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From the Editor



Ahmad Husari (*Chief Editor*)
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This fifth issue this year deals with several issues of interest to a wide range of readers in the region. A prospective study from Iraq assessed the role of inhibin B in the evaluation of male factor infertility as a prospective study. Semen analysis was performed according to World Health Organization guidelines. A total of 55 infertile (oligospermia) and 87 presenting for fertility (normal) evaluations were recruited. The mean serum inhibin B level was significantly ($P < 0.05$) lower in the oligospermia than normal group. The authors conclude that Inhibin B levels in fertile men and infertile men in Kurdistan region were similar but not identical to those reported in other geographic regions. Both inhibin B and FSH are useful markers of spermatogenesis.

A second paper from Iraq attempts to develop new, simple, cheap, fast, accurate, and sensitive colorimetric methods that can be used for the determination of thymol in drug in pure form as well as in pharmaceutical formulations. The methods are based on the reaction phenylenediamine with nitrite in acid medium to form diazonium ion, which is coupled with thymol in basic medium to form azo dyes, showing yellow color and absorption maxima at 467nm. Beer's law is obeyed in the concentration of 0.5-7 µg/ml. The methods were successfully applied to the determination of thymol in pharmaceutical formulations.

A prospective paper from Jordan examined the risk of adverse pregnancy outcomes in pregnant women with uterine fibroid and the effect of pregnancy on fibroid. A total of 36 women with uterine fibroid were studied. The age range was between 18 and 45 years. Twelve women had uterine fibroids diagnosed before pregnancy. In seventeen women, the fibroid was diagnosed for the first

time by routine antenatal ultrasound. In seven women it was detected only during cesarean section. Also there were fifteen women who had postpartum hemorrhage. The authors concluded that pregnancy with uterine fibroids results in poor outcome.

A retrospective review of case files of all patients who underwent evisceration from Nigeria to determine the pattern of evisceration at Federal Medical Centre. The commonest indication for evisceration was intraocular infection (46.7%), followed by ocular trauma (36.7%). Evisceration was common among the middle age group (41-50 years). Ocular trauma accounted for the highest number, 11 (36.7%) of all indications for evisceration in age group less than 20 years. The authors concluded that intraocular infection and ocular trauma were the main indications for evisceration at Federal Medical Centre, Birnin Kebbi. Ocular trauma accounted for the highest number of all indications for evisceration in the age group less than 20 years. The need to prevent ocular trauma among children in order to reduce the magnitude of evisceration is underscored.

A paper from Baghdad attempted to estimate serum chromium levels in obesity. Serum chromium concentration was measured by atomic absorption spectrophotometer. The results revealed that Serum chromium concentrations in obese patients were significantly ($P < 0.001$) lower ($0.011 \pm 0.013 \mu\text{g/L}$) than in normal control subjects ($0.441 \pm 0.021013 \mu\text{g/L}$). The Serum chromium concentration was negatively correlated with body mass index ($r = -0.87$), ($p < 0.001$). Therefore, the author suggested that chromium may play an important metabolic role in the development of obesity.

A retrospective study from Jordan looked at the association between RBC transfusion and mortality in critically ill children in the Pediatric Intensive Care Unit (PICU). Among 437 children in PICU over the period of the study 231 (52.8%) children were identified to have Hb $< 13\text{g/dl}$. Ages ranging between 3 days and 14 years (median age 5.6 years), were included in this study; they were put into two groups according to RBC transfusion. The authors concluded that RBC transfusion was significantly associated with increased mortality in critically ill children. Further research are needed to improve the outcome of RBC transfusion and to balance its benefit against the risks associated with it on critically ill children.

A case study from Oman looked at Intrauterine Contraceptive Devices with lost Strings. An unusual case of missing Intrauterine contraceptive device (IUCD) strings enclosed and wrapped in a thin transparent membrane due to intrauterine infection is reported.

Estimation of serum chromium levels in obesity

ABSTRACT

Chromium (III) concentrations in serum were assessed in obese patients to study the correlation between chromium and obesity. The body mass index (wt./ht²) was also measured to evaluate its relationship to the chromium level in obese individuals. Serum chromium concentration was measured by atomic absorption spectrophotometer. The results revealed that Serum chromium concentrations in obese patients was significantly ($P < 0.001$) lower ($0.011 \pm 0.013 \mu\text{g/L}$) than in normal control subjects ($0.441 \pm 0.021013 \mu\text{g/L}$). The Serum chromium concentration was negatively correlated with body mass index ($r = -0.87$), ($p < 0.001$). Therefore, the author suggests that chromium may play an important metabolic role in the development of obesity.

Key Words: Obesity, serum chromium, body mass index.

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Introduction

Chromium, an essential trace element required for normal carbohydrate, protein and fat metabolism, may improve impaired glucose tolerance [1, 2, 10], decrease elevated blood lipid concentrations and result in weight loss and improved body composition in some individuals, but results have been equivocal. As the active component of chromodulin, chromium has been suggested to potentiate the action of insulin, possibly insulin internalization and increasing insulin sensitivity [3, 4]. Signs and symptoms of chromium deficiency in mammals include glycosuria, neuropathy, encephalopathy, decreased insulin binding and receptor number and impaired immune response. While much of the research on the role of nutritional chromium has focused on its effects on blood glucose and lipid concentrations, the suggested beneficial effect of chromium on body composition was based on the rationale that chromium potentiates the functions of insulin and popular interest has centered on chromium as a means to increase muscle mass and reduce body fat in obese individuals. Mineral chromium, has drawn recent interest for its role in diabetes and obesity [5]. The link between these contradictory changes in body composition may be due to chromodulin's ability to potentiate insulin's effects on converting glucose to carbon dioxide or lipid. However, the effects of chromium picolinate (CP) supplementation on lean body mass (LBM), percent body fat and body weight, are equivocal. Evans [6], showed significantly increased LBM and decreased percent body fat in young males who supplemented with 200mg of chromium/day as CP and participated in a resistance training program. However, skin fold thickness measurements, which have a high rate of error, were used to assess body composition [7, 8]. Chromium (Cr) has been

examined in some animal studies and clinical studies for its anti-obesity effects [9]. Inadequate amounts of Cr may result in improper functioning of the metabolic process and lead to a number of physiological disorders that increase risk for diabetes and cardiovascular diseases including elevated circulating insulin, glucose, triglycerides, total cholesterol, reduced HDL-cholesterol and impaired immune function [11-13].

Materials and Methods

Subjects:

One hundred and fifty individuals of age 20-59 years were divided into three groups (50 each): normal (29 females and 21 males), overweight (29 females and 21 males), and obese (33 females and 17 males), based on their BMI.

Samples Collection and Preparation

About five milliliters of venous blood from patients and healthy subjects were drawn by utilizing disposable plastic syringes in the morning and transferred into sterile test tube. The blood was allowed to clot and centrifuged at 4000g for 10 minutes. Sera were separated and stored at -20°C until analysis.

Estimation of body mass index (BMI)

Body weight and height were measured and used to calculate the BMI, which was used as a measure of relative body weight.

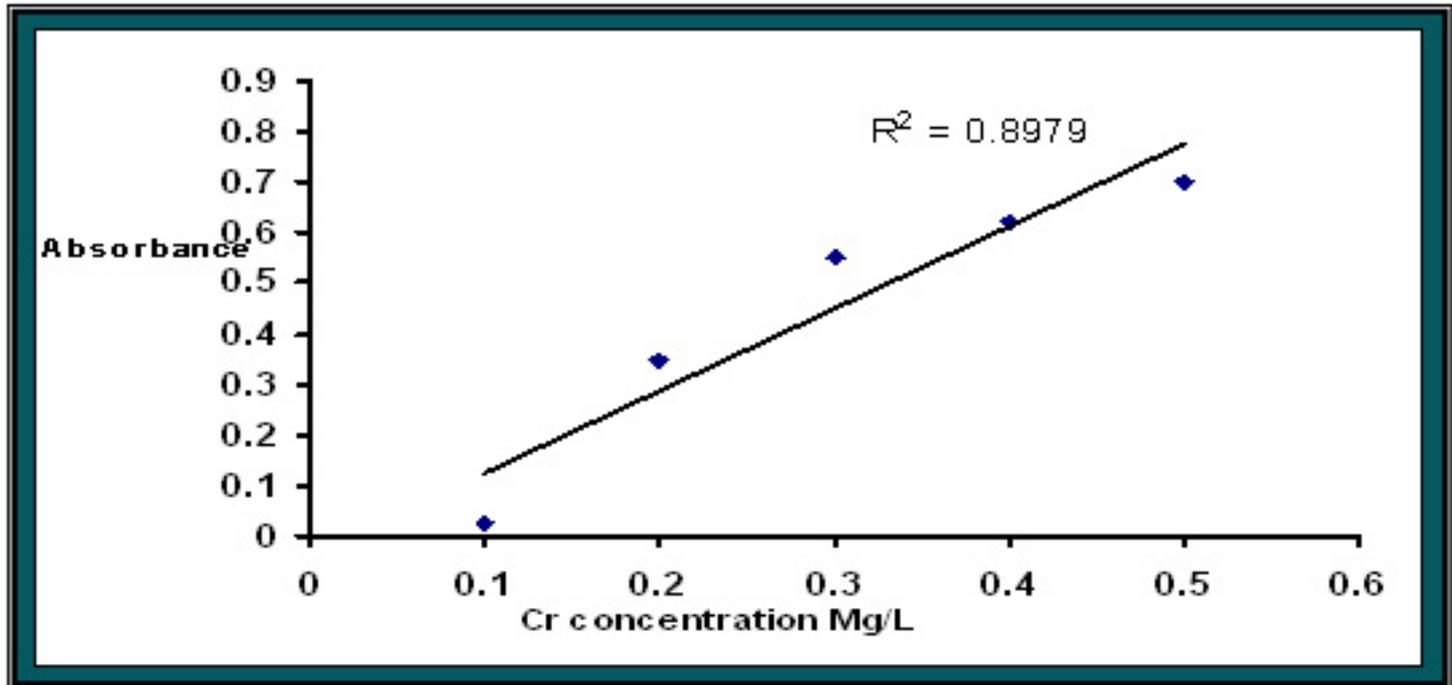


Figure 1: Standard curve for chromium

Estimation of serum chromium

Preparation of standard Chromium solution (1000ppb):

0.0075 grams of $\text{Cr}(\text{NO}_3)_2 \cdot 9\text{H}_2\text{O}$ was dissolved in 250ml of (0.02M HNO_3) solution then the volume was completed to 1 liter by (0.02M HNO_3) solution. Standard working Chromium solution (0.1, 0.2, 0.3, 0.4 and 0.5ppb) was prepared. Serum chromium was determined using Flame Atomic Absorption Spectrophotometer. Standards, samples and blanks were aspirated into a (Perkin Elmer 6000) atomic absorption spectrophotometer utilizing along-path air/acetylene burner and cathode lamp for chromium metal. Each sample was read three times at 1 second. When standard chromium concentrations were read by flame atomic absorption spectrophotometer, readings plotted against concentrations and a graphic representation is drawn for subsequent readings of serum samples; deionized water was used as a blank that gave zero readings. Serum samples then were aspirated into air /acetylene flame and read one by one and recorded. After each 5 samples of serum chromium, standards were read again while blank was tested before each sample. The concentration of chromium was found from the standard curve [14, 15].

Results and Discussion

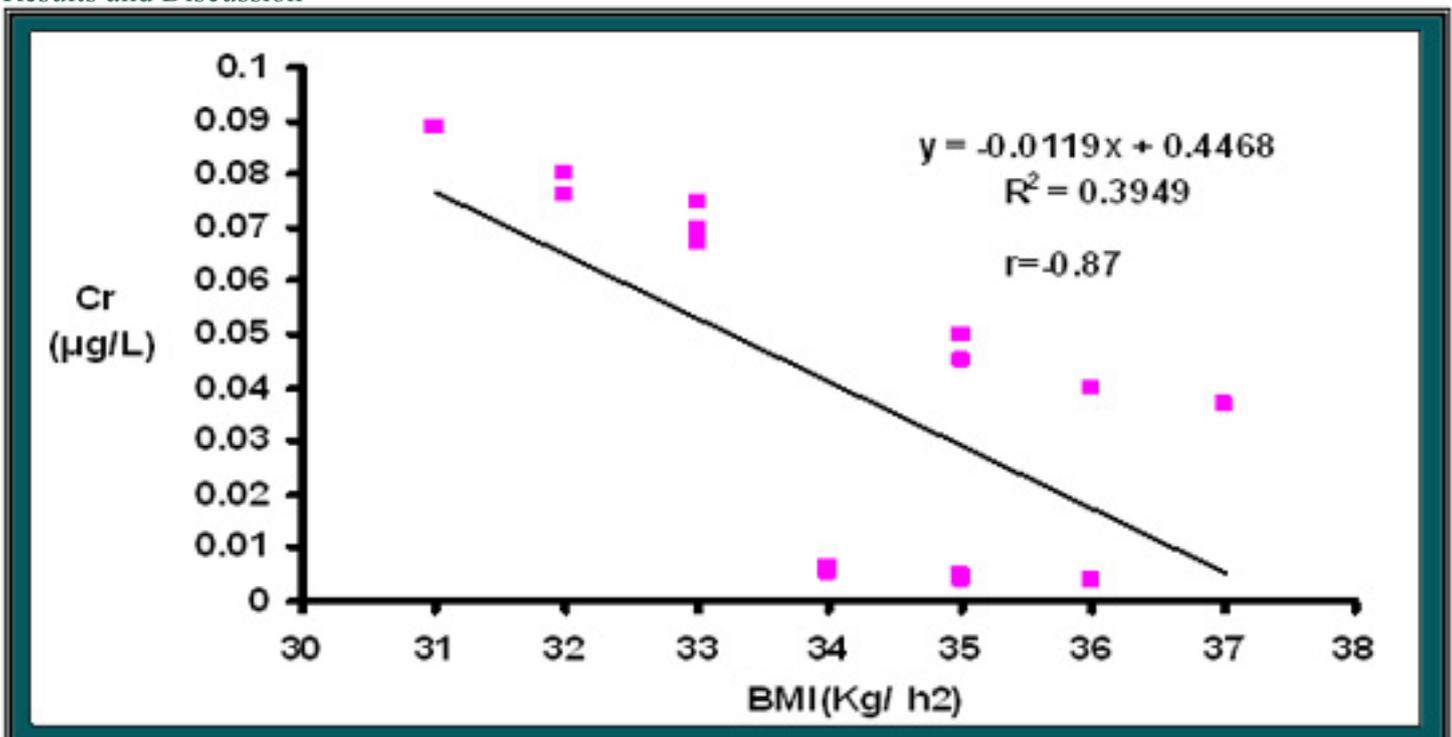


Figure 2: Correlation of chromium levels with body mass index in obese individuals

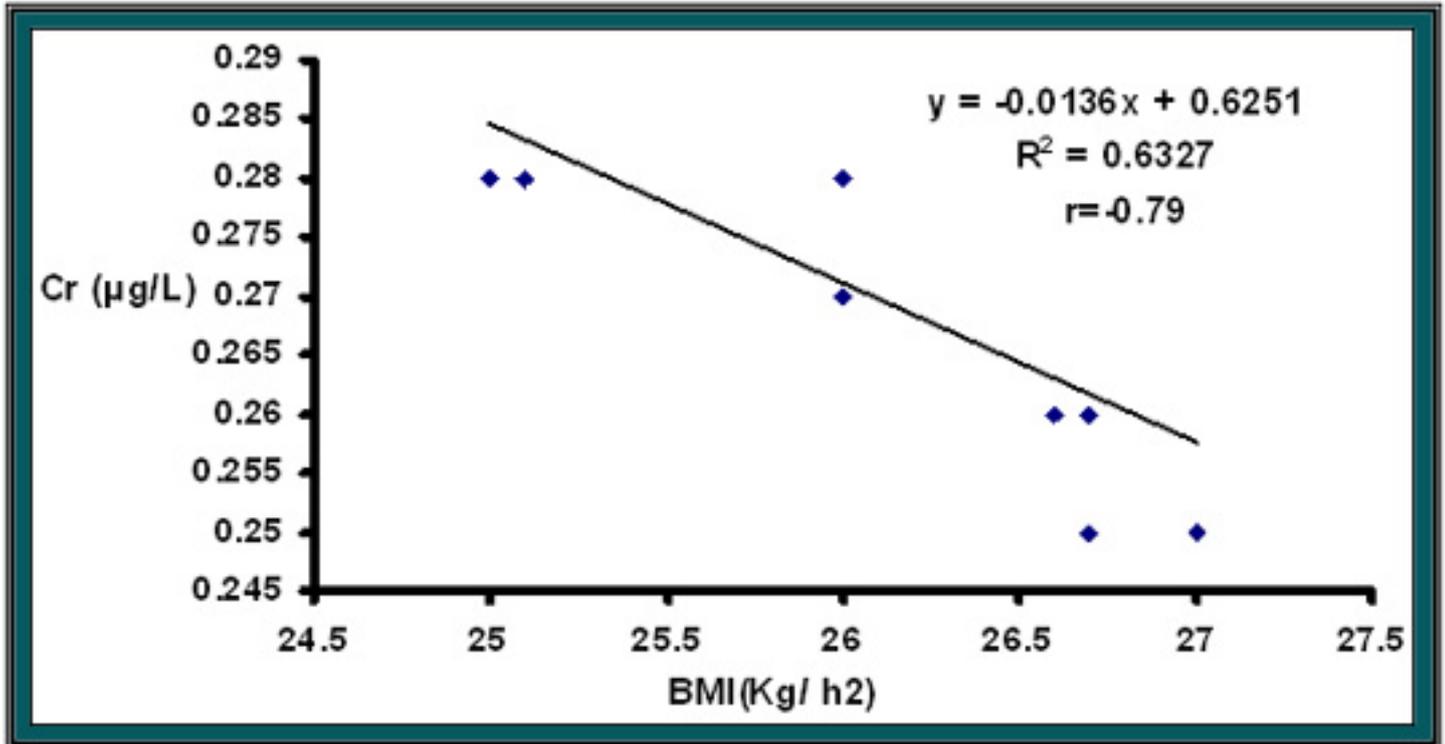


Figure 3: Correlation of chromium levels with body mass index in overweight individuals

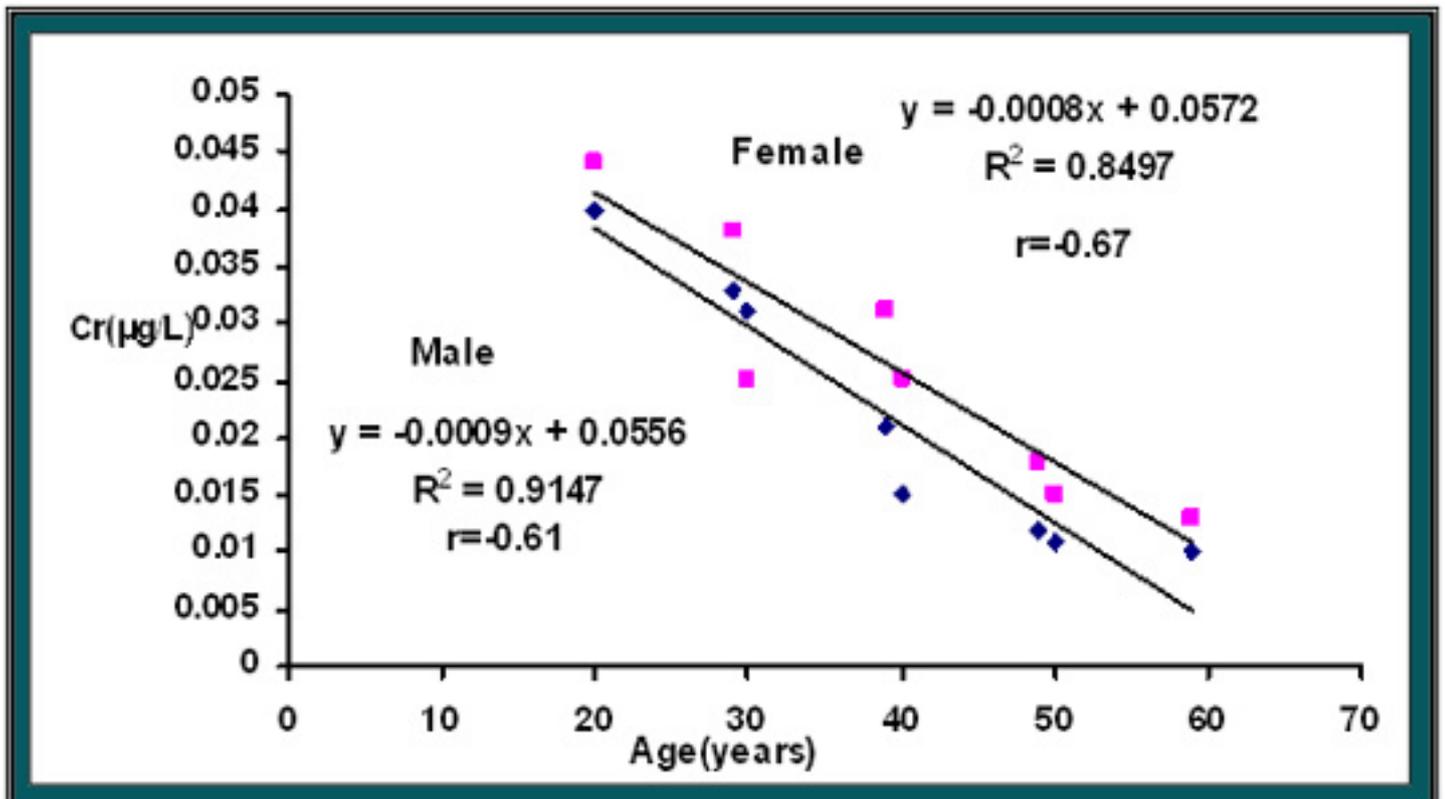


Figure 4: Correlation of chromium levels with age in males and females of obese individuals

The mean and standard error (SE) of serum chromium concentrations and BMI value of overweight, obese individuals and normal subjects are presented in Table (1). A significant decrease in serum chromium level was demonstrated in both overweight ($0.089 \pm 0.02 \mu\text{g/L}$) and obese individuals ($0.011 \pm 0.013 \mu\text{g/L}$), ($P < 0.001$) as compared with that of the normal subjects ($0.441 \pm 0.021013 \mu\text{g/L}$). The results of the present study revealed that BMI values of normal subjects was significantly lower ($21.3 \pm 0.64 \text{ Kg/h}^2$) ($p < 0.002$)

than the level in overweight ($27.4 \pm 0.92 \text{ Kg/h}^2$), obese individuals ($34 \pm 1.2 \text{ Kg/h}^2$) subjects. Chromium is an essential trace element and nutritional supplement that has garnered interest for use as a weight loss aid [16]. Purported benefits of supplementation include increased lean body mass, decreased body fat, and greater resting energy expenditure [17]. Chromium has been thought to be the active ingredient in glucose tolerance factor, a complex of molecules that includes glycine, cysteine, glutamic acid, nicotinic acid, and

