others in the choice set. If the selection criterion for a service is a 50 per cent or more probability that it will be selected by the panel of respondents, the left hand side, LN $p/(1\text{-}p)$ = 0, and a threshold cost/LY, may be calculated as:

If LE and X_i were unimportant—as in CEA—then b_3 = b_i = 0 and the threshold at which there was a 50 percent chance of service selection would be

$$Cost / LY = 1/b_1\left[a + b_2 Budget\right]$$

That is, the threshold would depend entirely upon the budget. When other variables are important, cost/LY is a function of these variables. Thus in the problematical case discussed in Section 2, where orthodox theory would leave a person to die (LE=0) if the cost/LY exceeded the threshold, the effect of the imminent death would increase cost/LY threshold according to the weight b_3/b_1.

Results from the analysis of 41,000 observations generated from 544 subjects indicated that the importance of LE resulted in very significant sharing. The results, more generally, show respondents, adopting a social perspective, were prepared to sacrifice almost one third of potential life years to achieve optimal sharing.

This exercise was illustrative. A wider range of social values could be tested and different combination rules employed. The general result would be the replacement of the fixed cost-effectiveness threshold of present CEA with a variable threshold which was de-

pendent upon the attributes of these receiving the service and the extent of sharing.

The chief obstacle to the development of such a framework, of course, is agreement upon health-related social goals and the creation of instruments for their measurement. The task is problematical in large part because, to date, there has been virtually no discussion in the mainstream literature of how social goals should be determined—*i.e.* what should be the criteria for the adoption of objectives.

4. CONCLUSIONS

There is an adage that for every complex problem there is always a simple solution which is wrong. The economic evaluation of health services is a complex problem and welfare theory is simple, elegant and beguiling. Minimalist extra-welfarism and CEA as practised encapsulate a single assumption with respect to the purpose of an NHS. We have argued here that this simplicity is, at best, misleading. Its perpetuation is undoubtedly related to three factors. First, there is an obvious need to ration the use of finite resources and current economic evaluation methods provide one way of doing this. Second, the implied methods and rationale incorporate elements that are undoubtedly important. Costs, budgetary costs and benefits, as presently defined, must clearly play a very large role in the decision process. Third, and as noted in the introduction, errors in theory will not lead to dramatic events. Bridges will not fall down and the stock exchange will not collapse. Rather, we will treat people unfairly but neither decision makers or patients will notice this. Very few Australians are aware of the huge discrepancies in the provision of services across the country but their distribution is nevertheless unfair.

We have argued that the root problem is a methodology based upon misleading and over-restrictive assumptions. The assumptions of welfare theory purport to be universally true and therefore applicable to the health sector. But direct evidence contradicts the universality of the behavioural assumptions and other assumptions needed to operationalise the theory are deeply problematical. The assumption of *only* health maximisation in extra-welfarism is even more restrictive than the assumptions of Welfare Theory. It is simply easier to operationalise. However, in this article we have not focused primarily upon the assumptions. Rather, the article has challenged the validity of the current approach another way. It has looked at some of the consequences of the assumptions and demonstrated that they are anomalous. As Popper [43] notes, theories may, in principle, be falsified not verified and the identification of anomalies is therefore an important challenge to a theory. However he also notes in practice, falsification can always be avoided by changing definitions, objectives or by making ad hoc repairs. The argument embedded in **Figure 1** for example and variations of it can allow welfare theory to evade falsification indefinitely, if that is the objective. However this will result in the type of disjunction between theory and practice which, we argue, already occurs as decision-makers approve services with a high cost/LY for a variety of "pragmatic reasons".

Finally, our critique does not imply that current work is worthless. The variables included in economic evaluation costs and benefits as measured are evidently of some importance. Rather, our criticism is that they are based upon a bad theory which is highly restrictive in the elements that it permits to be considered and which claim a universality that is not justified. We have suggested that a more pluralistic framework based upon some of the insights of decision theory will be less coercive and more flexible. Many- and possibly the majority of the recommendations of economic evaluation would remain unchanged. A more realistic framework could also overcome the anomalies discussed here.

A theory that is not true in one context may be applicable in another. In particular, a theory that is satisfactory in the supermarket (based upon self-interest, willingness to pay and consumer sovereignty) cannot simply be assumed to be true in the health sector. Deriving the authority of economic evaluation from the authority of 'economic theory' is unsatisfactory if the assumptions are not universally true in the context of positive analysis and if they lead to anomalies in normative theory. Despite this, present methods and theory satisfy the immediate goals of theorists and decision makers and are unlikely to change quickly. Policy makers want answers; their advisors benefit from the authority they have accreted from the theory. However, for the reasons outlined here, we believe that a better approach to theory exists and that decision makers should retain their pragmatism and treat with scepticism assertions that they should be guided exclusively by the net present value or cost/QALY rule. The evidence and argument here suggests that the underlying assumptions have implications that conflict with people's moral preferences and, in some instances are absurd.

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