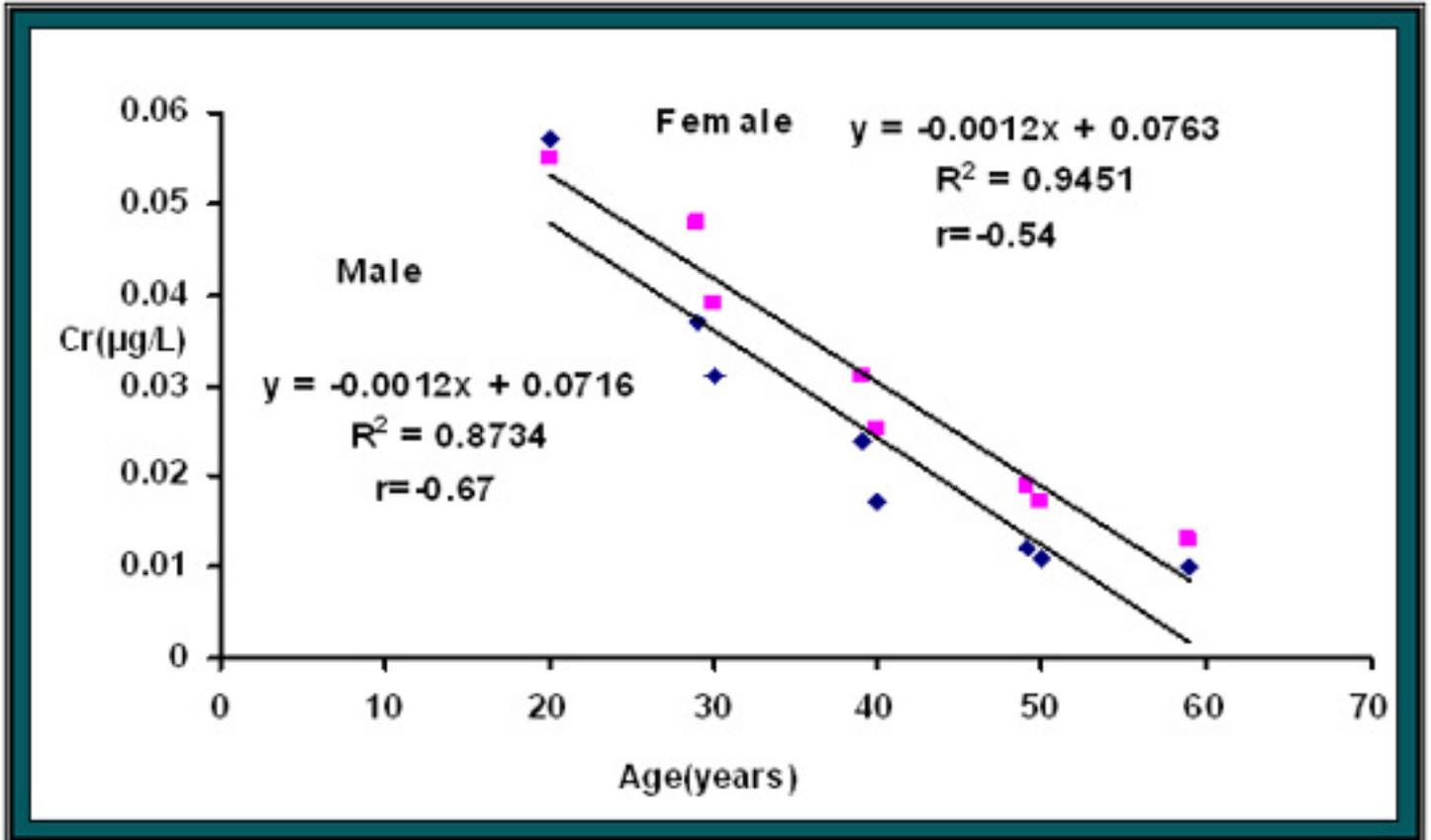


Figure 5: Correlation of chromium levels with age in males and females of overweight individuals

Parameters	Overweight n=50	Obese n=50	Control n=50	P value
Cr (µg/L)	0.089±0.02	0.011±0.013	0.441±0.021	0.001
BMI(Kg/ h ²)	27.4±0.92	34±1.2	21.3±0.64	0.002

Table 1: The mean ± SE of serum levels of chromium and body mass index (BMI) in Obese, Overweight and Control subjects

Parameters	Obese n=50		Overweight n=50		Control n=50	
	Female	Male	Female	Male	Female	Male
Cr (µg/L)	0.022±0.03	0.01±0.021	0.076±0.01	0.053±0.032	0.41±0.031	0.028±0.02
	0.011±0.013		0.089±0.022		0.441±0.021	
	Both		Both		Both	
P value	P<0.01		P<0.01		P<0.01	

Table 2: The mean ± SE of serum levels of chromium in Obese, Overweight and Control subjects according to gender

Parameters	Obese n=50		Overweight n=50		Control n=50	
	Cr (µg/L)	20-39	0.023±0.013	20-39	0.077±0.3	20-39
39-59		0.01±0.04	39-59	0.034±0.021	39-59	0.241±0.011
Both		0.011±0.013	Both	0.089±0.022	Both	0.441±0.021
P value	P<0.001		P<0.002		P<0.001	

Table 2: The mean ± SE of serum levels of chromium in Obese, Overweight and Control subjects according to gender

and chromium [18]. This complex of molecules found in high amounts in brewer's yeast and other foods functions synergistically to potentiate the effects of insulin [19-21] by increasing insulin binding to cells, up-regulating receptors, and suppress appetite and stimulate thermogenesis through sensitization of insulin-sensitive glucoreceptors in the brain [22]. Body fat distribution is related to insulin sensitivity; peripheral fat is more insulin sensitive than central fat found in the chest and abdomen [23]. A meta-analysis of 10 double-blind, placebo-controlled trials provides evidence of a relatively small reduction in body weight (1.1-1.2 kg over 10-13 weeks) in overweight and obese individuals receiving chromium picolinate [24, 25]. As shown in Figures (2, 3), serum chromium concentration correlated negatively with BMI value ($r = -0.87$), in obese individuals. There was also a significant negative correlation between serum chromium concentration with BMI value serum ($r = -0.79$), in overweight individuals. Chromium has been implicated as a cofactor in the maintenance of normal lipid and carbohydrate metabolism. In humans, high dietary intake of chromium over a long period lowered TC, triglyceride, and hemoglobin A1c in blood [26]. It was seen that two months of chromium supplementation resulted in a clinically useful increase in HDL cholesterol levels in men taking beta-blockers [27]. In a double-blind crossover study with humans, it was noted that levels of TC, LDL cholesterol, and apolipoprotein B (the principal protein of LDL) decreased significantly while the subjects were supplemented with chromium. The concentration of apolipoprotein A-I, the principal protein of HDL fraction, increased during the treatment. The HDL cholesterol level was elevated slightly, but not significantly, during chromium supplementation. In contrast, only apolipoprotein B was altered significantly during supplementation with the placebo [28, 29]. Table (3) shows the serum chromium concentrations in serum of overweight, obese individuals and normal according to age. A statistically significant age related decrease in chromium level was observed in overweight, obese individuals and normal subjects for both males and females. The serum Cr levels in the age group of 20-39 years were found significantly higher than those of the age groups of 39-59, 40-49, 50-59, years ($p < 0.001$). The same results were obtained by [30]. In our study, we observed that serum chromium levels decrease progressively with age with highest serum levels of these hormones present in adults and lowest in the elderly. Several factors have been described that influence the serum concentrations of trace elements. This

is evidenced by great variability in their serum concentrations from various populations. Some studies have shown that serum antioxidant concentrations are primarily influenced by such variables as sex, age, obesity, tobacco smoking, alcohol consumption and dietary intake [31]. Most factors cause a decrease rather than an increase in trace elements concentration. Decreased concentrations are related mainly to decreased nutritional intake, intestinal uptake and altered distribution while increased concentration is reported to result from excessive homeopathic intake, industrial or environmental exposure, smoking and administration of parenteral fluid [32]. The lower serum levels of chromium observed in the hypothyroidism males may be attributed to increased urinary excretion of this element in the males. The lower serum chromium levels in the males may also be associated with additional seminal loss of chromium in hypothyroidism males. Davies et al [33], observed significantly lower mean serum Cr levels in males than in females. Figures (4, 5) show the correlation of chromium levels with age in males and females of overweight and obese individuals. Both men and women showed a significant decrease in serum chromium levels with advancing age. There was a significant negative correlation between age and chromium levels in obese males ($r = -0.61$) and obese females ($r = -0.67$). Similarly serum chromium levels negatively correlated with age in overweight males ($r = -0.67$) and obese females ($r = -0.54$).

Age related physiological changes, drug therapy, modified dietary requirements and chronic diseases leading to or associated with enhanced consumption or excretion of trace elements might contribute to trace elements deficiency in the elderly [34]. A number of surveys have shown that Cr intake by old persons to be lower than the corresponding nutrient intakes; this may be attributed to changes in mineral bioavailability with aging [35]. Ageing has been previously associated with low intracellular Cr concentration, probably the consequence of insulin resistance due to ageing. Increased urinary loss of trace elements associated with ageing may be attributed to reduction in renal function with ageing. Low chromium, status has also been reported in the elderly population. A significant decrease in Cr values was also demonstrated in individuals within the age range of 91-110 years when compared with individuals within the age range of 60-90 years [36]. Ding et al., (2008) also demonstrated increases in the urinary Cr concentration in the diabetics and the control group

with increasing age. It also proved a positive correlation between age and serum and red cell chromium levels in women [37]. Figures 4 and 5 show the relationship of gender with serum chromium levels in obese and overweight patients. The mean serum levels of chromium levels in obese and overweight males was significantly lower than that of obese and overweight females ($P < 0.001$) (Table 2). The lower mean chromium levels in males in the 30- to 59-year age groups occur at the time of life when there are maximal differences in the male/female prevalence of coronary artery morbidity and mortality. It was found that men had a higher refined-carbohydrate intake than women in these age groups, which could account for the lower chromium levels in males observed in this study. It may also be that female hormones may contribute to the observed higher chromium levels in these tissues [38].

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Serum Levels of Male Oligospermia Glycoconjugate Inhibin B hormone and a-L-Fucose in Kurdistan (Iraq) populations

ABSTRACT

Objectives: To assess the role of inhibin B in the evaluation of male factor infertility as a prospective study. To determine normative levels of inhibin B and examine levels in relationship to FSH, sperm count, and motility in a cohort of fertile and infertile men from the Kurdistan region of Iraq.

Materials and methods: Semen analysis was performed according to World Health Organization guidelines. ELISA techniques were applied for serum levels of inhibin B (ng/L), LH (mIU/L), FSH (mIU/L), and testosterone (ng/ml) assays. A colorimetric procedure was followed for the fucose and protein bound fucose levels determination.

Results: A total of 55 infertile (oligospermia) and 87 presenting for fertility (normal) evaluations were recruited. The mean serum inhibin B level was significantly ($P<0.05$) lower in the oligospermia (18 ng/L) than normal group (24 ng/L). No significant differences were found in determination levels of fucose in both groups. Serum FSH and LH levels were significantly higher in oligospermia than in normal group.

Conclusion: Inhibin B levels in fertile men and infertile men in Kurdistan region were similar but not identical to those reported in other geographic regions. Both inhibin B and FSH are useful markers of spermatogenesis.

Keywords: Inhibin B, FSH, LH, fucose, male infertility, spermatogenesis

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Introduction

The World Health Organization defines infertility as the inability of a sexually active couple, not using contraceptive methods, to achieve pregnancy within one year. This condition affects 15% of couples, and the male is responsible in 50% of cases. Standard tests to study infertility include spermogram and hormonal profile(1). The prevalence of infertility varies widely, being less in developed countries and more in developing countries where limited resources for investigation and treatment are available. In addition, infertility is considered also a public problem. It does not affect the couples' life only, but it also affects the healthcare services and social environment(2). Recent studies have focused on semen quality of men in the general population; however, the interpretations of semen data, over time, has been hampered by significant intra- and inter individual differences in sperm counts, high levels of non response, inappropriate sampling procedures, and intra laboratory differences in methods(3). Some of these problems are difficult to overcome because semen analysis has to be performed soon after ejaculation, and the analysis must be performed in a laboratory at the site of collection, in contrast to blood analysis. Furthermore, in most semen quality studies, the participation rate is low, which could introduce selection bias. Blood samples are easier to obtain than ejaculates. Therefore, a valid serum biomarker of spermatogenesis is of particular interest for population studies(3). Sperm concentrations of less than 20 million/mL are classified as oligospermic. This figure probably derives mainly from the work of MacLeod and Gold, who found that only 5% of fertile men had sperm concentrations less than 20 million/mL(4). Recent study of fertile men generally support 20 million/mL as clinically useful although the new edition of the WHO semen analysis manual will suggest lowering the cutoff to 15 million/mL and also lower values for sperm motility and normal morphology(5). There is a correlation between sperm concentration and other aspects of semen quality. Both motility and morphology are usually poor with oligozoospermia(6). Hormones play a vital role in initiating and maintaining male reproductive function, yet it is not well

understood how variability in the levels of some hormones impact semen quality(7). Endocrine malfunctions are more prevalent in infertile men than in the general population, but are still quite uncommon. Hormonal screening can be limited to determining follicle stimulating hormone (FSH) in case of abnormal semen parameters (8). Inhibin b is a heterodimeric glycoprotein, and in men inhibin B is secreted from the testis as a product of Sertoli cells involved in the regulation of FSH secretion(9). Inhibin B is a modulator of FSH secretion by the pituitary and gametogenesis in the gonads. It is recognized that inhibin B regulate these processes by blocking the stimulatory actions of the structurally related proteins, activins(10). Serum inhibin B was recently suggested as a possible, more direct serum marker of spermatogenesis in men with testicular disorders(10). Although it is now known that inhibin B plays a crucial role in spermatogenesis, uniform normative data are elusive(11). a- L -Fucose is naturally found and it was identified in mammalian tissues and fluids located at non reducing ends of sugar chains of glycoconjugates (Glycoprotein and glycolipid)(12). Important roles for fucosylated glycans have been demonstrated in a variety of biological settings (13, 14). However, because of the diversity of fucose-containing glycoconjugates and the difficulties inherent in studying the biological function of carbohydrates, it is likely that many additional functions for fucosylated glycans remain to be uncovered. Fucose may present in the human body in three forms free, protein bound, and lipid bound(15). These three forms of fucose represent the total Serum fucose (TF). Successful fertilization requires the coordination of species signature events between the sperm and oocyte. It has been well documented that fertilization is a carbohydrate-mediated event (16). The aim of this study was to ascertain the levels of Inhibin B hormone and the fucose as a glycoconjugate content of the Inhibin B hormone.

Materials and Methods

Subjects (Sampling): Blood samples should be fast at least 9-12 hours. Blood samples (10 mL) were obtained from infertile patients (55) and fertile male (control group) (87) by vein puncture. Samples were allowed to clot at room temperature for 15 min. and then centrifuged at 2500 rpm for 15 minutes. Sera were removed for analysis directly or stored in plain tubes at -20 °C until analysis. Hemolysis and lipaemic sera were avoided.

Semen samples were collected by masturbation after 2 to 3 days of abstinence. Semen analysis was manually performed according to WHO criteria (WHO, 1999). Seminal volume, sperm density, and percentage of motile sperm were recorded. Determination of Inhibin B (INHb)

Principal of the assay: A kit assay manufactured by WKEA MED firm has been adopted in the determination of serum INHB, the protocol of the assay is the application of ELISA Technique in which a purified INHB antibody coated the micro titer plate wells which represents the solid-phase antibody. An addition of INHB (to be determined) to wells, combined INHB antibody which with HRP labeled, become antibody-antigen-antibody complex, a colored complex formation was obtained from the addition of TMB substrate solution.

Ab1+Ag+Ab2 Ab1-Ag-Ab2+ free moieties

The binding reaction was terminated by the addition of H₂SO₄ solution and the color change measured spectrophotometrically at $\lambda = 450$ nm. The concentration of the unbound INHB in the sample is proportional to the color intensity and can be determined by comparing the absorbance of the sample to the standard curve, automatically prepared.

Determination of the FSH hormone

Principle: The assay principal was as that of (Reyes et al, 1976 and Young D 1990)(17, 18) in which two-site immune enzymatic analysis is adopted. FSH present in the test samples is bound with the monoclonal Ab immobilized on a magnetic solid phase (beads) and enzyme-labeled monoclonal Ab in the test cups. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal Ab and are then incubated with a fluorogenic substrate 4MUP. The amount of enzyme-labeled monoclonal Ab that binds to the beads is directly proportional to the FSH concentration in the test sample.

A standard curve is constructed and unknown sample concentration calculated using the standard curve.

Determination of serum LH hormone

Principal: A similar protocol was applied in the determination of LH level and the only difference was the kit type. An STIA-PACK LH kit was applied in the determination assay, and the kit is specified for LH hormone determination (17, 19).

Determination of serum testosterone

Principle: The assay procedure was adopted from that of Teitz (1995)(20). A competitive enzyme immunoassay was applied as a technique for the determination of testosterone for both groups of patients and controls. Upon mixing by biotinylated Ab (AbB), enzyme-Ag conjugate and a serum containing the native Ag, a competition reaction results in between the native Ag and the enzyme-Ag conjugate (AgE) for a limited number of Ab binding sites. The enzyme activity in Ab bound fraction is inversely proportional to the native Ag concentration. The interaction of the binding assay is illustrated by the following equation:



Determination of serum total fucose

Principle: The principle depends on a direct reaction of concentrated sulfuric acid with serum components. The reactants combine with cysteine hydrochloride, and the color mixture measured at 396 nm and 430 nm. The differences in absorbance were directly proportional to methyl pentose content of the solutions(21).

Determination of serum protein bound fucose (PBF):

Principle-Protein Bound Fucose was determined according to Dische and Shettles method (21). A color product (chromophor) was formed by fucose in strong acid medium, which combines with color developer (cysteine hydrochloride); the color product with cysteine measured at 396 nm and 430 nm.

Determination of serum total protein (TP):

A kit depending Biuret method was used in determination of total protein. The principle of the reaction depends on the formation of the colored complex(22, 23).

Results

Table 1 (opposite page) reveals the physical and chemical properties of both normal and oligospermia semen as mean and 95% confidence interval. It was seen that semen liquefaction/min was significantly ($P<0.05$) decreased in oligospermia samples than normal. No changes were found in the PH value, while a slight difference was observed in the volume obtained.

Both sperm concentration per total ejaculate and sperm concentration per ml ejaculate were found to be decreased in oligospermia samples compared with those of normal. The decreases were significant ($P<0.05$) (Table 2).

Results obtained (Table 3), also revealed the levels of the endocrine hormones, Inhibin B, Testosterone, LH, and FSH in both oligospermia and normal groups. Significant differences ($P<0.05$) were found in all, except LH, determinations of oligospermia samples compared with those of normal.

Investigating the levels of fucose and protein bound fucose (Table 4), the results showed no significant differences between oligospermia samples and normal.

Discussion

The current studies show that there is no significant different in the volume of the ejaculate. The main reason is that there is no age difference between oligospermic patients and control group. Some of the methodologically stronger studies show decreases in semen volume of 3%-22%, likely when comparing 30-year-old men to 50-year-old men. Most studies examining fertility status suggest a relationship between male age and fertility (24). Low semen volume may be due to the drugs, chronic illnesses, abnormality in the urogenital system and incomplete ejaculation. Those cases were excluded in the current study(25).

The significance of high-volume ejaculates remains unclear. While sperm density may be decreased in high-volume samples, sperm motility remains unchanged (26). The seminal vesicles secrete the substance responsible for coagulation. Patients with congenital bilateral absence of the vas usually have absent or hypoplastic seminal vesicles. Semen in these patients does not coagulate, is acidic, and has a low volume. Secretions from the testis, epididymis, bulbourethral glands (Cowper's glands), glands of Littre (periurethral glands),

prostate, and seminal vesicles compose the normal seminal fluid (25).

Current study shows significant different in the liquefaction time being higher in the control group than the oligospermic patients. The effect that semen nonliquefaction has on male fertility remains unclear (25). Some patients with non-liquefying semen have normal postcoital test (PCT) results. In addition, sperm may be found in the cervical mucus before semen liquefaction(27).

Although liquefaction of semen may be induced by the addition of seminalin (a seminal protease) or α -amylase, there is no evidence that treatments with these agents increase fertility(28). The World Health Organization laboratory manual, last revised in 2010, states that the normal pH of semen ranges from 7.2 to 8.0 (29). Current study shows similar results with that of Harraway (2000) (30).

Semen PH is due to a balance between acidic prostatic secretions and alkaline seminal vesicle secretions. Low ejaculate volume with normal pH may be normal for some patients but may also indicate incomplete collection or retrograde ejaculation, whereas low ejaculate volume with an acidic PH suggests ejaculatory duct pathology or absent seminal vesicles(25). One commonly used method rates the sperm movement on a five-point scale. A rating of zero signifies no motility; 1 denotes sluggish or non-progressive movement; 2 refers to sperm moving with a slow, meandering forward progression; 3 signifies sperm moving in a reasonably straight line with moderate speed; and 4 indicates sperm moving in a straight line with high speed (31).

The most common category of sperm movement is reported. An alternate system places the sperm into four categories. "A" signifies rapid progressive motility; "B," slow or sluggish progressive motility; "C," non-progressive motility; and "D," no motility. In this system, the percent of sperm falling into each category is reported(29).

It is unnecessary and of no proven prognostic value to determine motility parameters at repeated time points after seminal collection. This is a non-physiologic measurement because sperm leave the semen and enter the cervical mucus within minutes of deposition within the vaginal vault (32).

Cases in which the semen sample demonstrates all non-motile sperm or less than 5% to 10% motility may be due to ultrastructural defects, in which case the sperm are alive but have defects in the flagella. Although immotile, these sperm may appear morphologically normal. Alternatively, nonmotile sperm may be dead, in which instance the patient is said to demonstrate necrospermia (25).

This is a non-physiologic measurement because sperm leave the semen and enter the cervical mucus within minutes of deposition within the vaginal vault (32).

Parameter	Normal (n= 87)	Oligospermia (n= 55)	P value
Volume in milliliter	3.7 (3.2-4.1)	3.4 (2.8-3.9)	
Liquefaction in minute	51 (41-60)	38 (31-44)	S
PH	8.6 (8.5-8.7)	8.7 (8.6-8.8)	

Table 1: Physical and Chemical Properties of the semen, Means and 95% Confidence Interval
S - Refers to the significant difference from the control group (P < 0.05).

Parameter	Normal (n= 87)	Oligospermia (n= 55)	P value
Sperm concentration/total ejaculate	198(164-233)	18.2(13-23)	S
Sperm concentration/ml of ejaculate	53(45-60)	5.3(4.0-6.0)	S
Progression %, means and 95 CI			
• Static	44 (40-80)	62 (56-68)	S
• Non progressive motility	21 (19-23)	19 (16-23)	
• Progressive motility	34 (30-38)	18 (14-22)	S
Velocity %, means and 95 CI			
• Rapid	29 (25-33)	13 (9-17)	S
• Medium	18 (16-19)	15 (12-17)	S
• Slow	9 (7-10)	10 (8-12)	
• Static	44 (40-48)	60 (55-66)	
WHO %, means and 95 CI			
• Fast progressive (type a)	10 (8-11)	4 (2-5)	S
• Slow progressive (type b)	25 (20-30)	13 (10-17)	S
• Non-progressive (type c)	21 (19-23)	19 (16-21)	
• Immotile (type d)	44 (40-48)	57 (50-63)	S
• a+b	35 (31-39)	18 (13-22)	S
• a+b+c	57 (53-61)	38 (33-43)	S

Table 2: Concentration and motility analysis of the sperm in millions, means and 95 % Confidence Interval
S - Refers to the significant difference from the control group (P < 0.05).

Hormones	Normal (n= 87) Mean(95% CI)	Oligospermia (n= 55) Mean(95% CI)	P value
Inhibin ng/l	24 (19-22)	18 (17-20)	S
Testosterone ng/ml	8.8 (1.5-16)	4 (3.5-5)	S
LH mIU/l	4.3 (3.9-4.7)	5.4 (4.3-6.4)	
FSH mIU/l	4.6 (4.0-5.2)	9 (6-12)	S

Table 3: Hormonal analysis of the Normal and Oligospermic patients
S - Refers to the significant difference from the control group (P < 0.05).

Parameter	Normal (n= 87) Mean (95% CI)	Oligospermia (n= 55) Mean (95% CI)	P value
Total Fucose mg/dl	14.5 (13-16)	14.2 (13.4-15.1)	NS
Protein bound fucose mg/dl	13.6 (11.5-15.7)	13.4 (11.7-15.1)	NS

Table 4: Total fucose and protein bound fucose analysis of the Normal and Oligospermic patients
NS = Non significant different from control group (P>0.05).

Cases in which the semen sample demonstrates all non-motile sperm or less than 5% to 10% motility may be due to ultrastructural defects, in which case the sperm are alive but have defects in the flagella. Although immotile, these sperm may appear morphologically normal. Alternatively, nonmotile sperm may be dead, in which instance the patient is said to demonstrate necrospemia (25).

This is a non-physiologic measurement because sperm leave the semen and enter the cervical mucus within minutes of deposition within the vaginal vault (32).

In this study, we sought to determine normal levels of inhibin B in men undergoing vasectomy and levels in men presenting for infertility evaluation. Defining normative values for inhibin B is especially important because few studies have focused on this issue, and those that did, often assessed inhibin B values among men in European or other countries(11).

Our result showed similar, but not identical, reference data to prior reports of inhibin B in fertile men (33). Several other studies have found mean inhibin B levels among men of proven fertility to exceed 180 pg/mL (34, 35).

A reason for the discrepancy in normative data for inhibin B levels in fertile men may be the lack of assay standardization. There is no international reference preparation for inhibin B. Inhibin B is made as a research assay and not subject to the same rigorous laboratory controls as an assay made for clinical use. Furthermore, there are two research inhibin B assay protocols in use, one with an extensive boiling pretreatment process (Oxford Bioinnovations, UK) and one with treatment simultaneous with sample incubation (Diagnostic Systems Laboratories, Webster, TX). It is conceivable that differences in inhibin B results between studies may be explained, at least in part, by differences in assay method. Inhibin B levels were significantly related to sperm count, a finding that corroborates data reported in other studies(36-39).

Although we did not conduct semen analyses in fertile men in this study, prior reports have shown that serum inhibin B levels reflect sperm density and fecundity in healthy men (40).

There are controversial reports regarding the efficacy of inhibin B over FSH as a marker of spermatogenesis. Most studies suggest an improved predictive performance for inhibin B over FSH for sperm count(9, 33, 41), but some disagree (42).

Regardless, combined serum marker testing with inhibin B and FSH gave the highest predictive power for spermatogenesis(34).

Our somewhat smaller study showed no advantage for inclusion of FSH measurements with inhibin B for predicting sperm count. Previous publications have described

associations between inhibin B and sperm concentration or total sperm count, and some have also stated that inhibin B and sperm counts are better correlated at lower inhibin B levels(3, 7, 41, 43).

In addition to a negative correlation with FSH, circulating inhibin B shows a positive correlation with sperm concentration in the ejaculate. This was originally demonstrated in men with a range of reproductive function, from highly-selected semen donors to men(44), with azoospermia(45) and has since been confirmed in a large population-based study (46), in a group of men of proven fertility (47), as well as in infertile men(48).

The recovery from hormonal suppression of spermatogenesis also illustrates the interrelationships between spermatogenesis, inhibin B and FSH(49).

All three parameters (FSH, inhibin B and sperm concentration) rise during recovery and, at the end of that period, the physiological inverse relationship between inhibin B and FSH is restored. These data indicate that once pubertal testicular maturation has occurred and the normal compliment of spermatogenic cells is established, it is the number of Sertoli cells and their relationship with their compliment of germ cells that is the prime determinant of inhibin B production(1) .

The dependence of sperm production on the number of Sertoli cells(50), makes separation of the relative contributions of these two components difficult(43).

Our data confirm that serum inhibin B and FSH levels correlate well with sperm concentration and thus support their role as serum markers of spermatogenesis. In accordance with(51), we observed significantly lower serum inhibin B levels and higher FSH levels in the formerly cryptorchid infertile men, compared with the Inhibin B and testosterone originate from different types of cells in the testis. Even so, studies reported that both have a positive correlation with testicular function(52, 53).

In our study, we also found that inhibin B levels were positively correlated with testosterone levels, supporting the hypothesis that Leydig cells might influence inhibin B secretion. However, testosterone was not significantly correlated with sperm parameters in our study. This is probably because spermatogenesis occurs in a different cell population in the testis. Several authors proposed that some unidentified factors produced by Leydig cells may modulate the inhibin B production in the tubular compartment of the human testis(33).

Recent evidence has indicated that the gonadal control of the pituitary secretion of follicle stimulating hormone (FSH) may differ from that of luteinizing hormone (LH). Since it has been shown that in both prepubertal and adult, male and female rats peripheral levels of FSH can be selectively suppressed by preparations which contain inhibin-like

material, and since inhibin seems to be a short-term regulator of FSH concentrations, a physiological role for inhibin seems possible when secretory patterns of FSH and LH diverge during a short period of time (54).

The existence of two gonadotrophic hormones FSH and LH, originally proposed by Fevold et al. (1931) is now generally accepted. The role of FSH and LH in male animals has been extensively reviewed (55, 56).

In short, LH binds specifically to receptors in the membranes of the Leydig cells in the testis and stimulates androgen production by these cells. In contrast FSH has been shown to bind to cells of the seminiferous tubules, especially to the Sertoli. Since processes involved in gametogenesis and folliculogenesis are induced by both FSH and LH it is conceivable that the release of both FSH and LH is dependent on one hypothalamic releasing hormone luteinizing hormone releasing hormone (LHRH)(57), however, under many pathophysiological or experimental conditions diverging secretion patterns of FSH and LH have been observed(55, 58)

It is known that testicular and ovarian steroids are capable of controlling the secretion of LH and FSH in a number of circumstances. Therefore it could be possible at the observed diverging secretion patterns of FSH and LH are caused by a specific combination of steroids and, in experiments, also by a specific route and time of administration of steroids. In the human male a number of clinical conditions exist in which a selective rise in urinary or serum levels of FSH with normal levels of LH, can be observed. In these conditions the Leydig cells appear normal and are also regarded as functionally intact, since normal levels of testosterone are found. The germinal epithelium however is damaged or destroyed. This situation is found in the Sertoli-cell only syndrome, and oligospermia; elevated levels of FSH with normal levels of LH are also found after certain viral infections, irradiation or treatment with cytotoxic drugs(55, 59).

This finding was observed in our study, in that we found a significant elevation in FSH levels while that of LH remain within the normal level or slightly increased(60).

Dealing with the results of the study, inhibin can be involved in regulation of gonadotrophin secretion under physiological conditions. Measurement of inhibin levels could possibly also be used as a tool in the diagnosis of infertility in men with disturbances in spermatogenesis(55).

Generally, the data presented are evaluated. Inhibin can be detected, and apparently plays a physiological role in males especially when the number of Sertoli cells increases. Yet, it is discussed that the impurity of the preparations containing inhibin and the limitations of the bioassays used are major factors which prevent the full substantiation of the inhibin hypothesis(56).

A problem in speculations about the future applicability of inhibin is the fact that it is not known whether disturbances in inhibin feedback represent a symptom or a cause of a pathological condition. If a disturbance in inhibin secretion is a cause, the study of inhibin might open new perspectives in treatment of fertility disorders. If a disturbance in inhibin secretion represents a symptom, measurement of levels of inhibin might be used as a parameter for the number and/or physiological condition of the cells supporting gametogenic elements(58, 59).

It has been recognized that spermatogenesis required glycoconjugates in which two moieties of importance (the fucose and sialic acid) play a significant role in fertility. It has been proved that a novel class of glycosphingolipids was observed composed of eight fucosylated molecules present in fertile but not in infertile mutant mice. This class is expressed differentially in testicular germ cells(61).

More importantly, the neutral subset of this new glycolipids class strictly correlates with male fertility. These data implicate polyunsaturated, fucosylated glycolipid as essential for spermatogenesis and male mouse fertility (62).

GDP-fucose synthesis, which facilitates the incorporation of L-fucose into protein, the importance of fucosylation has been exemplified by the case of human congenital disorders of glycosylation (CDGs). GDP-L-fucose is formed either by the de novo or salvage pathways, which in Golgi serves as a substrate for the glycosylation mediated by different fucosyltransferases. Mice deficient in FX, an enzyme synthesizing GDP-fucose from GDP-mannose as part of the de novo pathway, exhibit infertility due to the deficiency of cellular fucosylation (63).

The scatter plot, (Figure 1, top of next page) indicates graphically whether there is a linear relationship between inhibin B and fucose. A numeric indicator of such a linear relationship is the sample correlation coefficient (r) of inhibin B and fucose. Among the patient group significant correlation with linear regression was found between fucose level and inhibin ($r = 0.037$). A suggestion was made that such correlation can give an idea about the association of inhibin B, a fucose content glycoprotein in inhibin B, with fucose. It was observed that inhibin B was significantly decreased at the same time fucose was decreased even though its decrease was slight.

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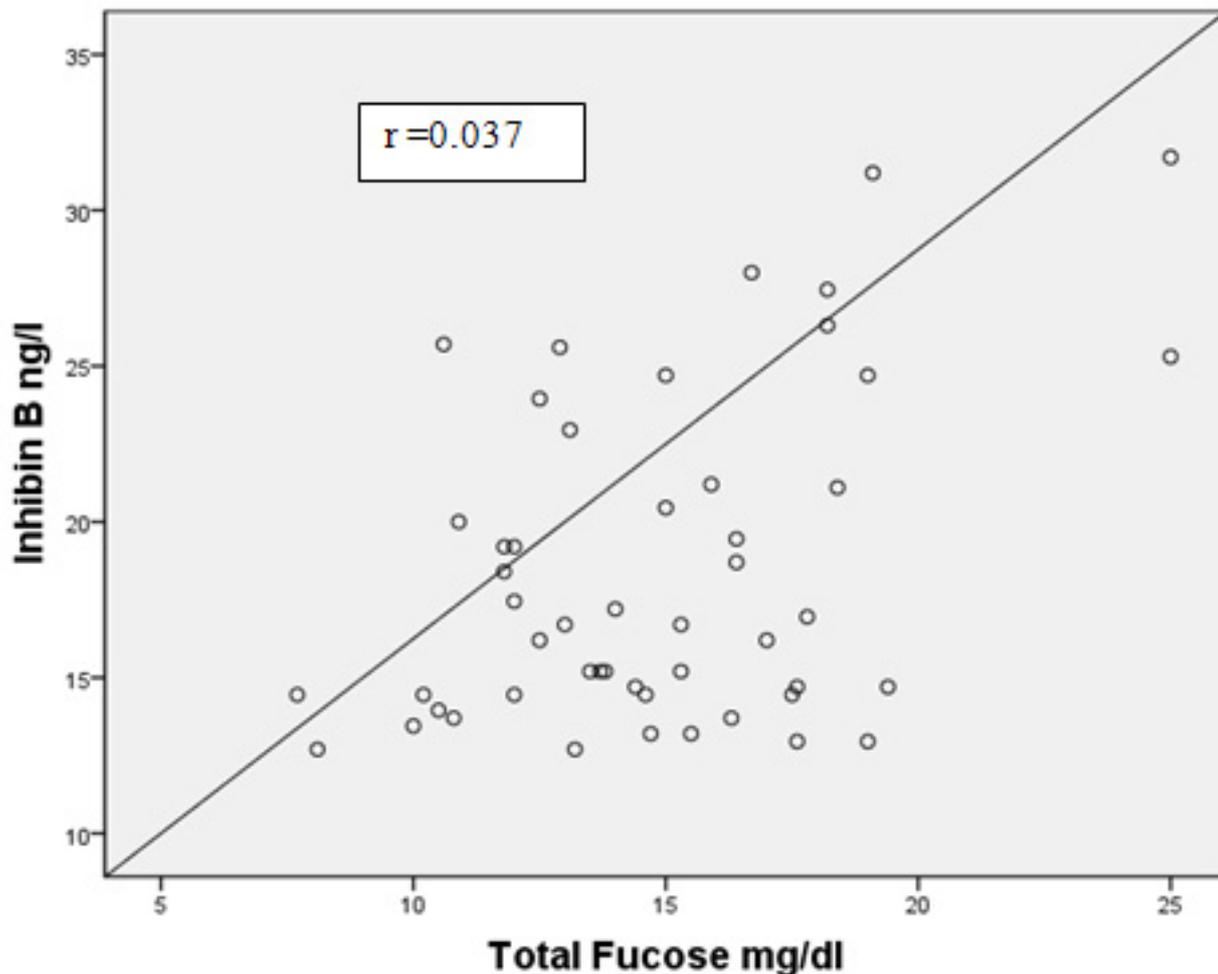


Figure 1: A significant correlation was found between inhibin B and fucose levels in oligospermic patients

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Mortality in Critically ill Children Post Red Blood Cell Transfusion

ABSTRACT

Objective: The aim of this study is to find if there is an association between RBC transfusion and mortality in critically ill children in the Pediatric Intensive Care Unit (PICU).

Setting: At a single center, mixed medical and surgical, 18 beds Pediatric Intensive Care Unit (PICU) of Queen Rania Hospital for Children (QRHC).

Patients and Methods: This study retrospectively analyzed the medical records of all patients who were admitted to PICU between August, 31, 2010 to September, 1, 2011 to identify the children who have Hb<13g/dl. Age groups between 3 days to 14 years were included in this study. Patients were put into two groups; the first group were children who received red blood cell (RBC) transfusion and were compared with the other patients in the second group who did not receive RBC transfusion. All surgical patients were included in this study except neurosurgical and cardio surgical patients whose operations were done outside QRHC. Any children known to have chronic anemia were excluded from this study. The hemoglobin value for each patient was recorded on admission within the first 6 hours of admission as base line.

Results: Among 437 children in PICU over the period of the study 231 (52.8%) children were identified to have Hb <13g/dl, with age ranged between 3 days and 14 years (median age 5.6 years), who were included in this study. They were put into two groups according to RBC transfusion. Transfused patients (group 1) and non-transfused patients (group 2) were estimated at 132 (57.2%), 99 (42.8%) respectively.

Mortality was estimated for both groups, (28.6%, versus 11.7 %) odds ratio 3.87, and 95% confidence interval: 2.34 to 5.16, $p<0.001$) for transfused patients and non-transfused patients respectively. Increased number of blood transfusions were associated with significant increase in mortality ($p<0.001$).

Conclusion: RBC transfusion is significantly associated with increased mortality in critically ill children. Further research is needed to improve the outcome of RBC transfusion and to balance its benefit against the risks associated with it in critically ill children.

Keywords: Mortality, RBC transfusion, critical, children

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Introduction

Transfusion of packed red blood cells is a complex biological product prepared from donated blood, and is unique in many respects when compared with other health interventions. RBC transfusion remains an essential and frequently performed medical intervention. (1)

Anemia is common in patients who have experienced major blood loss from physical trauma, those who are chronically ill, or those who have recently undergone major surgery. (2)

Few studies address the most common pediatric problems for which transfusions are used and most recommendations are extrapolated from adult data. There is clearly a need for additional carefully designed studies of transfusion therapy in children. (3)

Up to half of children in the ICU are given a red blood cell transfusion to counter anemia and improve oxygen delivery. (4)

Red blood cell (RBC) transfusions are a common therapy in critically ill and injured children. There are multiple risks associated with RBC transfusions, including transfusion-transmitted infections, transfusion-related acute lung injury, hemodynamic compromise, intravascular volume overload, acute hemolysis, and immunosuppression that increased mortality in critically ill children. (5, 6)

Transfusion of red blood cells is increasingly linked with adverse outcomes in critically ill children. (7)

Several studies have shown that patients, who receive transfusions, stay in the hospital longer and are generally more ill than patients who did not receive a transfusion. (8)

Some studies have even shown a greater likelihood of death in extensively transfused patients. (9)

Anemia is a common problem in critically ill children admitted to PICU, and is usually treated by blood transfusion on mortality in critically ill children is poorly defined.

A complete understanding of why blood transfusions can cause increased risk for some patients is not entirely known. (10) Transfusions can be lifesaving in critically ill patients but are not without risks. (11)

So this study was conducted to highlight the frequent RBC transfusions in critically ill children at PICU and estimate their mortality and to compare them with mortality in the non-transfused critically ill children.

Patients and Methods

This descriptive study retrospectively analyzed the medical records of all patients aged between 3 days to 14 years who were admitted to PICU between August, 31, 2010 to the September, 1, 2011. Their medical records were reviewed to identify the children who have Hb <13g/dl, and were enrolled in this study. They were put into two groups; the first group of children received red blood cell (RBC) transfusion and were compared with the other patients, in the second group who did not receive RBC transfusion. All surgical patients were included in this study except the neurosurgical and cardio-surgical patients whose operations were done outside QRHC. Patients with gestational age less than 37 weeks and birth weight less than 2.5kg, and children known to have chronic anemia were excluded from this study, and these included patients with sickle cell anemia, thalassemia, chronic renal failure and anemia related to chronic disease.

Hb was recorded for all children on the same day of admission and within the first 6 hours and every day during their stay in the PICU. Number of RBC transfusions was recorded; medical and progression notes before and after transfusion also were recorded by specialist doctors and well trained nurses.

The volume of RBC was calculated according to the age, weight and hemoglobin level.

Patient's Hemoglobin values within the first 6 hours of admission were recorded as the baseline value; the lowest hemoglobin during admission to PICU was recorded as the nadir Hb.

Results

Among 437 children who were admitted to PICU over the period of the study, after exclusion, 231 (52.8%) children were identified to have Hb <13g/dl with ages ranging between 3 days and 14 years (median age 5.6 years), were included in this study. Of them 125 (54.1%) were female and 106 (45.8%) were male. They were put into groups according to Hb level and age as seen in Table 1 (opposite page).

Children were put into two groups according to RBC transfusion.

Group 1: Transfused children,

Group 2: Non transfused children (see Table 2).

Numbers of transfused patients and non-transfused patients were 132 (57.2%), and 99 (42.8%) respectively.

The median period between the first and next transfusion was 3.3 days.

There was no significant difference in mortality rate according to the gender in both groups.

The number of mechanically ventilated patients recognized to have Hb<13g/dl was 109 (47.2%) children; transfused versus non-transfused patients was estimated at 78 (71.6%), and 31 (28.4%) respectively. There was no significant difference in mortality rate in both transfused and non transfused children on mechanical ventilation.

Mortality was estimated for both groups, (28.6%, versus 11.7 %); odds ratio 3.87, 95% confidence interval: 2.34 to 5.16, p<0.001) for transfused patients and non-transfused patients respectively it was higher for transfused patients.

Mortality for children with Hb below 7g/dl was higher in non transfused than transfused patients, while mortality for children with Hb<7-9g/dl for both groups transfused and non transfused, was almost the same rate and we found increased mortality in transfused children by increasing Hb level as seen in Table 2 and Table 3.

The mortality rate for each group was estimated as seen in Table 3.

The number of blood transfusions varies among transfused children: 91 (39.4%) children, 33 (14.3%) children, 21 (9%) children, 5 (2.2%) children, 3 (0.8%) children were transfused RBC 1, 2, 3, 4, 5-7 times respectively during their admission to PICU. Increased number of blood transfusions was associated with significant increase in mortality (p<0.001).

Discussion

The hemoglobin threshold for blood transfusion and transfusion volume varies among European pediatric intensive care physicians, for the same patients. (12)

The optimal hemoglobin threshold for erythrocyte transfusions in critically ill children is still unknown. (4)

According to WHO, anemic range of Hemoglobin (Hb), value of children aged between 0.5-4 years, 5-12 years, 12-15 years, Hb value are 11g/dl, 11.5g/dl, 12g/dl respectively. (13)

Critically ill patients receive an extraordinarily large number of blood transfusions. Between 40% and 50% of all patients admitted to intensive care units receive at least 1 red blood cell (RBC) unit during their stay, and the average is close to 5 RBC units. (14-16)

Hb level (g/dl)	Age: 3-30 days	1 month-3 months	3-6 months	6-12 months	1-3 years	3-6 years	6-14 years	Total
5-7	2	3	2	1	2	1	3	14
>7-9	5	5	6	7	4	5	4	36
>9-11	7	11	5	7	9	11	7	57
>11-13	15	21	15	19	17	15	22	124
Total	29	40	28	34	32	32	36	231

Table 1: Hemoglobin level in children according to their age

Hb(g/dl) level	Number of children	Group(1): Transfused children	Group(2): Non transfused children	Number of deaths in transfused children	Number of deaths in non transfused children
5-7	14	11	3	4 (4/11=36.4%)	2 (2/3=67%)
>7-9	36	29	7	12 (12/29=41.4%)	3 3/7=42.8%
>9-11	57	34	23	18 18/34=53%	7 7/23=30.4%
>11-13	124	58	66	32 32/58=55.2%	15 15/66=22.7%
Total	231	132(57.2%)	99(42.8%)	66 (66/132=50%) (66/231=28.6%)	27 (27/99=27%) (27/231=11.7%)

Table 2: Children groups according to RBC transfusion and their mortality at each Hb level

Anemic groups	Mortality related to each group	Mortality related to total Critical children
Group(1):	66(66/132=50%)	66/231 (28.6%)
Group(2)	27(27/99=27%)	27/231 (11.7%)
Total mortality	93/231 (40.3%)	93/231 (40.3%)

Table 3: Mortality rate in both groups

The number of units of RBCs appears to correlate with increased mortality, so the number of units transfused should be as few as possible. (17, 18)

In this study the more frequent blood transfusions were associated with increased mortality in critically ill children ($p<0.001$).

Taylor et al found in their study a significant increase in mortality for patients who received transfusions compared with patients who did not receive a transfusion. (19)

A critical review of 6 studies shows an association between red blood cell transfusion and increased mortality. (20) Tara Ann Collins after the analysis of these studies found an association between RBC transfusion and increased mortality rates. Although in two studies disease state was adjusted so RBC transfusion correlated with decreased mortality. (20)

There has been growing interest and efforts to limit RBC transfusion. Although a so called restrictive RBC transfusion strategy has been shown to improve morbidity and mortality in critically ill adults, there have been relatively few studies on RBC transfusion performed in critically ill children. (10)

Some data suggests that blood transfusion increased rates of mortality in critically ill patients. (21)

Vincent JL and colleagues concluded both ICU and overall mortality rates were significantly higher for transfused vs. non-transfused patients (ICU: 18.5% vs 10.1%, respectively ($p < 0.001$)). (22) In another study for the same author the mortality of the transfused propensity matched patients was 22.7% vs. 17.1% for the non-transfused patients ($P = 0.02$). (23)

In this study after adjusting propensity score for transfusion, increased risk of mortality was significantly higher when transfusion of RBC at Hb level equal or above 9g/dl and decrease mortality when transfusion occurred at Hb level below 8g/dl. The mortality rate in transfused children and non transfused children was 28.6%, vs. 11.7% respectively ($p < 0.001$). According to this study the mortality in transfused children was twice and more than in non transfused children.

Rauen CA. according to the multicenter, randomized, controlled clinical trial, showed that a restrictive transfusion strategy, defined as transfusion when the hemoglobin level is less than 7 g/dL, decreased rates of mortality as compared with the liberal transfusion strategy, defined as transfusion when the hemoglobin level is less than 10 g/dL. (24)

The Surviving Sepsis Campaign and many institutions recommend using a hemoglobin level of 7 g/dL as a transfusion trigger in ICU patients. (25)

A single-center retrospective study of transfusions in critically ill children by Kneyber and colleagues also found increased mortality and morbidity in children who received a transfusion, and all of their transfusions were with leuko-reduced blood. (26)

Scot T. Bateman and colleagues in the first large, multicenter, prospective study of anemia, blood loss, and transfusion practices in critically ill children found that critically ill children are at significant risk for developing anemia and receiving blood transfusions and concluded transfusion in the PICU was associated with worse outcomes. (16)

Transfusion Requirements in Critical Care (TRICC) study and a Cochrane review of this and other smaller randomized controlled trials conclude that, for most patients, RBC transfusion is not indicated unless the haemoglobin concentration decreases below 7-8 g/dl.(27-29)

In the absence of acute bleeding, hemoglobin levels of 7 to 9 g/dl are well tolerated by most critically ill patients and a transfusion threshold of 7 g/dl is appropriate. (30)

Most of the studies advise RBC transfusion at Hb level < 7g/dl. (22, 31-35)

Subjects with Hb concentrations below 6 g/dL almost always require transfusion therapy. In stabilized patients with Hb values between 6 and 10 g/dL, the decision whether to transfuse is based on an evaluation of clinical status; patients with values above 10 g/dL rarely require transfusion. (36)

C. Contillo Lacroix J, et al. conclude that in critically ill but stable children the use of a restrictive strategy involving a hemoglobin threshold of 7 g/dL for red-cell transfusion can safely reduce both the number of transfusions and the rate of exposure to red cells, thereby diminishing the chance of adverse outcomes of transfusion. (4)

Transfusions are still associated with risks and although their benefits are established in limited situations, transfusion decisions in critically ill children should be based on individual patient's characteristics rather than generalized triggers. (10) The reason why critically ill children were not transfused when Hb was below 7g/dl was the first one aged 3 months had autoimmune hemolytic anemia and direct Coombs test was positive so blood transfusion increased his RBC hemolysis and a further dropping in Hg level; the second one had severe heart failure post dilated cardiomyopathy.

The morbidity and mortality may be increased in patients who receive blood transfusions. Therefore, the decision to transfuse should be individualized, based on a rational approach and taking into account physiologic variables in addition to the hemoglobin value. This strategy should limit unnecessary exposure to RBCs. (37)

The reasons for increased mortality in critically ill children post blood transfusion is still not clear and will demand further studies in the future.

However some studies tried to find associations between RBC transfusion in critically ill children and increased morbidity and mortality. Oliver Karam et al in their study found an independent association between more prolonged RBC unit length of storage and increased morbidity and worse outcome in critically ill children. (38) Samuel Diomário et al found in their study RBC transfusion is associated with increased oxidative damage markers and reduced interleukin-6 levels in critically ill patients and a significant relationship between levels of post-transfusion carbonylated proteins and mortality. (39)

This study highlights the mortality in critically ill children post transfusion of RBC. It was mainly conducted for mortality not morbidity, as this needs another study, and it would be best to be a prospective study and hopefully in

future further research about RBC transfusion in critically ill children will be conducted.

Limitations of this study

It was conducted at a single center of PICU and it is not a large study. This limits the general application of this study to other pediatric care units and it is a retrospectively descriptive study, because data were retrospectively analyzed. Causality cannot be determined. Other limitations to this study is that the data were restricted during hospitalization to PICU so the mortality rate was estimated only during their stay at PICU and no follow-up after discharge from PICU, as the children were discharged from PICU will be stable and usually their clinical condition has improved and mortality after discharge is unlikely to be related to RBC transfusion. However the purpose of this study is to highlight RBC transfusions and their association with mortality in critically ill children.

Study message: Try your best effort to treat anemia, decrease bleeding tendency and decrease blood withdrawal to decrease the requirements of blood transfusion in critically ill children.

Conclusion

RBC transfusion is significantly associated with increased mortality in critically ill children. Further research is needed to improve the outcome of RBC transfusion and to balance its benefit against the risks associated with it in critically ill children.

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The effects of salbutamol , methylprednisolone and their combination on the histology of trachea in rabbits

ABSTRACT

Background and objectives: The combinations of a long-acting β_2 -agonist to low-to medium doses of inhaled corticosteroids, has become the preferred treatment for moderate persistent and severe asthma in adults and children. This study was conducted to determine the effects of salbutamol, methylprednisolone, and their combination on the histological features of normal tracheal tissue in rabbits.

Methods: Forty six rabbits were divided into two groups, control rabbits (n=10) and experimental rabbits (n=36). The experimental group was subdivided equally into three groups that received 0.5mg/kg salbutamol injection for six days weekly, 1.5mg/kg methylprednisolone injection 2 times weekly and combination of the 2 drugs with the same doses and the same time intervals respectively. The samples of the middle part of the trachea were collected 3 weeks and 6 weeks following the beginning of drug administration and the samples were subjected to histological (H&E) and histochemical (PAS stain) procedures.

Results: The salbutamol group demonstrated discontinuities among the epithelial cells, an increase in the number of goblet cells together with reduction in the thickness of the smooth muscle. On the other hand, the methylprednisolone group showed metaplasia of the epithelium in many sites together with increase collagen fibers formation in the lamina propria and prominent increased in the number of chondrocytes. The combination of the 2 drugs resulted in limited changes in the epithelium in few regions, with a higher number of chondrocytes when compared with the control group. The intensity of PAS stain was concentrated in the apical cytoplasm of the epithelial cells in the methylprednisolone group and in the perichondrium in the combination group.

Conclusion: Salbutamol and methylprednisolone produced many histological changes in the trachea and the combination of the 2 drugs limited these changes.

Key Words: Salbutamol, Methylprednisolone
Histological features, tracheal tissue.

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Introduction

Salbutamol is used widely for the treatment of bronchial asthma and in obstetrics for the prevention of premature labour (1). The possible clinical side effects after the administration of salbutamol include muscle tremor, palpitations, muscle cramps during intense exercise, and headache (2).

Goblet cell hyperplasia is a prominent feature in animals with atopic asthma and salbutamol enhances goblet cell hyperplasia and airway hyperresponsiveness in rats receiving salbutamol (3). Tamaoki et al. in 2004 stated that the β_2 -adrenoceptor receptor agonist stimulates proliferation of airway epithelial cells and produces airway wall thickening in vivo via mitogen activated protein (MAP) kinase-dependent pathway, and these effects are prevented by inhaled corticosteroids (4).

Long-term corticosteroid treatment has been shown to induce pronounced atrophy of the gut and uterine smooth muscle. Furthermore, corticosteroids could affect connective tissue, inducing skin atrophy and marked microvascular changes (5). The anti-inflammatory properties demonstrated for inhaled corticosteroids in biopsy studies on asthmatic patients include reduction in numbers of mast cells, reduction in numbers and activation of eosinophils, reduction in numbers and function of T- lymphocytes, improved epithelial morphology and reduction in epithelial cell activation, and some degree of reversal of airway remodeling (6).

The combinations of a long-acting β_2 -agonist to low-to medium doses of inhaled corticosteroids, has become the preferred treatment for moderate persistent and severe asthma in adults and children (7). The early treatment with steroids, alone or in combination with β_2 -adrenoceptor agonists, may be highly beneficial to prevent or reduce the extent of airway wall remodeling by limiting inflammation at an early stage. Airway wall thickening, which is largely due to smooth muscle proliferation and hypertrophy, is thought to be an important target for anti-inflammatory drug action. As β -agonists may interact favorably with corticosteroids,

combination therapy may be particularly advantageous in preventing or lessening airway wall remodeling (8).

The beneficial effect of inhaled corticosteroids in the treatment of asthma was studied by Hoshino et al in (1998). They found that 4 months of treatment with inhaled beclomethasone dipropionate (BDP) 500 µg twice daily led to significant reductions in the number of both mast cells and eosinophils, also significant decreases reported in the number of activated eosinophils and mast cells in biopsy samples in the epithelium and lamina propria from patients treated with BDP for atopic asthma (9). These findings confirm the results of earlier studies, in which BDP significantly reduced the number of eosinophils in the epithelium and lamina propria of biopsy samples from asthmatic patients (10).

This study was conducted to determine the effects of salbutamol, methylprednisolone, and their combination on normal tracheal tissue in rabbits as very few studies have dealt with the in vivo effects of these two drugs on normal trachea in rabbits.

Materials and Method

Experimental design

The study was performed on 46 male rabbits. Their weight ranged from 1.2-1.8 kg. They were kept in the animal house of the college of medicine /HMU and were fed barley and vegetables in a suitable room temperature (25C).

The rabbits were divided into two groups, experimental (n=36) and control group (n=10). The experimental group was subdivided equally into three subgroups, (12 rabbits for each). The first subgroup received intramuscular injection (IM) of (0.5) mg/kg of salbutamol for six days weekly. The second group received 1.5mg/kg methylprednisolone IM injection twice a week, while the third group received a combination of both drugs in the same doses and time intervals.

Three weeks following the beginning of the experiment, half the number of each experimental subgroup (n=6) rabbits were scarified. Transverse section from trachea of each animal was collected exactly at the middle part of the trachea by using operating scissors. Six weeks following the beginning of the experiment the remaining rabbits of each experimental subgroup were scarified in the same way and a transverse section of trachea was collected from each rabbit. The specimens were kept in 10% formalin for preservation until time of tissue preparation.

Sections of the trachea were also collected from rabbits that didn't receive any type of drugs (control group) in the same way to obtain baseline data of the normal rabbit trachea at light microscopical level. All of the specimens were subjected to Hematoxylin and Eosin PAS stain procedure and examined and photographed using light microscope (Olympus, CH3OR200 Japan and Motic Digital Microscope).

Results

The histological preparation of the trachea of the control group rabbits showed great similarity to the trachea of the human being. The different levels of the nuclei of the pseudostratified epithelia and the cilia can be easily demonstrated using high power. The goblet cells with their ovoid shape are present between the epithelial cells together with many basal cells. All cells were rested on a well defined thick basement membrane that separate the epithelia from the underlying lamina propria which is consists of loose connective tissue filled with many blood vessels and inflammatory cells, especially lymphocyte (Figure 1 - opposite page).

Histological findings of 3 weeks salbutamol groups

The injection of salbutamol for three weeks resulted in different changes in the histological appearance of the trachea. Generally the epithelia demonstrate many discontinuities and the underlying lamina propria demonstrated many congested blood vessels. The epithelial tissue of trachea which is usually pseudostratified ciliated epithelia seems to change their appearance approaching simple columnar epithelia with elongated basally located nucleus that were rested on a single squamous basal layer. In addition numbers of goblet cells seemed to be slightly increased; the underlying connective tissue demonstrated many congested blood vessels and reduction of the thickness of the layer as a whole. The basement membrane that separate between epithelia and connective tissue appeared to be obviously thicker than in the control group, (Figure 2 - opposite page).

Histological finding of 6 weeks salbutamol group

The histological changes that were demonstrated after three weeks injection of salbutamol became more obvious in this group such as the discontinuity of epithelia, the thick basement membrane, and presence of many congested blood vessels. The change of pseudostratified ciliated epithelia to simple columnar epithelia rested on single squamous basal layer became more obvious. The thickness of the underlying lamina propria appeared to be reduced greatly in many regions (Figure 3 - page 28).

The thickness of the trachealis muscle again appeared to be decreased in their thickness especially at the site that devoid the presence of c-shape cartilage, (Figure 4). Using the cytoplasm of the lining epithelia was completely basophilic and the apical portion showed complete positive reaction to PAS stain even for the lining epithelia over the trachealis muscle which resulted in great difficulty in detecting the goblet cells which demonstrated an obvious PAS positive reaction and centrally located nucleus (Figure 5).

Histological finding of 3 weeks methylprednisolone group

Generally the lining epithelium preserves its pseudostratified columnar feature in many locations while few sites showed the metaplasia of the epithelium to simple columnar to cuboidal epithelium that appeared thinner than in the salbutamol group. The mucosal secretory cells (goblet cells) appeared to increase in number and obviously filled with mucous materials that showed the typical foamy appearance. The underlying lamina propria showed a great reduction in the inflammatory cells

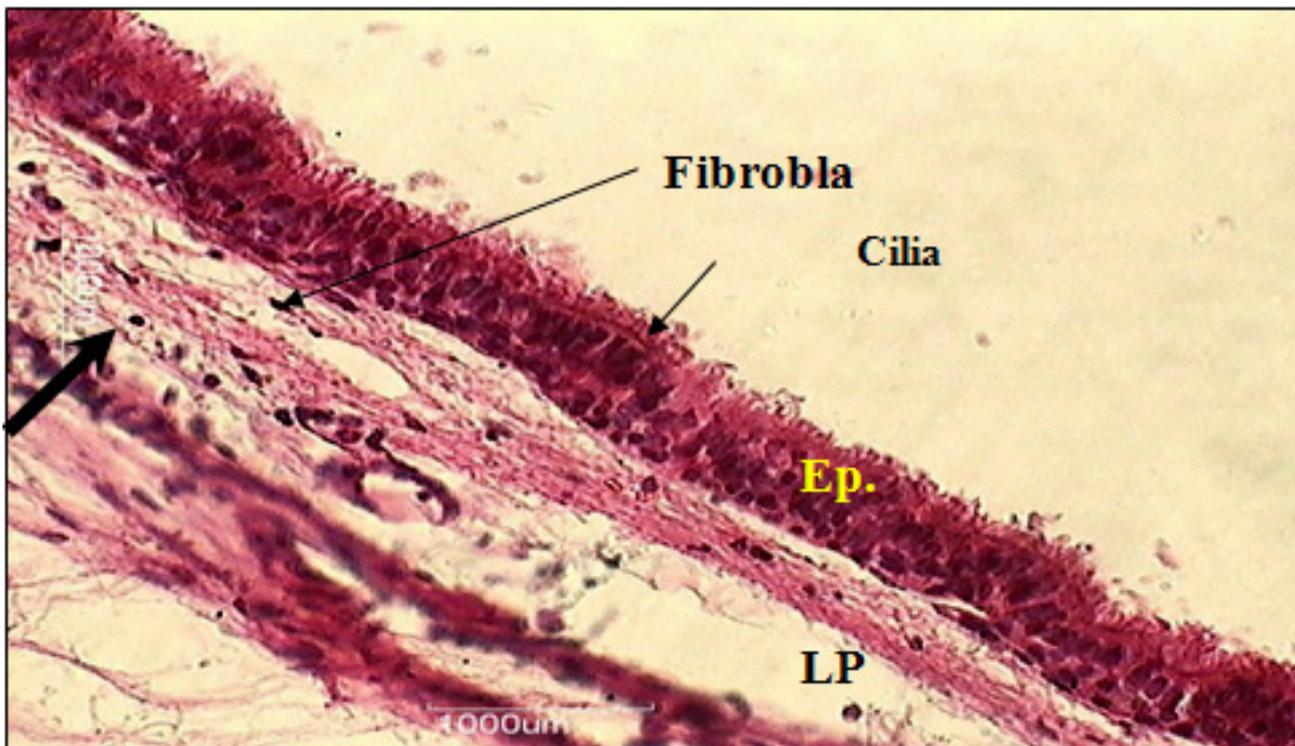


Figure 1 : Photomicrograph of trachea of control rabbit shows the different level of nuclei of epithelial lining (Ep.) and the cilia can be easily demonstrated. Thin collagen fibers are accompanied by fibroblast cells in the underlying lamina propria (LP) together with many lymphocytes (thick arrow). H&E. 400X

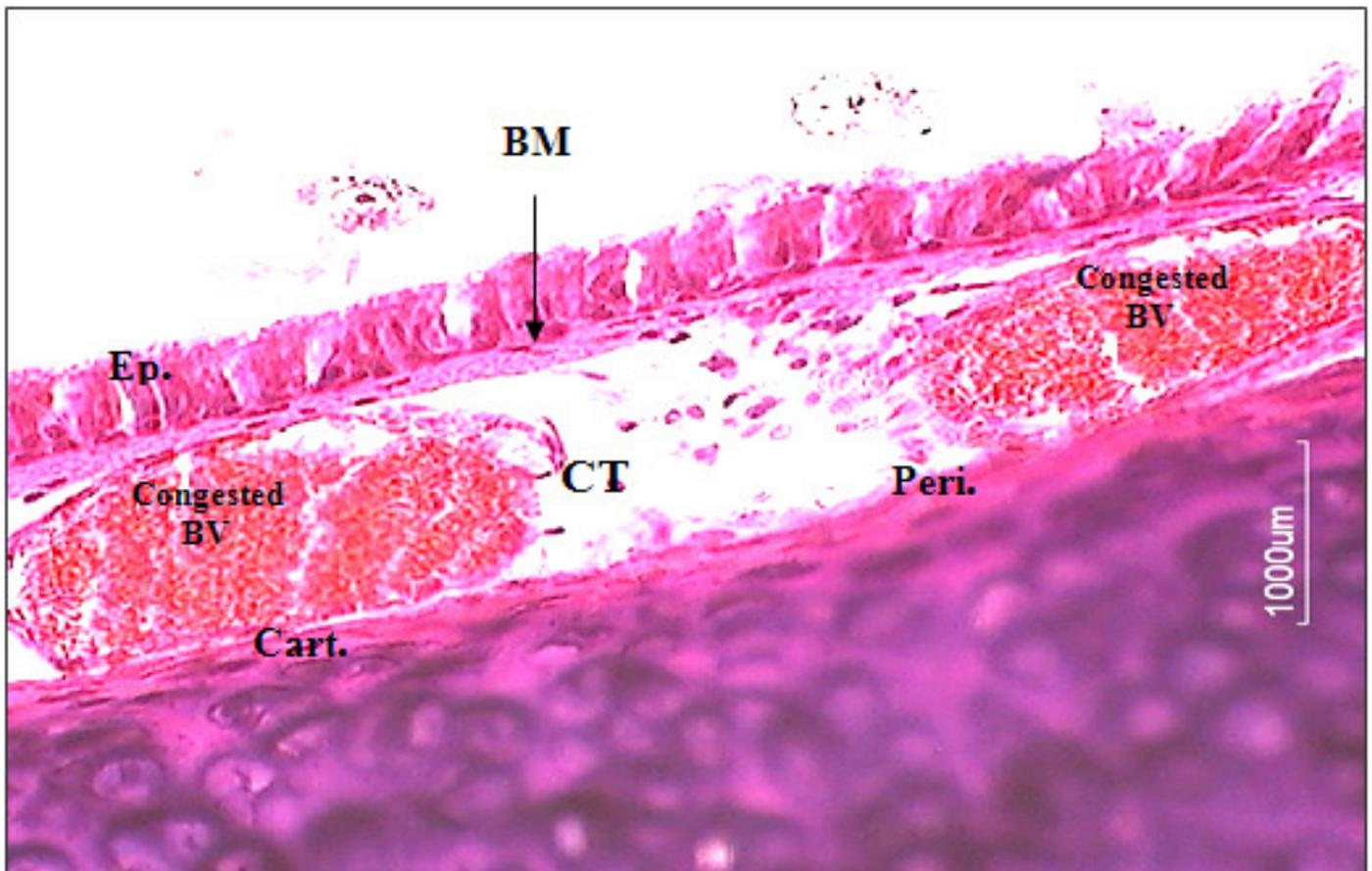


Figure 2 : Photomicrograph of trachea of rabbit 3 weeks after salbutamol injection shows the epithelial discontinuity, underlying connective tissue with many congested blood vessels (BV), and reduction of the thickness of the layers as a whole. The basement membrane (BM) which separate between the epithelium (Ep.) and connective tissue (C.T) appears to be obviously thick. H and E.400X

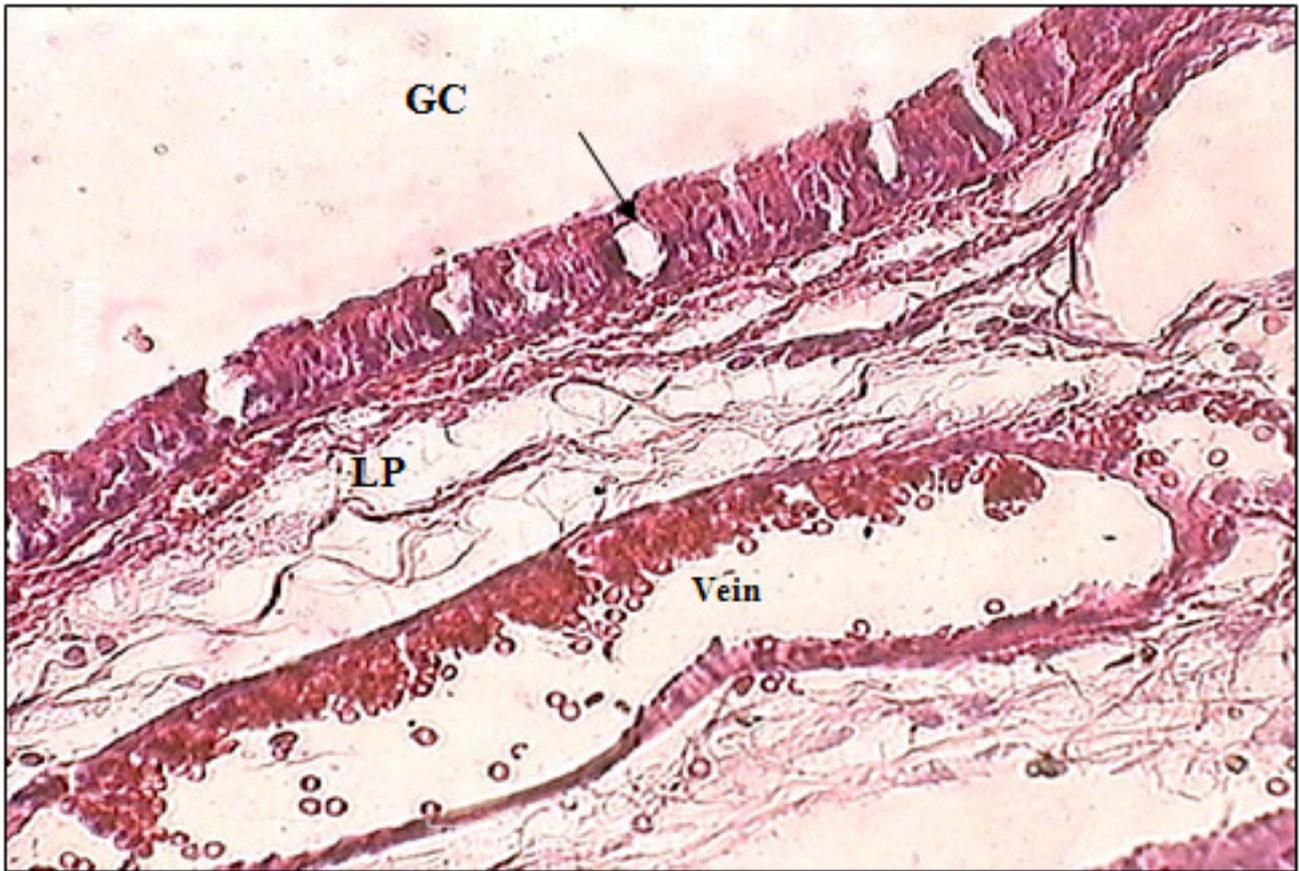


Figure 3 : Photomicrograph of trachea of rabbit 6 weeks after salbutamol injection, shows the discontinuity of epithelium (star) many goblet cells (GC) and many congested blood vessels (BV) in the lamina propria (LP) . H&E. 400X

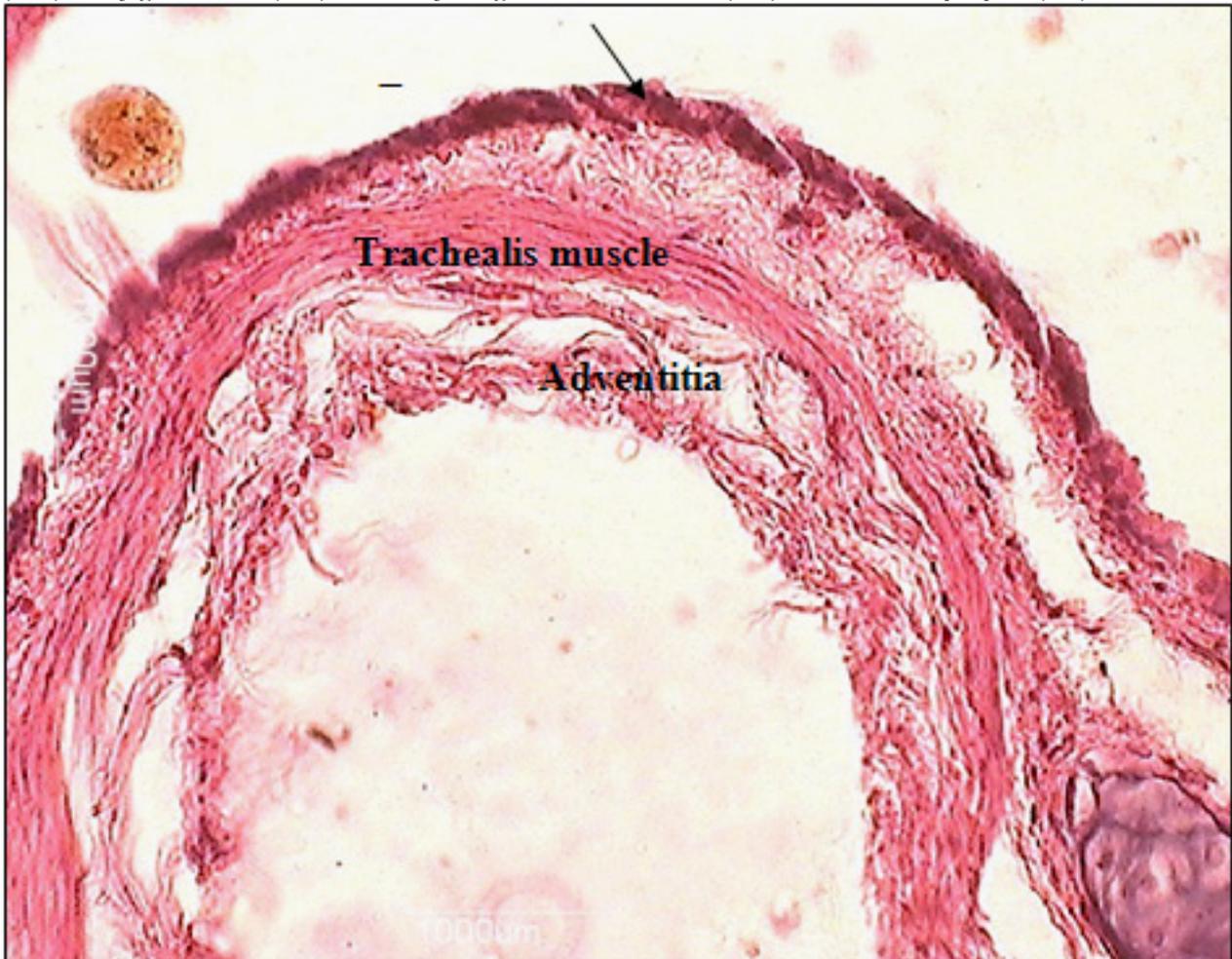


Figure 4: Photomicrograph of trachea of rabbit 6 weeks after salbutamol injection, shows the reduction in the thickness of trachealis muscle. H&E.400X

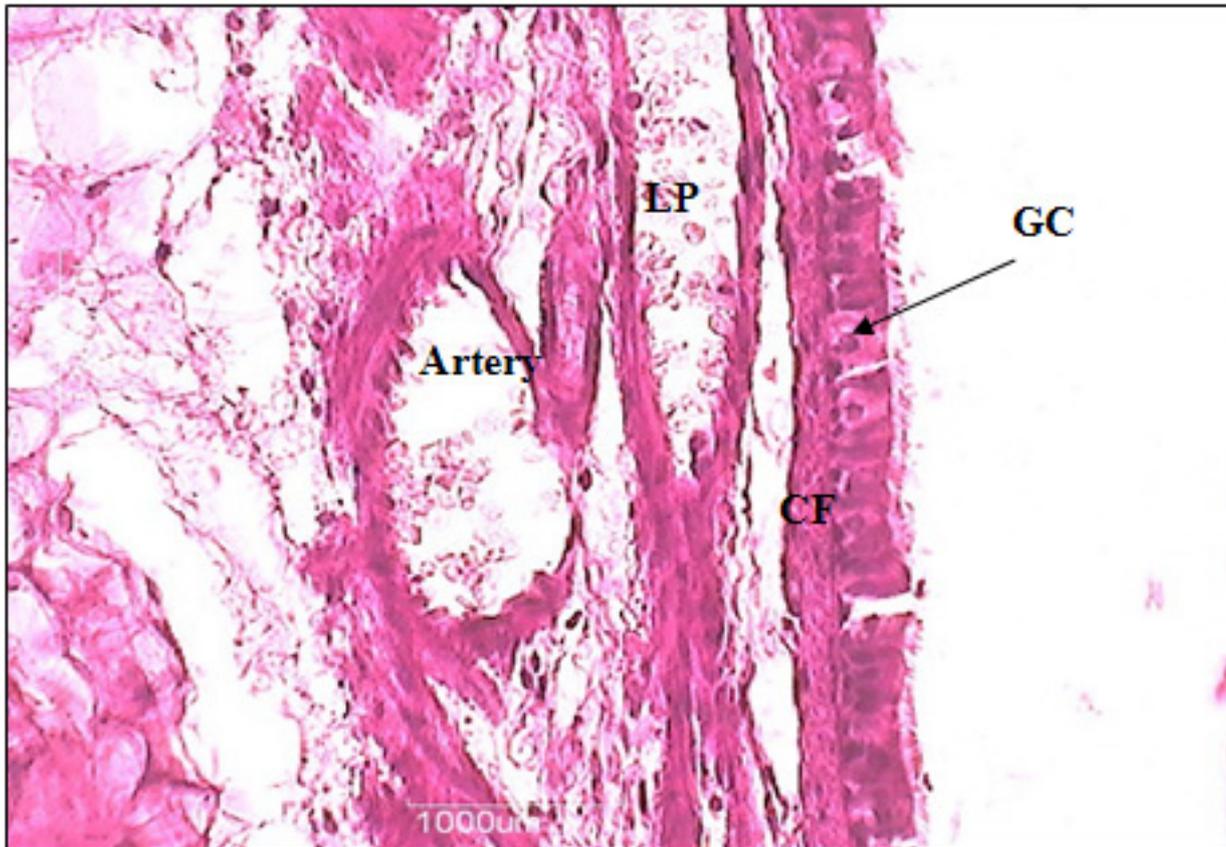


Figure 5: Photomicrograph of trachea of rabbit after 6 weeks salbutamol injection, shows the basal cytoplasm of the lining epithelia is completely basophilic and the apical portion shows complete positive reaction to PAS stain. The nucleus of goblet cells (GC) occupy a central location, the lamina propria (LP) filled with PAS positive congested blood vessels wall. (PAS stain 400X)

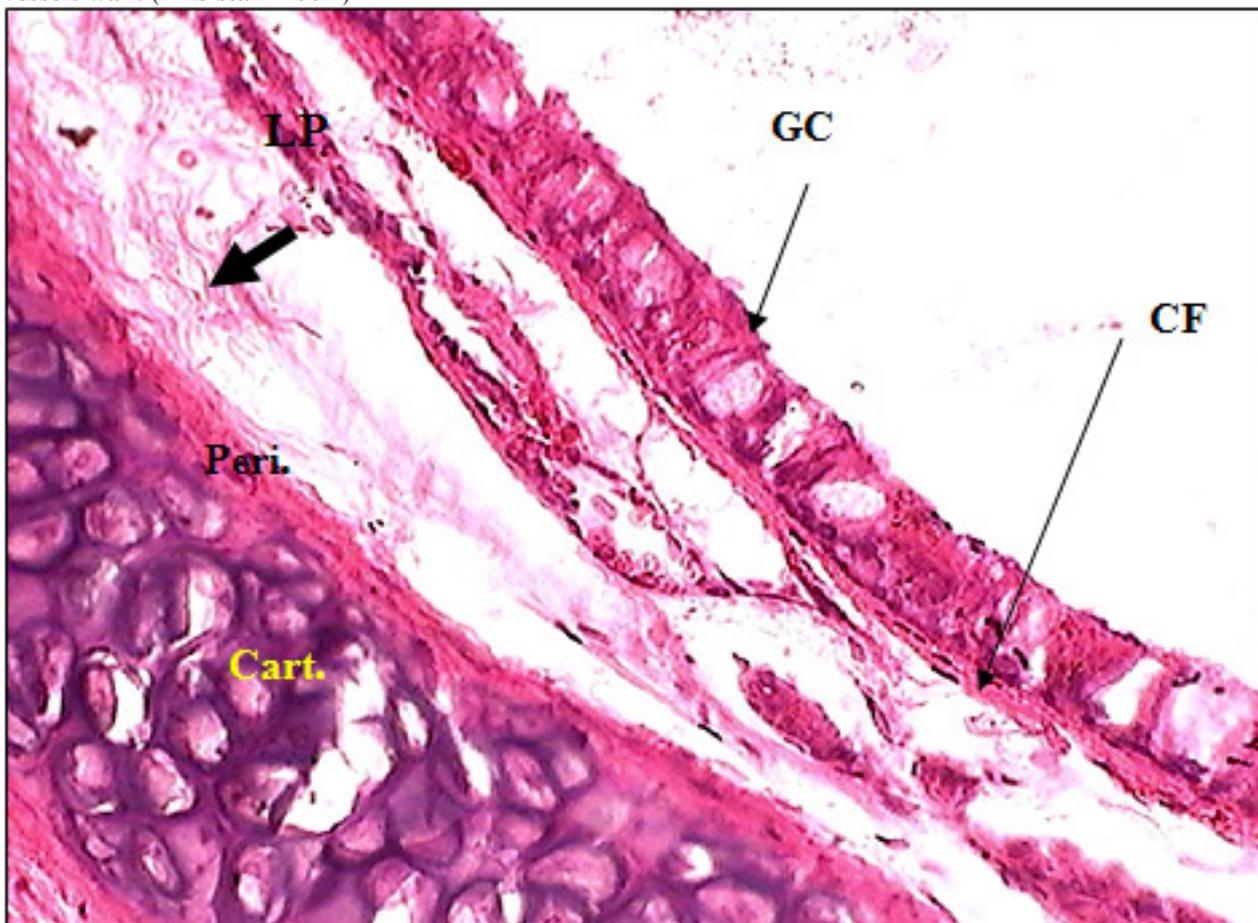


Figure 6 : Photomicrograph of trachea of rabbit 3 weeks after methylprednisolone injection, shows epithelia (Ep.) with many goblet cells (GC) that filled with mucose material and have a foamy appearance. H&E.400X
BV: blood vessel, Cart: cartilage, CF: collagen fiber, Peri : Perichondrium.



Figure 7 : Photomicrograph of trachea of rabbit 3 weeks after methylprednisolone injection. The chondrocytes in some regions show many opened face nuclei with lack of capsule around the lacunae which indicated low activity in the formation of GAG, Cart: cartilage, Peri: perichondrium. H&E.40

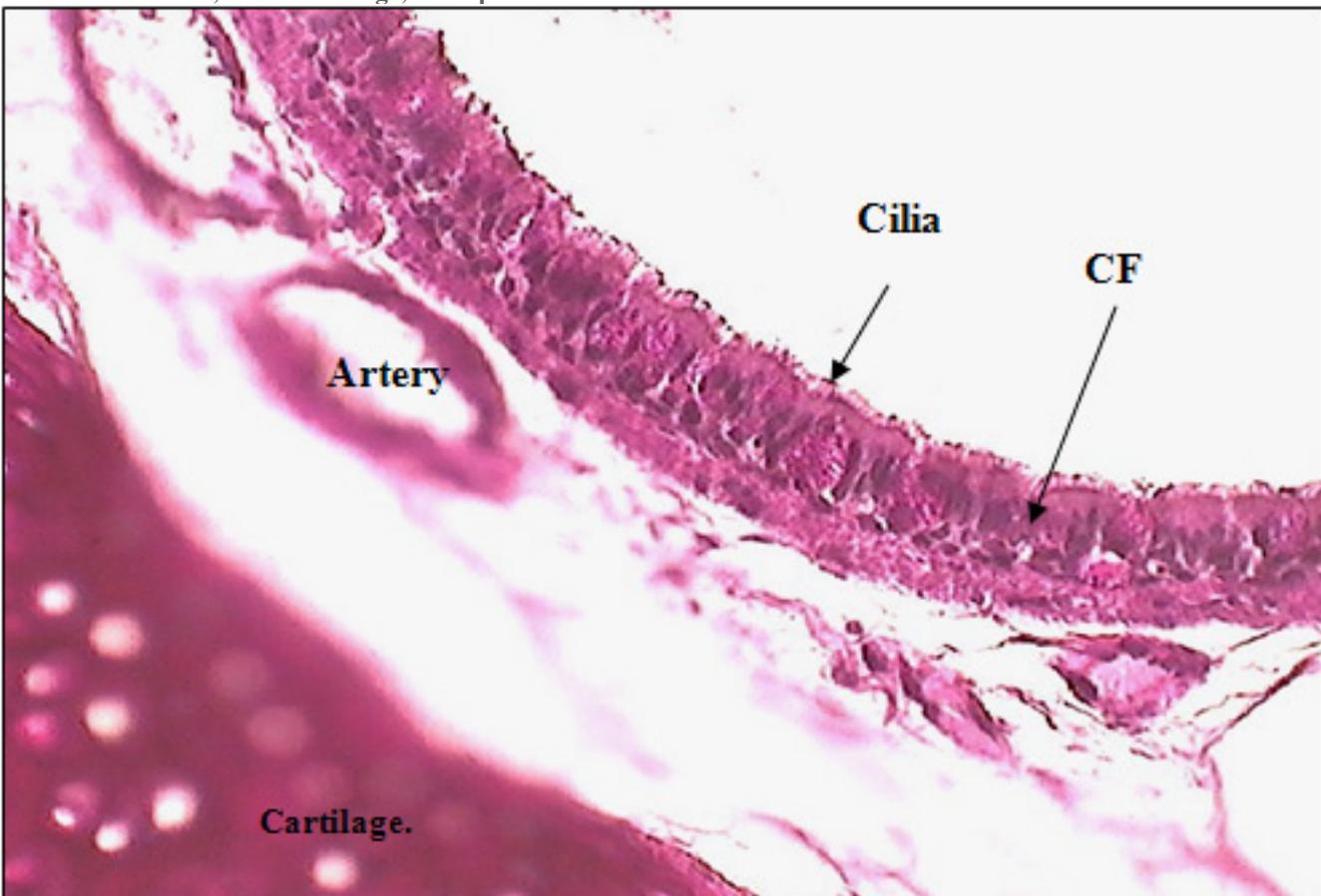


Figure 8 : Photomicrograph of trachea of rabbit after 3 weeks Methylprednisolone injection. The lining epithelia (Ep.) of this group demonstrate the usual pseudostratified columnar epithelia with cilia together with many PAS positive goblet cells (stars), CF: collagen fibers. PAS. 400X



Figure 9 : Photomicrograph of trachea of rabbit 6 weeks after methylprednisolone injection. The underlying lamina propria (LP) shows a normal thickness with few inflammatory cells, and a considerable number of cross sections of new blood vessels. (BV) especially within the thick collagen fiber (CF) with budding of new blood vessels (BV), Cart.: cartilage, Peri.: Perichondrium. H&E.400X

(lymphocytes) and an increase in the lay down of collagen fiber. There was a reduction in the number of blood vessels which appeared to have normal wall layers and were not decongestant when compared with the salbutamol group and even when compared with the normal group. Below the epithelium and directly under the basement membrane a thick band of newly formed collagen fiber was demonstrated in different locations (Figure 6). The chondrocytes of the hyaline cartilage showed high degree of mitotic division with the formation of many new isogenous groups which resulted in a crowded appearance of chondrocytes and reduction in the amount of extracellular material. In some regions the chondrocytes show many opened face nuclei with no capsule around the lacunae which indicates high activity in the formation GAG, (Figure 7). The lining epithelia of this group demonstrates the usual pseudostratified columnar epithelia with cilia together with many PAS positive goblet cells that filled with granular materials, (Figure 8).

Histological finding of 6 weeks methylprednisolone group

The epithelial lining of the trachea of this group showed the histological feature of pseudostratified ciliated epithelia with presence of goblet cells in different sites. The underlying lamina propria showed a normal thickness of loose connective tissue with few inflammatory cells together with the presence of an obviously thick band of collagen fibers directly under the basement membrane and within this band a budding of many newly formed blood vessels were demonstrated (Figure 9).

Histological finding of 3 weeks combination of methylprednisolone and salbutamol group

Trachea of this group showed some histological differences from the normal group. The epithelia had the normal pseudostratified columnar ciliated appearance which was rested on a thick basement membrane with the presence of many goblet cells among them. As a result of deeply eosinophilic cytoplasm of epithelial cell, the presence of goblet cells become more obvious and their nuclei occupy a central location. The underlying lamina propria which consists of loose connective tissue showed a small number of inflammatory cells when compared with the normal group but at the same time it contained many blood vessels which were congested in many locations but not as in the salbutamol group (Figure 10 - next page).

Generally the PAS reaction in the tissue of the trachea is less intense. The goblet cells can be demonstrated easily since the reaction of the epithelia was less intense and only deep positive reaction was seen in their apical cytoplasm and in their cilia. Using PAS stain the basal cells were completely negative to this stain and were easily demonstrated as a group of cells rested on the basement membrane with slightly wider base than the apex (pyramidal in shape).The underlying lamina propria showed the localization of discontinued collagen fibers directly below the basement membrane. Most of the blood vessels in the lamina propria appeared to be normal with thin walls slightly positive to PAS stain, (Figure 11 - next page).

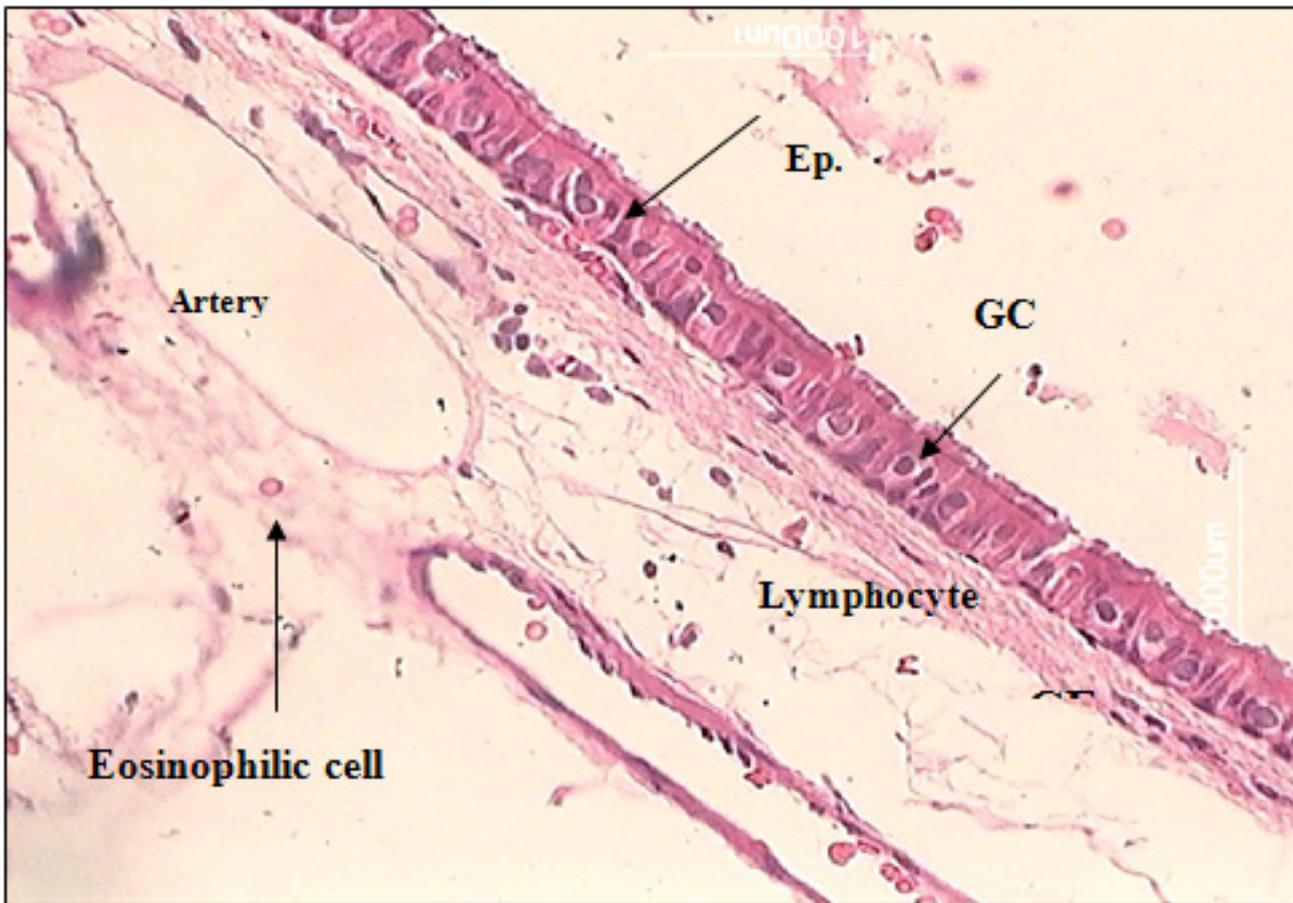


Figure 10 : Photomicrograph of trachea of rabbit after 3 weeks combination of methylprednisolone and salbutamol injection, shows deeply eosinophilic cytoplasm of epithelial cell (Ep), and the presence of goblet cells (GC).Lymphocytes and eosinophilic inflammatory cells were present in the lamina propria (LP) in small numbers with many blood vessels (BV) and thick band of collagen fibers (CF) beneath the basement membrane H&E.400X

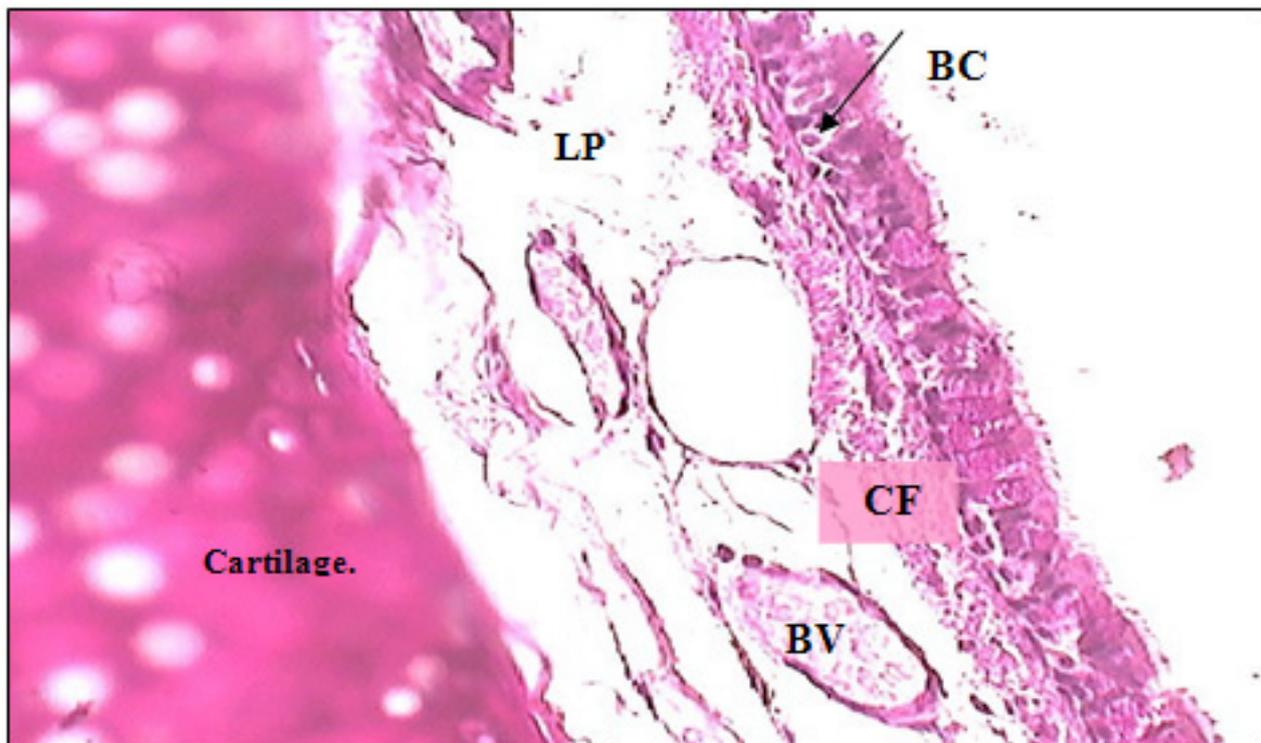


Figure 11 : Photomicrograph of trachea of rabbit after 3 weeks combination of methylprednisolone and salbutamol injection shows the goblet cells (dots), the basal cells (BC) rested on the basement membrane with their pyramidal shape, the discontinuity of collagen fiber below basement membrane, the blood vessels (BV) shows PAS positive reaction in their smooth muscle wall. PAS. 400X,

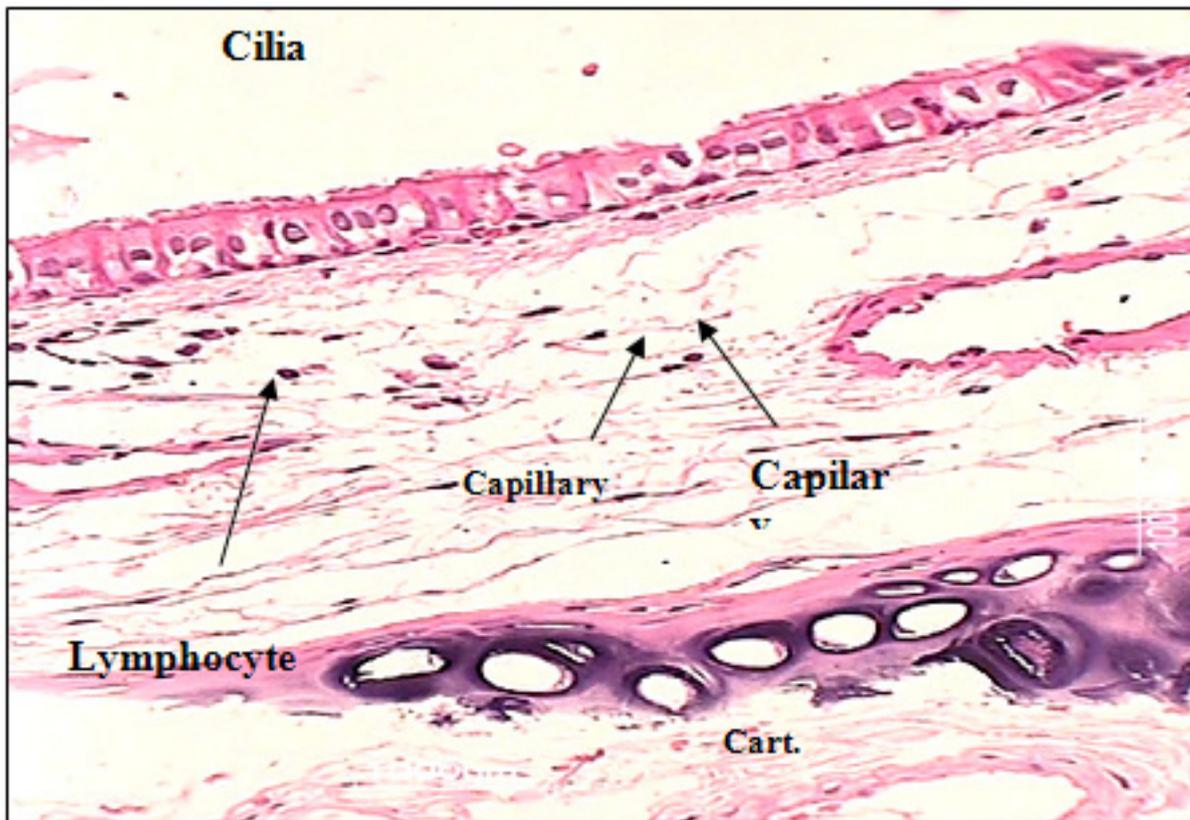


Figure 12 : Photomicrograph of trachea of rabbit after 6 weeks combination of methylprednisolone and salbutamol injection shows many goblet cells (GC) with the nucleus located centrally in most of them, the underlying lamina propria (LP) show the budding of many new small blood vessels (BV) few inflammatory cells. H&E.400X.

Histological finding of 6 weeks combination of methylprednisolone and salbutamol group

Pseudostratified ciliated epithelia was detected easily in most of the locations with deeply stained eosinophilic cytoplasm similar to that of 3 weeks combination group. In a few regions, the epithelia seemed to be a single columnar layer that is rested on single squamous epithelia which have flat nuclei resting directly on the basement membrane. The underlying lamina propria showed the budding of many new small blood vessels especially adhering to the basement membrane and generally the lamina propria seems to be highly vascular with inflammatory cells more prominent when compared with the methylprednisolone group, (Figure 12 - above).

Discussion

The histological preparation of the trachea of the control group rabbits showed the normal histological features of trachea with great similarity to that of human being. The mucosa consists of pseudostratified columnar ciliated epithelium with many goblet cells that are rested on a thick basement membrane. The cartilage had the typical features of hyaline cartilage with the presence of many isogenous groups of chondrocytes. This histological description was also stated by Vajner et al (2001) who studied the changes of glycoconjugates contained in tracheal goblet cells in rabbits (11).

The injection of salbutamol for three week resulted in different changes in the histological appearance of the trachea. The epithelial tissue of trachea seems to change

their features approaching simple columnar epithelia with the presence of many spacing or discontinuities between the cells, together with a slight increase in goblet cells. In the area of trachealis there was an obvious reduction in the thickness of the smooth muscle. The histological changes that were demonstrated three weeks after the injection of salbutamol became more obvious in the 6 weeks salbutamol group. The thickness of the lamina propria appeared to be reduced greatly in many regions, and the cartilage demonstrated areas of fibrosis in many locations that were devoid of chondrocytes and replaced by a region of highly eosinophilic fibers. These results are in an agreement with Konradova et al (2000) who has studied the trachea of white rabbits 30 minutes after inhalation of 2 puffs of the aerosol of salbutamol. (12).

The results also agree with Kamachi et al (2001) who stated that salbutamol induced more than a two fold increase in the number of goblet cells in rat after 4 weeks of 0.5mg/kg/day salbutamol subcutaneous administration (3). The same histological changes were obtained by Tomlinson et al, (1994) who reported that salbutamol has a direct inhibitory effect on mitogen-induced proliferation of airway smooth muscle cells grown in culture (13), while it disagree with Nishimura et al (2002), who showed that salbutamol stimulates the proliferation of human airway smooth muscle epithelia after it obtained a human bronchial epithelium which incubated with salbutamol for 48 hours in vitro (14). This disagreement could be due to different methods of cells exposure to salbutamol. The result of this study disagrees with Libretto,(1994) who stated that the inhalation of 1000mcg of salbutamol aerosol twice daily for three months did not produce any morphological changes in the lung, trachea of dogs. This

disagreement may be due to the different dose and different way of administration (15).

In the salbutamol group, the lining epithelia showed discontinuity in many locations and the number of goblet cells appeared to be increased and their size appear larger than the control group. In addition, the neighboring epithelia demonstrate PAS positive reaction throughout their cytoplasm. This result is in agreement with the study of Vanjer et.al, (2001) who found that the ultrastructural finding in the tracheal epithelium after the oral administration of salbutamol indicated the overstimulation of the majority of secretory elements resulting even in their damage and degeneration, fifteen minutes post exposure (16).

Histological finding of the 3 weeks methylprednisolone group showed that the lining epithelium preserved its pseudostratified columnar shape in many locations while few sites showed the metaplasia to simple columnar in some locations. The goblet cells appear to increase in number and the underlying lamina propria showed a great reduction in inflammatory cells, and reduction in the number of blood vessels with obvious increase in lay down of collagen fibers. The chondrocytes showed a high degree of mitotic division with the formation of many new isogenous groups and reduction in the amount of extracellular material while the trachealis muscle demonstrated no histological changes.

Methylprednisolone injection for six weeks confirmed the changes which were seen in the three weeks group. In addition the changes became more obvious especially the increase in chondrocyte numbers of hyaline cartilage. This result agrees with Pavlovic et al (1998) who observed that steroid treatment caused a change in the epithelial structure of trachea that appeared monostratified in rats after administration of triamcinolone 1.2mg/kg/day for seven days (17).

The active goblet cells found in this study may agree with the study of Yoshiaki et al (2008) who found that the administration of prednisolone subcutaneously at doses 12.5, 25 and 50mg/kg for 4 days in rats caused increases in the number of goblet cells and mucosal thickening was apparent in the trachea and bronchi (18). The result of this study also agrees with Barnes (2000) who found that the inhaled corticosteroids also improved epithelia morphology and reduced epithelial cell activation with some degree of reversal airway remodeling (19). While our results disagree with Keeley et al (1987) who found that corticosteroid treatment decreases connective tissue synthesis after administration of dexamethasone in rats, this disagreement may be due to the difference in species (20).

The histological findings of 3 weeks combination of methylprednisolone and salbutamol group did not greatly differ from control group. In spite of that, some locations demonstrated areas of variations where epithelial lining appeared to consist of superficial columnar cells and basal cuboidal cells. The underlying lamina propria showed

reduction in the inflammatory cells when compared with the normal group but at the same time it contained many congested blood vessels. The administration of the two drugs for six weeks caused a pronounced increase in goblet cells, the nucleus of which seemed to be located centrally in most of them. The underlying lamina propria showed the budding of much new small blood vessels especially next to the basement membrane with fewer inflammatory cells when compared with control group. These results agreed with the study of Stewart et al, (1997) in which they found that early treatment with steroids in combination with β_2 -adrenoreceptor agonists may be highly beneficial in that this approach may prevent or reduce the extent of airway wall remodeling by limiting inflammation at an early stage (8). The results also agreed with the study of Tse et al., (2003) who found that β_2 -agonists alone may increase eosinophil survival, whereas corticosteroids reduce survival though opposing effects on apoptosis (21). Similarly β_2 -agonists may increase the late response to allergen and the number of eosinophils recruited into the airways, whereas corticosteroids have an apoptosis effect. Thus corticosteroids have the capacity to prevent any potentially adverse inflammatory consequences of chronic β_2 -agonist therapy. On the other hand, Orsida et al (2001) showed that the long-term benefits on combination therapy had beneficial effects on the structural changes in the airway that occur in chronic asthma. One of these changes is an increase in airway blood vessels and the combination therapy significantly reduced the number of blood vessels in the airway mucosa (22).

Conclusion

Salbutamol and methylprednisolone produced many histological changes in the trachea and the combination of the two drugs limited these changes.

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New Diaz Coupling Reactions for Visible Spectrophotometric Determination of Thymol in Pharmaceutical Preparations with phenylenediamine as the coupling reagent

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ABSTRACT

The purpose behind the present study is to develop new, simple, cheap, fast, accurate, and sensitive colorimetric methods that can be used for the determination of thymol in drug in pure form as well as in pharmaceutical formulations. The methods are based on the reaction phenylenediamine with nitrite in acid medium to form diazonium ion, which is coupled with thymol in basic medium to form azo dyes, showing yellow color and absorption maxima at 467nm. Beer's law is obeyed in the concentration of 0.5-7 µg/ml. The molar absorptivity and Sandell's sensitivity is 1.438x10⁴ L mole⁻¹ cm⁻¹, 0.0095 µgcm⁻². The optimum reaction conditions and other analytical parameters were evaluated. The methods were successfully applied to the determination of thymol in pharmaceutical formulations.

Keywords: Determination, thymol, phenylenediamine, Coupling Reactions

Introduction

Thymol use Antimicrobial preservative; antiseptic. Thymol is 5-methyl-2-(methylethyl) phenol. It is colorless crystals with an aromatic odour; melting point, about 50° (1, 2). The Ancient Egyptians used thymol and carvacrol in the form of a preparation from the thyme plant, because of their ability to preserve mummies. Thymus oil is derived from thyme (*Thymus vulgaris* L.), which is popular for respiratory disorders and there is good evidence on its efficacy. Other pharmacological activities such as anti-inflammatory, antimicrobial, antiviral and antioxidant effects were also reported (59). Thyme essential oil was characterized by the presence of terpinene (4.3%), p-cymene (23.5%), carvacrol (2.2%), and thymol (63.6%), which composed 93.6% of the total oil (3-5). In literature, there are many ways to determine thymol by different analytical techniques, such as liquid chromatography(6), Spectrophotometric method(7-9) and titrimetric method(10). Claudia et al. (11) used solid-phase microextraction-gas chromatography technique to determination thymol and some volatile compounds in human plasma, automated headspace with a limit of quantitation (LOQ) of 8.1ng/ml. The purpose of the present study is to describe the simple Spectrophotometric analytical methods for determining, thymol by using phenylenediamine as the coupling reagent.

Experiment

Chemicals and Reagents

All chemicals used were of analytical reagent grade and double distilled water was used for preparing the reagent solutions. Thymol standard material was provided from Drug Industries and Medical appliances-(SDI) in Sammara, Iraq. It was of (99% purity) and obtained from (BDH) and standard solution of 100 ppm thymol which was freshly prepared by dissolving 0.01gm of Thymol with distilled water to 100 ml. A standard solution of 100 µgml of phenylenediamine (99.8% purity) was freshly prepared by dissolving 0.01gm of phenylenediamine in 20ml absolute ethanol and then diluted with distilled water to 100 ml, Sodium nitrite (99.8% purity) and stander solution of 1% was prepared. [1M] Sodium carbonate of (98% purity) was prepared, 100 ppm of various interferences and [1M] of HCl, sulfuric acid, acetic acid, nitric acid, phosphoric acid, sodium hydroxide, potassium hydroxide, potassium carbonate and ammonium hydroxide were used.

Equipment:

All spectral and absorbance measurements were done by using a Computerized UV-Visible, Shimadzu T60U Spectrophotometer, with 1cm matched quartz cells.

General procedure:

Aliquots of the working standard solution of thymol (1-10 µg/ml) were transferred into a series of 10 ml calibrated flasks. For all the methods 0.5 ml of 1% sodium nitrite and 0.5 ml of 1M HCl were added and the solutions were shaken thoroughly for 5 minutes to allow the diazotization reaction to go to completion. Add 0.2 ml of 1% sulfamic acid, stir the solutions for 5 min and add 0.6 ml of 100 µg/ml phenylenediamine ester. Then add 0.6 ml of 1M sodium carbonate solution and the contents were diluted to 10 ml using double distilled water and mixed well. After 5 minutes, the absorbance of the yellow azo dyes was measured at 467 nm against the reagent blank.

Procedure Assay of mouth washing solution Lastarim antiseptic

The average concentration of thymol in Lastarim antiseptic drug container was (0.06gm in 100 ml) so, 40 ml from the container was dissolved in distilled water into a 100 ml volumetric flask, and finally diluted to the marked level with distilled water to obtain 240. µg ml⁻¹. Further appropriate solution (100 µg ml⁻¹) was made by using distilled water. Two different concentrations of this mouth washing solution were analyzed in five replicate by analytical spectrophotometric procedures.

Results and Discussion

Spectral characteristics

Method was involving the diazotization of phenylenediamine, followed by the coupling with thymol in alkaline medium. The absorption spectra of the yellow azo dyes formed have absorption maxima at 467 nm. Figure 1 shows the spectra of yellow product formed against the reagent blank. So, the maximum absorption at 467 nm is used in all subsequent experiments.

Study of the Optimum Reaction Conditions

The effect of various parameters on the absorption intensity of the dye formed was studied and the reaction conditions are optimized.

Effect of acidity on diazotization

The effect of acidity on the diazotization reaction was studied. 0.5 ml of Varian acids, such as HCl, HNO₃, H₂SO₄, CH₃COOH and H₃PO₄ were examined. It was found HCl gave the maximum absorbance. The range 0.1-1ml of [1M] HCl was examined. The minimum time required for diazotization was 5 minutes. Diazotization was carried out at room temperature and the optimum acidity for the formation of diazonium ion was fixed to 0.5ml of [1M] HCl.

Effect of sodium nitrite

The optimum concentration of sodium nitrite solution was found to be 0.3 ml of 1% solution of sodium nitrite.

Effect of Base:

It was found that the presence of a base led to increase the intensity of the produced product, therefore some bases such

as NaOH, NH₄OH, KOH, K₂CO₃ and Na₂CO₃ are examined and it was found that Na₂CO₃ gave maximum absorbance intensity, so, Na₂CO₃ was selected. The effect of Varian volumes from 0.2-0.9 ml of [1M] sodium hydroxide solutions was examined. The investigations showed that 0.6 ml of sodium hydroxide gave high sensitivity and was selected in subsequent experiments.

Effect of coupling agent

The effect of varying the concentration of coupling reagent was studied using the proposed procedure and adding 0.1-1.0 ml of 100 ppm of phenylenediamine to a series of drug solutions. It was found that maximum and stable color was formed with 0.6 ml of phenylenediamine 100 ppm solution in final volume of 10 ml.

Calibration Graph

Employing the conditions described in the procedure, a linear calibration graph for thymol is obtained (Figure 2), which shows that Beer's law is obeyed over the concentration range of 0.5-10 µg.ml⁻¹ with correlation coefficient of 0.9991 and an intercept of 0.0146. The conditional molar absorptivity and Sandell's sensitivity of the yellow product formed was found to be 1.438x10⁴ L.mol⁻¹.cm⁻¹, 0.0095 µgcm⁻² respectively.

Effect of Reaction Time:

The colour intensity reached its maximum after the drug thymol had been reacted immediately in the presence of sodium hydroxide with diazotization solutions of phenylenediamine and became stable after 5 minutes.

Therefore, 5 minutes development time was selected as optimum in the general procedure. The colour obtained was stable for at least 24 hours.

Effect of Order of Addition

To obtain optimum results, the procedure of order addition of chemical on the colour intensity is given and explained in Table 1.

Table 1 shows that order addition of No.2 gave high color intensity of azo dye, otherwise a loss in colour intensity was observed. So the procedure No.2 was selected as optimum in the general procedure.

Effect of Temperature

The resulting product of the proposed method was studied at different temperatures. The results indicate that the absorbance values remain constant in the temperature range 0-45°C, whereas, at higher temperatures the absorbance value decreases, indicating the dissociation of the product on prolonged heating. The coloured product was stable for more than 24 hours at room temperature (25°C). Therefore room temperature is selected in this method.

Effect of Organic solvents

The effect of organic solvents such as ether, chloroform, benzene, acetonitrile, ethanol, methanol and distilled water

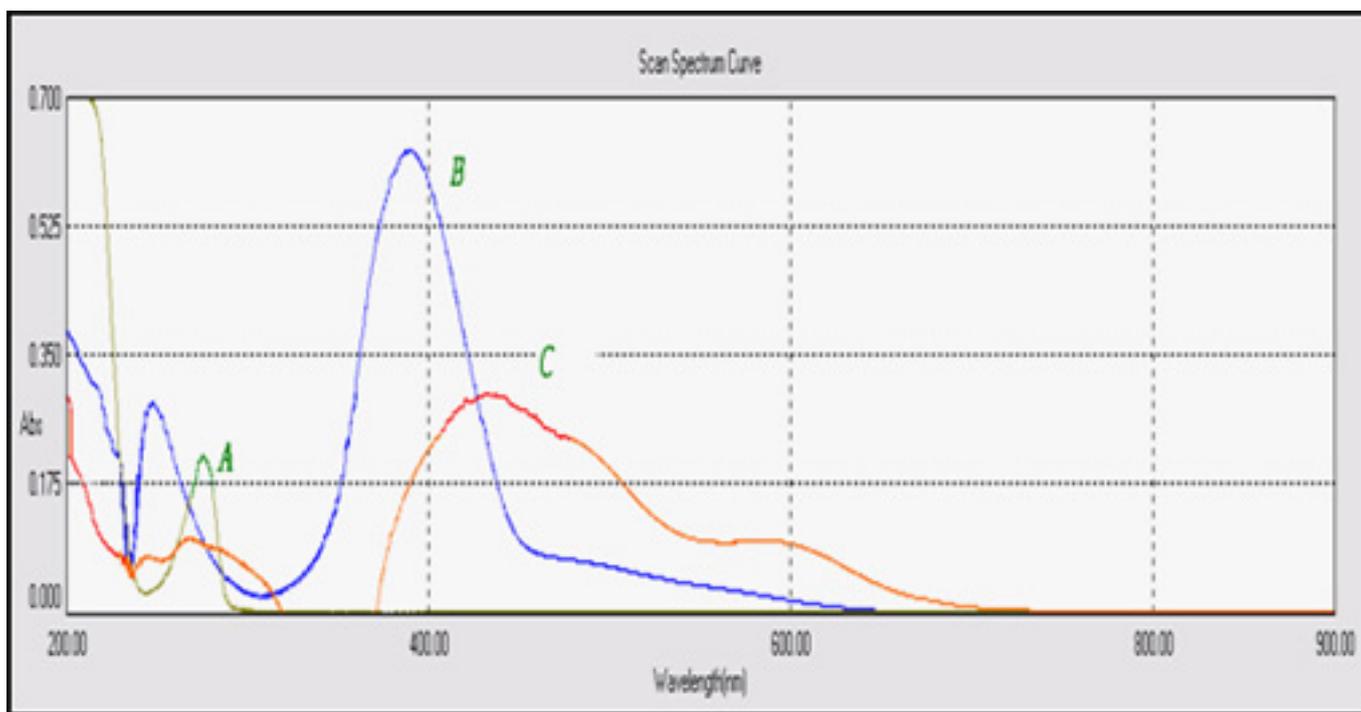


Figure 1: (A) Absorption spectrum of thymol, (B) Absorption spectrum of phenylenediamine, (C) Absorption spectrum of the diazo dye formed

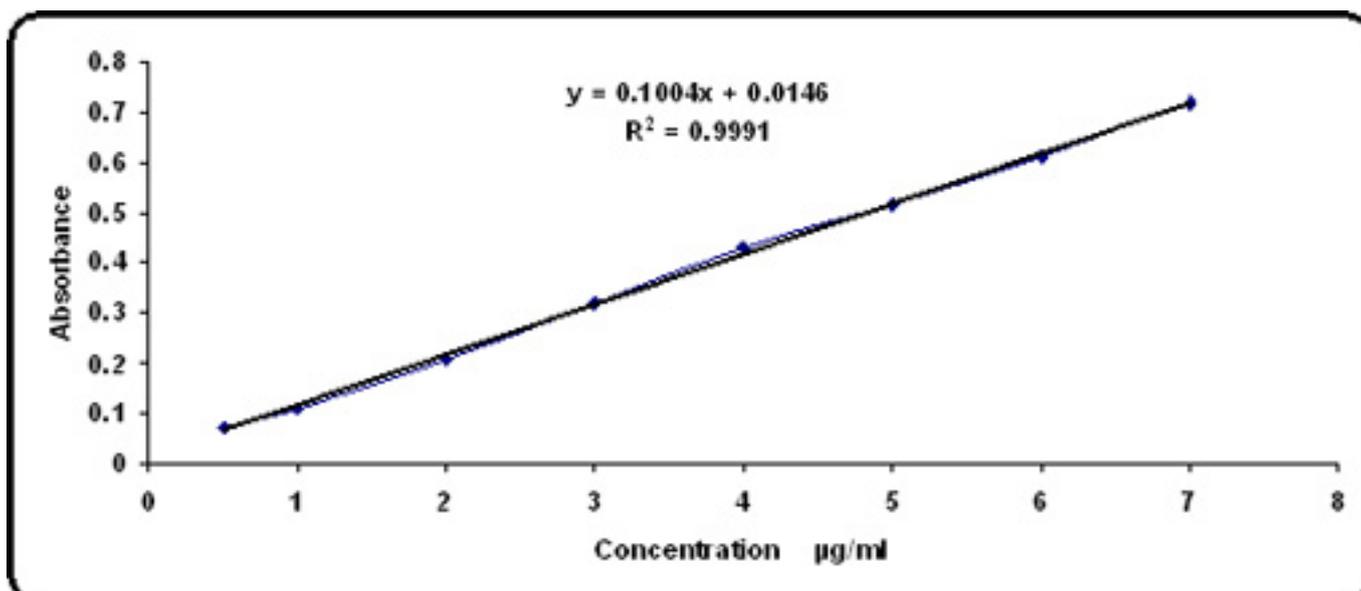


Figure 2: Calibration graph of thymol

No.	Order of addition	Absorbance
1	R+H+N+F+T+ B	0.634
2*	T+H+N +F+R+B	0.777
3	T+H+N+T+B+F	0.734
4	T+H+N+F +B+R	0.763

2* : Thymol(T), HCl(H), NaNO₂(N), Sulphamic acid(F), Reagent (R), NaOH(B)

Table 1: The effect of order addition on absorption intensity of products color

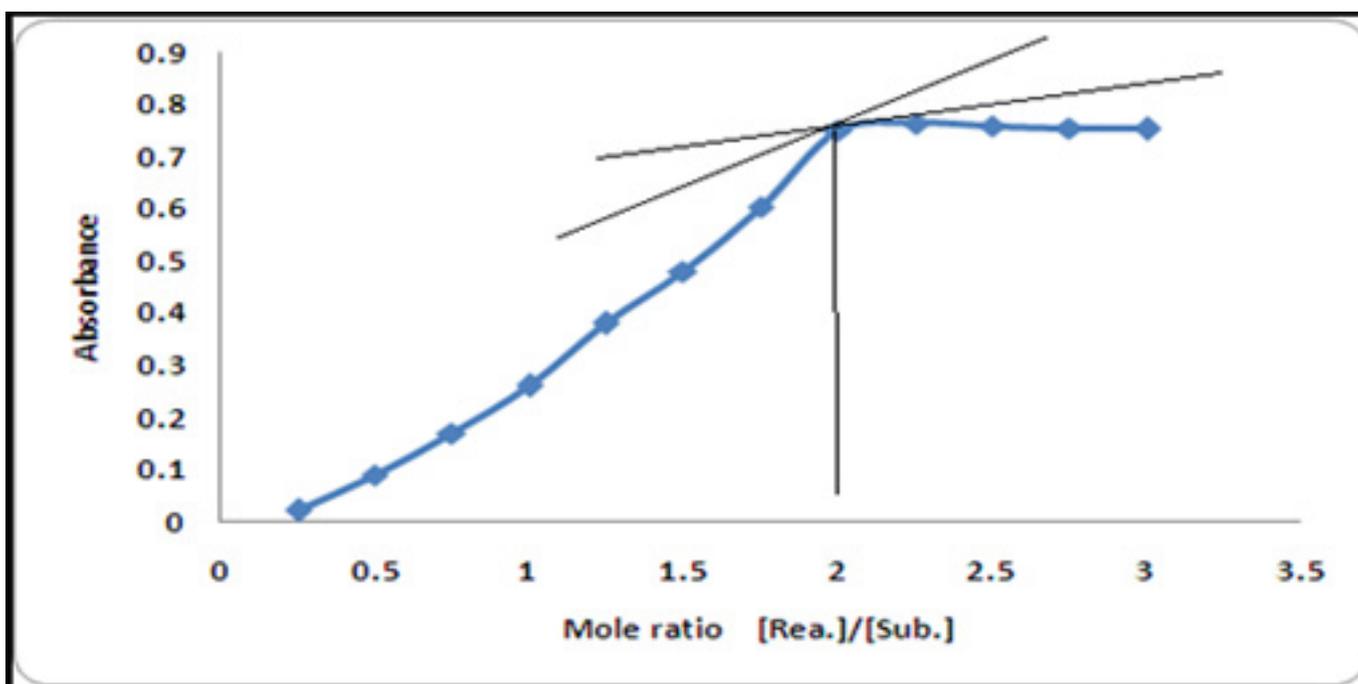


Figure 3: Molar ratio method of Thymol with phenylenediamine

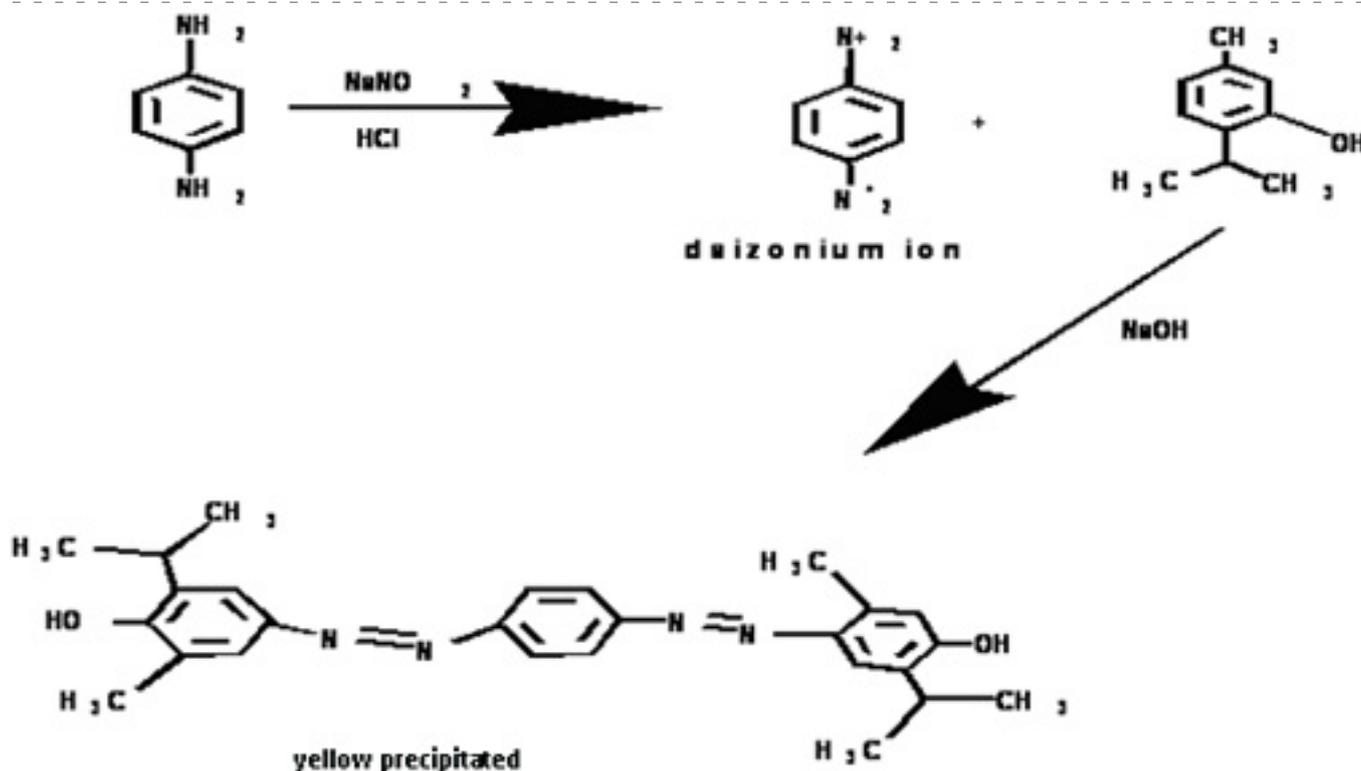


Figure 4. Probable product formation of diazo dye

Thymol Taken $\mu\text{g/ml}$	Thymol found $\mu\text{g/ml}$	* Recovery % Rec%	Average recovery % Rec%	% E Relative Standard error	Average Relative Standard error %E	Relative Standard Deviation* RSD%
5	4.95	99.0	99.9	1	0.96	0.646
6	6.08	101.3		1.33		0.771
7	6.96	99.4		0.57		0.555

* Average of five determinations

Table 2: Accuracy and precision of proposed methods

thymol Sample	Thymol µg/ml		Recovery%	Average recovery%	Relative Standard Deviation [‡] %
	Taken	Found	Rec%	Rec%	RSD%
Mouth wash ^a Listerine	5	4.91	98.2	99.9	0.56
	6	6.13	100.2		0.455
	7	7.11	101.5		0.382

* Mean of five determinations.

a. Marketed by India.

Table 3: Application of the proposed method and pharmaceutical preparations for determination of thymol

were studied by using them in the dilution and measuring the absorbance which was found to be 0.0251, 0.002, 0.013, 0.053, 0.129, 0.235 and 0.589 respectively. We used distilled water because it had the best absorbance for its abundance.

Effect of Interference

The effect of foreign species of organic compounds were studied by adding different amounts of species such as toluene benzoic acid, lactose, fructose, starch, sodium carbonate and sodium acetate, then measuring the absorbance of a dye solution containing 1 ml of 100 ppm of thymol. The color was developed following the recommended procedure described earlier. The results showed that benzoic acid of the common compound does interfere. It was observed that the lactose, fructose, starch, sodium carbonate and sodium acetate were not interfering with the determination at levels found in dosage form.

Structure of the Dye

The stoichiometry of the reaction between Thymol and phenylenediamine was investigated using mole ratio method (12, 13); the results obtained (Figure 4) shows that 1:2 drug to reagent was formed at 463 nm.

The product formed was water soluble, the stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amount of thymol and phenylenediamine. The average conditional stability constant of the dye in water under the described experimental conditions was 1.155×10^4 .

Precision and Accuracy

To evaluate the accuracy and precision of the methods of pure drug analyzed, each determination was repeated five times at three different concentrations.

The results shown in Table 2 indicate that satisfactory precision and accuracy could be attained with the proposed method. The RE (%) and RSD (%) values were less than 1% which indicates the high accuracy.

Analytical application

The proposed method has been used of Listerine drug containing thymol (Mouth wash) and they gave good accuracy and precision as shown in Table 3.

Conclusions

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing thymol showed no interference from the common excipients. The proposed method does not require temperature control or the solvent extraction step. Hence, these methods could be considered for the determination of thymol in the quality control laboratories.

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Type of method	Reagent	Colour of the dye	λ_{\max} nm	ϵ , L mole ⁻¹ cm ⁻¹	Linear range $\mu\text{g.ml}^{-1}$	RSD (%)	Ref.
Colorimetric	SNP ⁻ -NH ₂ OH	green	700	2.7×10 ⁴	0.1 – 14	<1	14
Colorimetric	N,N –diethyl-p-phenylen diamine hydrochloride		605	1.9x 10 ³	0.4-11	<1.5	15
Spectrophotometric	phenylenediamine	Yellow	467	1.438x10 ⁴	0.5-7	<1	Proposed methods

* SNP : Sodium nitroprusside

Table 4: Comparison of thymol determination in the proposed method and other literature methods

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Pregnancy outcome with fibroid uterus

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Introduction

Uterine fibroids are the most common female genital tumors(1). These common tumors are clinically apparent in 20% women of reproductive age and may be present in as many as 70% of uteri removed at hysterectomy. Their incidence is increased in women of Afro-Caribbean origin(2). The incidence is decreased with prolonged use of the oral contraceptive pill as well as with increasing numbers of term pregnancies(3).

Approximately 50% of fibroids are asymptomatic and it is unclear as to why some do produce symptoms and others do not(4). Association of uterine fibroid and pregnancy is not very common. It has been observed that 1-4% of pregnancies are associated with fibroid(5). Overall, 80% of uterine fibroids observed during pregnancy remain the same size or become smaller during the course of pregnancy(6).

Fibroid have been associated with various complications during pregnancy like abortion, preterm labor, abdominal pain due to generation, abruption, intrauterine growth restriction, obstructed labor and postpartum hemorrhage(7). Much of this opinion is based on a small series of case reports published. Here we present a series of thirty six cases of pregnancy with fibroids in our area in patients attending this hospital in the north of Jordan.

Methods

This is a prospective study of pregnancy outcome in 36 women with uterine fibroid. The study was conducted between the 1st of August 2008 and the end of July 2011 at Prince Rashid Bin-Al-Hassan Military hospital in the north of Jordan. During this period a total of 18,450 births took place at this hospital. All cases were from a local geographically defined obstetric population.

Maternal demographics, obstetrical and medical history, delivery outcome and complications were evaluated. After the diagnosis, all women were advised bed rest. Iron supplementation and folic acid were also given. Pregnancy and the fibroid were monitored periodically, both clinically and by ultrasound.

Also perinatal outcome like LBW, IUGR, preterm baby, congenital malformations and perinatal mortality were documented for each patient. All neonate deliveries between 28 and 37 weeks were considered as preterm and neonates less than 2.5 kg were taken as low birth weight.

ABSTRACT

Objectives: To examine the risk of adverse pregnancy outcomes in pregnant women with uterine fibroid and the effect of pregnancy on fibroid.

Methods: This is a prospective study of pregnancy outcome in 36 women with uterine fibroid during a period of three years between August 2008 and July 2011 at Prince Rashid Bin-Al-Hassan Military Hospital, Irbid, Jordan.

Results: During the study period (2008-2011), there were 18,450 hospital deliveries, and 36 women with uterine fibroid delivered during this period. The age range was between 18 and 45 years. Twelve women had uterine fibroids diagnosed before pregnancy. In seventeen women, the fibroid was diagnosed for the first time by routine antenatal ultrasound. In seven women it was detected only during cesarean section. Also there were fifteen women who had postpartum hemorrhage.

Conclusion: Pregnancy with uterine fibroids results in poor outcome.

Key words: Uterine fibroid, pregnancy, outcome

Results

During the study period (2008-2011), there were 18,450 hospital deliveries, and 36 women with uterine fibroid delivered during this period, and Table I shows the distribution of characteristics of mothers with respect to their age and period of gestation at time of diagnosis. The mean age for sampled patients was 27.5 years, with a standard deviation of 5.1 years. The age range was between 18 and 45 years.

Period of gestation at the time of diagnosis is also shown in Table 1. Twelve women had uterine fibroids diagnosed before pregnancy. In seventeen women, the fibroid was diagnosed for the first time by routine antenatal ultrasound. In seven women it was detected only during cesarean section.

Regardless of mode of delivery, 19 women delivered normally with 3 women with forceps delivery and 14 women delivered by cesarean section.

Complications that occurred to pregnant women with uterine fibroid are shown in Table 2. Four women aborted before 20 weeks; also there were fifteen women who had postpartum hemorrhage and eight women had preterm labor. There was one stillbirth due to prematurity.

Discussion

Uterine fibroids are observed in pregnancy more frequently now than in the past because many women are delaying child bearing till their late thirties, which is the time for greatest risk of fibroid growth. Also the use of ultrasonography has improved the diagnostic capability of detecting small fibroid and has increased our knowledge of fibroid in pregnancy(8).

Presence of fibroid during pregnancy is potentially a serious problem. However, to date, the extent to which leiomyoma affects pregnancy is not well established and assessments of the magnitude of pregnancy complications remain inconsistent(9). Though in some cases, it does not affect the outcome of pregnancy, in many cases it leads to problems like abortion, preterm labor, PPROM, postpartum hemorrhage, and obstructed labor(10). Fibroid in close proximity to the placenta are more likely to be associated with early pregnancy loss.

Uterine leiomyoma commonly affects women in their childbearing years. The mean age for sampled patients in our study was 27.5 years which is comparable to other studies(11,12). In most of our patients, the fibroid was diagnosed for the first time by routine antenatal ultrasound. In seven women it was detected only during cesarean section. Therefore, in contrast to prior studies that may underestimate the prevalence of fibroids(13), in this study, most of the pregnant women received early routine ultrasonographic screenings for uterine fibroid. The timing of the ultrasound screening might be an issue because fibroids could be difficult to detect during the second and third trimesters when the placenta and fetus were enlarging(14).

Regarding mode of delivery, we found no effect of uterine fibroid in the mode of delivery except for obstetric indications

or for women with large fibroid which had higher rates of cesarean delivery before onset of labor as seen by Vergani et al(15).

Pregnancy along with a fibroid is a high risk pregnancy which may lead to complications with unequal gravity. As seen in our study, there is increased incidence of postpartum hemorrhage along with associated complication of anemia, which was consistent with studies by Noor et al(16). Also our results were in line with prior studies by Coronado et al(17) and Qidwai et al(18), which consistently found that women with uterine fibroid were at an increased risk for preterm infants.

Our data is too small to draw any conclusions on the effect of fibroid on pregnancy or vice-versa. But the majority of the authors agree that mean gestational age at delivery is significantly lower in patients with fibroid uterus(19).

Conclusion

We conclude that pregnancy with fibroid results in poor outcome, so we suggest that clinicians intensively monitor women with uterine leiomyoma during pregnancy.

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Characteristics	Numbers
Age (years)	
18-25	11
26-35	14
36-45	9
Period of gestation (weeks)	
< 13	6
14-20	7
25-36	4
>36	7

Table 1: Age and period of gestation at diagnosis

Complications	Number
Abortion before 20 weeks	4
Antepartum hemorrhage	5
Postpartum hemorrhage	15
Preterm delivery	8
PPROM	7
Stillbirth	1
IUGR	2
Low birth weight	6

Table 2: Complications in 36 pregnant women with fibroid

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Evisceration in Federal Medical Centre, Birnin Kebbi, Nigeria

ABSTRACT

Background: To determine pattern of evisceration at Federal Medical Centre, Birnin Kebbi, Nigeria.

Materials and Methods: A retrospective review of case files of all patients who underwent evisceration in the Eye Unit of Federal Medical Center, Birnin Kebbi, Nigeria from January 2004 - December 2011. The following information was extracted: biodata, indication for evisceration, eye affected and complication of the surgery.

Results: There were 22 males and 8 females with M:F ratio 2.8:1. The mean age of the study population was 36.87years (SD 21.2), with age range of 5-80 years. The commonest indication for evisceration was intraocular infection 14 (46.7%), followed by ocular trauma 11 (36.7%). Evisceration was common among the middle age group (41-50 years). Ocular trauma accounted for the highest number 11 (36.7%) of all indications for evisceration in age group less than 20 years. Ocular trauma in both children and artisans occurred only in male patients.

Conclusion: Intraocular infection and ocular trauma were the main indications for evisceration at Federal Medical Centre, Birnin Kebbi. Ocular trauma accounted for the highest number of all indications for evisceration in age group less than 20 years. The need to prevent ocular trauma among children in order to reduce the magnitude of evisceration is underscored.

Keywords: Evisceration, Birnin Kebbi, Ocular trauma, eye infections

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Introduction

Evisceration is a procedure in which the intraocular contents are removed while the sclera, tenon's capsule, conjunctiva, and optic nerve are preserved.(1) Evisceration is one of the commonest ocular destructive surgeries performed worldwide. Von Graefe first advocated the use of evisceration in the presence of severe endophthalmitis as a means of preventing intracranial spread of infection. (2) Over the year's indication for evisceration has expanded to include both infectious and non-infectious intraocular inflammation resulting in total loss of vision with no potential for any useful vision.(3)

The demographics and indications for evisceration in Africa are likely to differ significantly from those in developed countries due to difficulties in prompt access to ocular medical treatment. Evisceration has psychological, economical and social impact on both patients and their relatives. Patients commonly experience symptoms of depression after enucleation or evisceration.(4)

The practitioners need to be prepared for these symptoms and should treat or refer patients to the appropriate specialist.

Federal Medical Centre Birnin Kebbi is the largest single provider of ophthalmic care in Kebbi State. The population of Kebbi state in 2006 was estimated at 3,137,989.(5)

Knowledge of the pattern of evisceration in the eye unit of Federal Medical Centre will go a long way in improving eye care delivery in Kebbi State, Nigeria.

Materials and Method

This was a retrospective study over an 8 year period (1st January 2004 to 30th December 2011). It involved all consecutive patients who had evisceration at the Eye Unit of Federal Medical Centre Birnin Kebbi. The chart review was performed of patients who had evisceration using eye clinic emergency register, ward admissions register and theater operations register of the Eye Unit. The following information was extracted: biodata, indication for evisceration, eye affected, treatment offered before presenting at the hospital and complications following evisceration. Excluded are the patients who previously had eviscerations from the referral hospital. The data was entered and analyzed by SPSS 16.0 statistical software package.

The Ethical Approval to carry out this study was obtained from Research Ethical Committee of Federal Medical Centre, Birnin Kebbi, Nigeria.

Results

A total of 30 patients had evisceration within the study period. There were 22 males and 8 females with M: F ratio 2.8:1. The mean age of the study population was 36.87 years (SD 21.2), with range of 5-80 years (Table 1). The left eye was eviscerated in 20 out of 30 eyes.

The commonest indication for evisceration was intraocular infection 14 (46.7%), followed by trauma 11 (36.7%). Evisceration was common among the middle age group (41-50 years). Ocular trauma accounted for the highest number of indications for evisceration in age group less than 20 years (Table 2). All the 4 children less than 4 years old in our study had their eyes eviscerated due to severe irreparable penetrating eye injuries.

Ocular trauma was the indication for evisceration in all the artisans. Ocular trauma in both children and artisans occurred only in males. Intraocular infection was the commonest indication for evisceration among farmers (57.1%), followed by housewives (28.6%) (Figure1).

Postoperatively, one patient developed severe scleritis and extrusion of ocular implants. As many as 46.7% of the patients used traditional eye medication (TEM) and 40% used eye drops purchased from the counter before presenting at the hospital for evisceration. One patient had evisceration on account of panophthalmitis developed after ocular surgery (following ocular trauma) that was done at the referral hospital. Only 10% of the study patients accepted an artificial eye after evisceration.

Discussion

Intraocular infection and trauma accounts for 83.3% of indications for evisceration in our study. This was in agreement with other reports from developing countries. (6, 7) In a northern Indian study, 78.6% of all eviscerated eyes were reported to be due to panophthalmitis while in another 21.3% were due to irreparable globe injury.(2) The percentage of infection responsible for evisceration in our

study is higher than what was reported by Nwosu from Onitsha, Nigeria.(8) This may probably be due to a high number of patients with infective ocular condition attending our hospital. The high incidence of infective causes in our study probably reflects the low socioeconomic condition of the people and poor access to eye care in the community. The bad effect of traditional eye medication (TEM) on the cornea is well documented worldwide.(8, 9) Our study reported 46.7% of the patients used TEM and 40% used eye drops purchased from the counter before presenting at hospital for evisceration. This was slightly different from a previous study from Onitsha(8) where 37.5% of their patients had used traditional eye medications (TEM) while another 53.5% had used eye drops (purchased over the counter). In a rural Indian study investigating the role of TEM in the management of corneal ulcers, the authors reported that 47.7% of the studied patients had applied TEM prior to presenting at the eye centre.(9) Intraocular infection was the commonest indication for evisceration among farmers (57.1%) in our study. This could probably be due to socioeconomic status and TEM uses in these group. Severe irreparable penetrating eye injuries were responsible for evisceration in all the children less than 4 years. This was quite disturbing and could be due to poor supervision of children playing at home and school. This situation needs further study. The entire trauma in both children (0-15years) and artisans occurred in male patients, highlighting the aggressive and adventurous nature of the male gender.

Limitation of the study

The limitation of this study was the fact that it was a retrospective study, however the study would be useful in improving eye health care services thereby reducing avoidable eye loss to evisceration and its consequences.

Conclusion

Intraocular infection and trauma were the main indications for evisceration in our study. Evisceration was common among the middle age group and Trauma accounts for highest number of indication for evisceration among children. Therefore there is need for preventive measures in terms of adult supervision of children at play and public enlightenment on ocular effect of trauma.

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Age (years)	Gender		Total
	Male (%)	Female (%)	
0-10	5 (16.7)	1 (3.3)	6 (20.0)
11-20	1 (3.3)	2 (6.7)	3 (10.0)
21-30	3 (10.0)	0 (0.0)	3 (10.0)
31-40	3 (10.0)	1 (3.3)	4 (13.3)
41-50	4 (13.3)	3 (10.0)	7 (23.3)
51-60	3 (10.0)	0 (0.0)	3 (10.0)
61-70	2 (6.7)	1 (3.3)	3 (10.0)
71-80	1 (3.3)	0 (0.0)	1 (3.3)
Total	22 (73.3)	8 (26.7)	30 (100.0)

Table 1: Age and Sex distribution

Age (years)	Indications for Evisceration				Total
	Trauma	Intraocular infection	Painful blind eye	Staphyloma	
0-10	5	0	0	1	6
11-20	2	0	0	1	3
21-30	1	2	0	0	3
31-40	1	0	2	1	4
41-50	0	7	0	0	7
51-60	2	1	0	0	3
61-70	0	3	0	0	3
71-80	0	1	0	0	1
Total	11	14	2	3	30

Table 2: Indications for evisceration by age

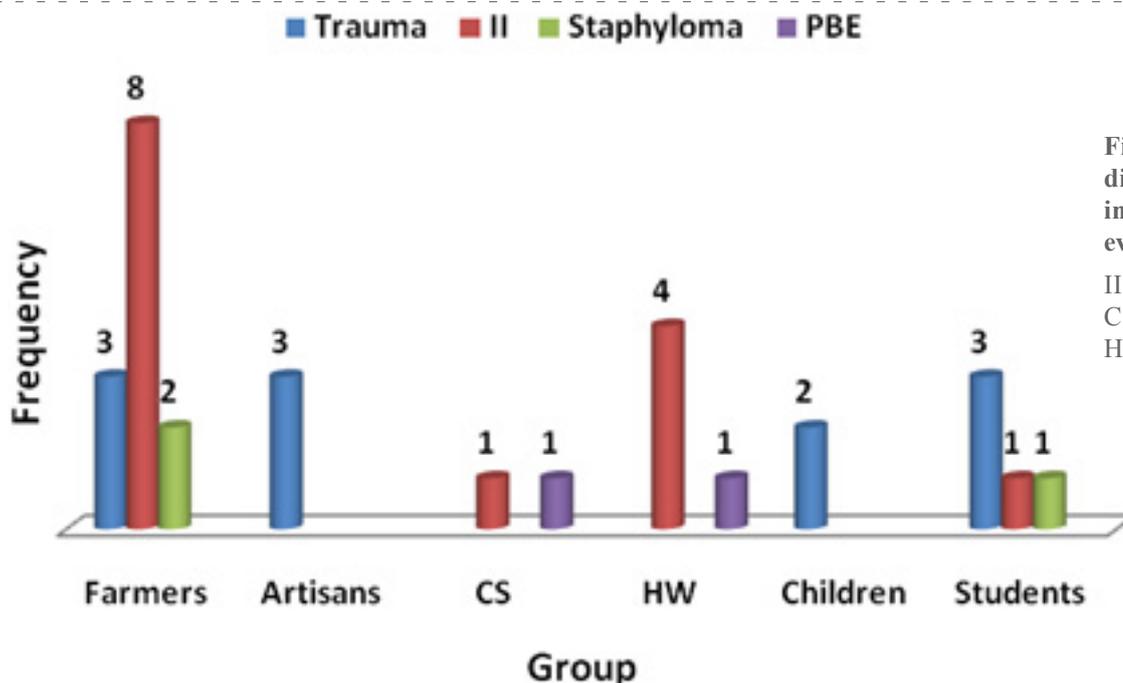


Figure 1: Group distribution of indications for evisceration

II, intraocular infection; CS, civil servant; HW, housewives

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Intrauterine Contraceptive Device with lost strings : A case report

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ABSTRACT

Intrauterine devices are an increasingly popular method of contraception used worldwide. Currently Copper -T 380A is the preferred intrauterine device (IUD) used in Oman. It is reversible, long acting, convenient method of birth spacing and acts immediately. It provides ten years protection against pregnancy and does not interfere with intercourse and there is immediate return to fertility on removal. This method is suitable for lactating mothers as there is no effect on the quality and composition of breast milk. It is also used as an emergency contraceptive method. Effective counseling is needed for acceptance and continuation of this method.

An unusual case of missing Intrauterine contraceptive device (IUCD) strings enclosed and wrapped in a thin transparent membrane due to intrauterine infection is reported. Reporting of this case is done to highlight the risk of pelvic infection associated with this method of contraception. Pelvic infections are largely polymicrobial and mainly linked to the insertion technique of the device.

Key words: Aerobic bacteria, intrauterine device, infection, lost strings, misplaced IUCD

Introduction

The Copper T- 380A IUD looks like the letter “T”. This design has proven to be highly effective, safe and adaptable (Figure 1 - next page). Copper IUDs are made of plastic with a copper wire round the stem and copper caps on the arms making the system highly effective due to the large surface area of copper. Less the surface area of copper as seen in other IUDs, higher is the failure rate noted (Figure 2 - page 41). Mechanism of action is by preventing fertilization by causing an inflammatory or foreign body response which causes cellular or biochemical changes in the endometrium and uterine fluid. It decreases the number of sperm reaching the fallopian tube and their capacity to fertilize the egg. IUDs can be inserted any time when a woman is not pregnant.

Infection has been the main problem that women face using the IUD all over the world, besides minimal side effects like mild cramps, irregular or heavy menstrual bleeding. Very rarely, serious complications of concern can be uterine perforation, pelvic inflammatory disease predisposing to infertility and endometritis. Ghazal et al (1) reported a higher rate of isolated bacteria among IUD users compared to non users with no overall significance in relation to duration of use. In the same study 50% of the positive culture results were bacteria and 40% were potentially pathogenic. Beta hemolytic streptococci was the most frequent bacteria and Escherichia coli was the most pathogenic organism isolated.

The IUCD user should know the importance of checking the IUCD strings to be sure that the device is in place. The most common reason for missing threads is the slipping of the strings up into the cervical canal and an uncommon reason may be that the IUCD has partially or completely perforated the uterus or become embedded in the uterine wall. Limited side effects of this contraceptive method are cramps, spotting, heavy menstrual flow, infection and infertility. Complications such as perforation, heavy bleeding, abnormal spotting and smelly discharge may occur rarely. The most common infections are acute endometritis and pelvic inflammatory disease.

Case Presentation

This article presents a case involving missing strings of IUCD in a 32 years para three referred to Nizwa referral hospital from a local health centre with history of IUCD insertion two years back in a private clinic. She was not able to feel the threads since two months and there was no history of spontaneous expulsion of the device. There were no complaints of painful abdomen, dysuria, backache or menstrual irregularities. Abdominal palpation and speculum examination were normal. Endocervical and high vaginal swabs were collected for gram staining and culture testing. Cervical smears were sent for histopathological examination. Transabdominal scan showed the IUCD was placed well in position in the uterine cavity. She was informed that the

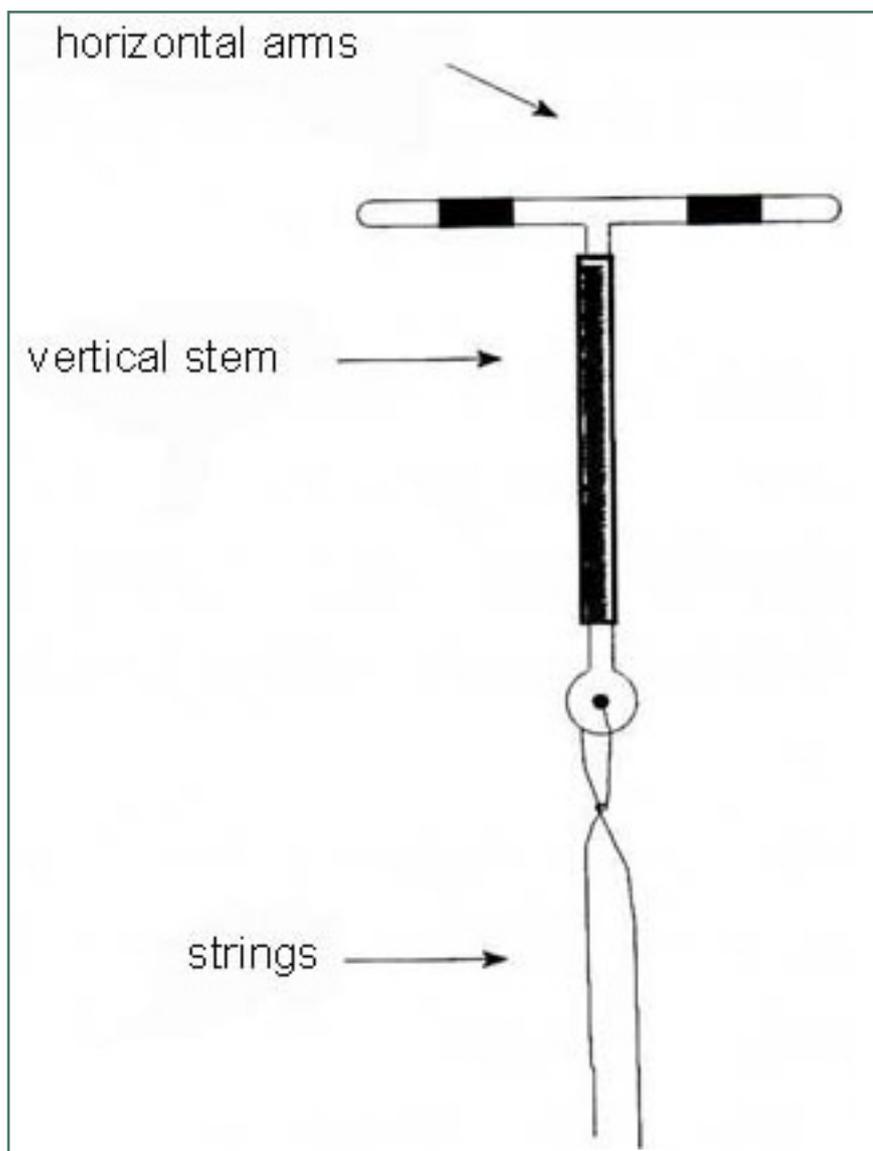


Figure 1: Copper-T 380A

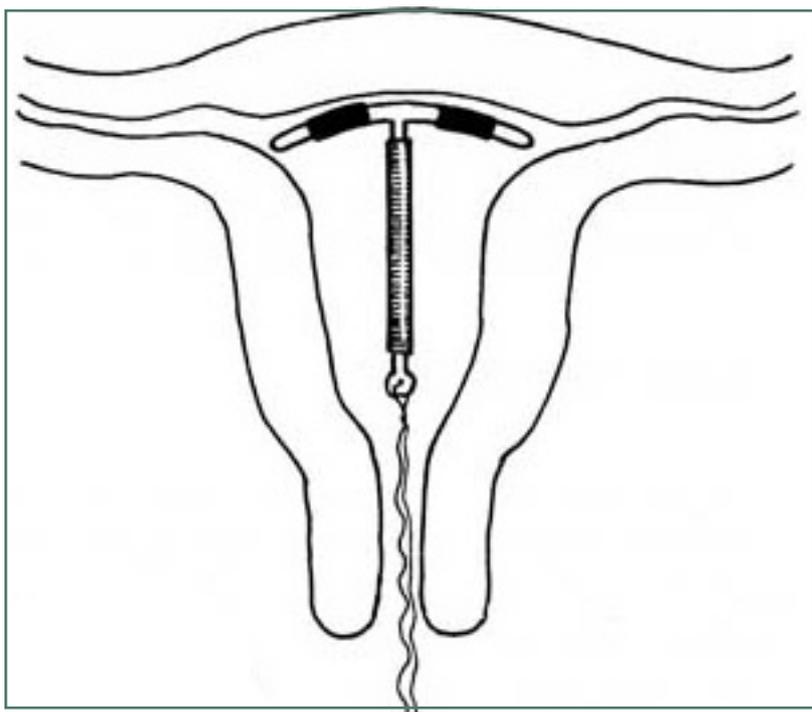


Figure 2: Copper-T 380A in uterus

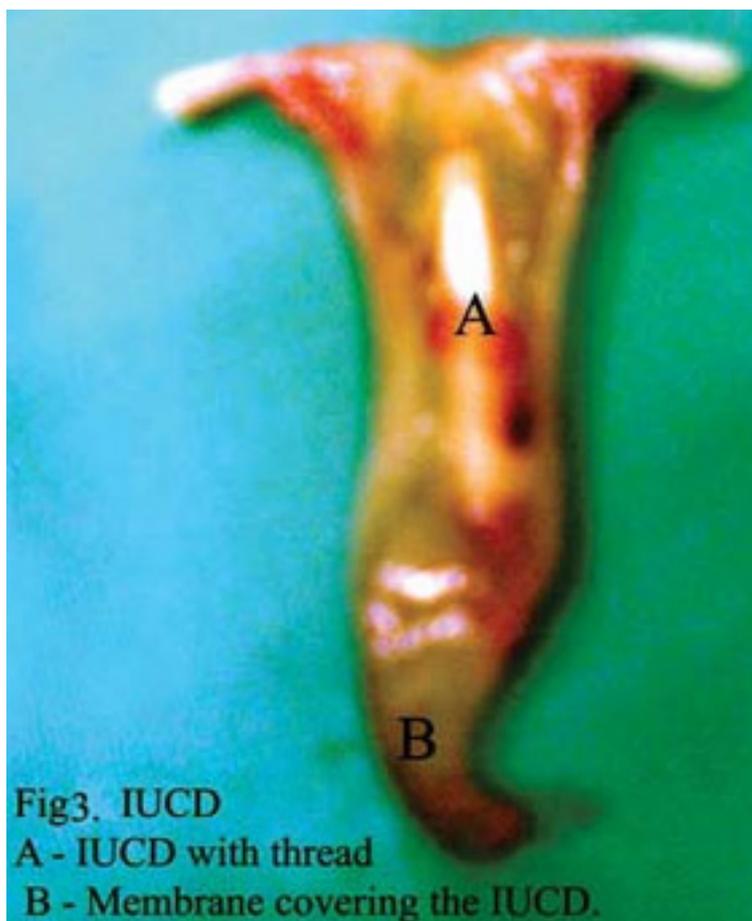


Figure 3

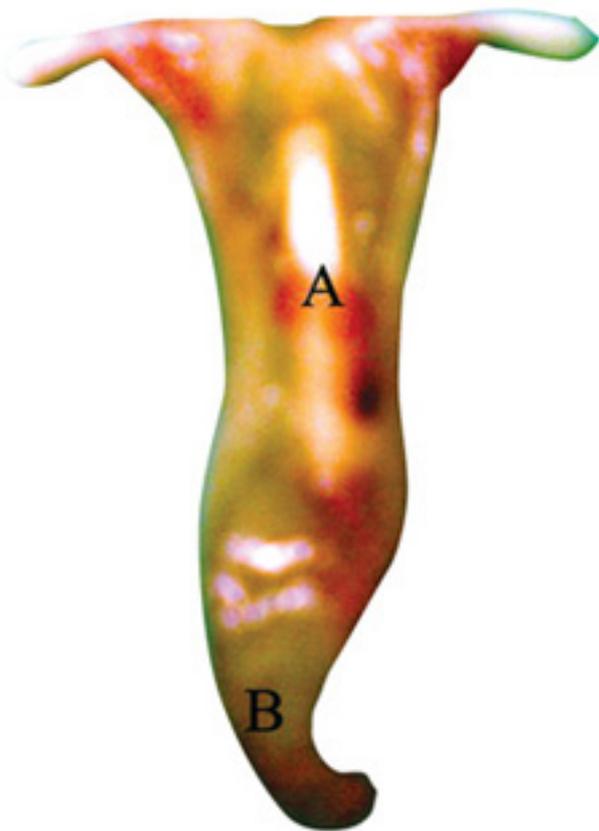


Figure 4

IUCD was not misplaced but the IUCD strings were missing. She preferred removal of the IUCD and refused continuation of this method. The device was removed under general anaesthesia by dilatation and curettage. The Copper T- 380A was surprisingly found to be covered by a transparent, gelatinous thin membrane all around and the thread was pulled inside this membrane (Figures 3, 4). This was attributed to some intrauterine infection resulting in the formation of membrane around the device. The only positive culture noted was a heavy growth of Group B Streptococcus in the vagina. The rest of all cultures from the uterine cavity and from the IUCD were found negative.

Discussion

Birth spacing is an integral part of the maternal and child health (MCH) service in Oman. IUCD is seen easily on X-ray as it is made up of molded polyethylene plastic coated with barium. A plastic thread is attached to the device which is used for locating and removal of the IUCD. IUCD insertion should be provided by trained service providers as the technique can introduce ascending infection through the vagina largely during the first year due to colonization of the device. The tail portion of the IUD which is composed of plastic monofilament surrounded by a nylon sheath may be a primary source of transmission of pathogenic microorganisms. The polypropylene fibres colonize pathogenic bacteria that are found in the human vagina and encourage bacteria to migrate into the uterine cavity. Organisms which contaminate IUDs are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Neisseria gonorrhoea*, and *Candida albicans* (2). In some studies the upward migration of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* across abraded monofilament polypropylene tailstrings of intrauterine contraceptive devices was experimentally proved(3). Different factors affecting flora of the female genital tract such as pH and estrogen depends on the female age. Aerobic and anaerobic bacteria of the female genital tract can adhere to IUD and account for most of the ascending infections. Organisms harboring the female genital tract are mainly *Streptococcus*, *Staphylococcus* and *Beta haemolytic Streptococcus*. There is a close relationship between aerobic bacteria, IUD use and bacterial vaginosis (4). A recent study showed that IUD use even increases the growth of *Trichomonas vaginalis* (5). IUD is considered as any other device or catheter to develop infections associated with foreign body. An Iraqi study found a strong association of *Klebsiella* with IUD use 14.5%, and much less association with isolation of *E-coli* and *Staphylococcus aureus* 4.35% and 8.7% respectively (6). Many surveys have revealed that 75% of the IUDs in women with reproductive tract infections were covered with microorganisms organized into biofilms (2). Biofilm is formed by the adherence of various microorganisms by their fimbriae, pilli, flagella and extracellular polymeric substance (EPS) to various surfaces like wood, glass, food products and in our case to the plastic surface of the IUCD. This biofilm is enclosed in a matrix of

polysaccharide material. This film is responsible for chronic bacterial infection and infection on medical devices as IUDs resulting in pelvic inflammatory disease(7). This could have been the same phenomenon as observed in our case.

If the strings are lost the possibilities of broken strings, withdrawal into the uterus, expulsion, uterine perforation and translocation of the device has to be explained by the provider (8). Women with misplaced IUCD may present with pregnancy (intrauterine/extrauterine), vaginal bleeding, pelvic pain or may be asymptomatic for many years (9). Ultrasound, abdominal X-ray, intra venous urogram (IVU), urine analysis, culture and sensitivity and at times CT scan of abdomen is required to confirm the correct diagnosis especially in cases where there is suspicion of perforation of the IUCD with migration into any abdominal viscus. Perforation by copper containing device can cause inflammatory reaction due to release of cytokines, and degradation of extracellular matrix caused by matrix metalloproteinase. Literature reviewed showed a number of cases with IUCD translocated to various organs but in around 60% of missing strings the device is found in the uterine cavity as in our case (10).

Conclusion

IUD use is limited due to concerns about the risk of developing reproductive tract infection. Various studies have proved higher IUD related diseases by the opportunistic pathogens in the female genital tract. There is always easy access of vaginal bacteria to the upper genital tract as part of device projects through the cervical canal.

Abbreviations: IUCD (Intrauterine contraceptive device), IUDs (Intrauterine devices), IUD (Intrauterine device)

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