



## THE RELATIONSHIP BETWEEN SEMINAL INTERLUKIN-6 (IL-6), ZINC CONCENTRATION, BLOOD HORMONAL PROFILE, AND SPERMOGRAM PARAMETERS AMONG JORDANIAN INFERTILE MALE WITH AND WITHOUT VARICOCELE

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### ABSTRACT

The potential relationship between IL-6, zinc, hormonal profiles and spermogram parameters among Jordanian infertile male in relation to varicocele was investigated. Seminal fluid and serum from 162 infertile males, including 48(29.6%) without varicocele and 114(70.4%) with varicocele divided to grade (I) 18(11.1%), grade (II) 48(29.6%), and grade (III) 48(29.6%). Routine semen analysis was performed and the level of Zn was estimated by atomic absorption. Seminal levels of IL-6 detected by Enzyme Immunoassay were analyzed and correlated to seminal fluid parameters as well as to serum hormonal profile of Estradiol (E2), testosterone (T) and prolactin (PRL) detected by competitive chemoluminescent enzyme immunoassay. A significant differences appeared in seminal fluid ( $p=0.006$ ), non progressive motility ( $p=0.05$ ), and abnormal morphology ( $p=0.046$ ) among infertility groups and subgroups. The mean concentration of Zn among infertile male with varicocele grade II showed the lowest value in comparison to grade I and III, and also in male without varicocele. Correlation was found between seminal volume ( $p=0.001$ ), leukocytes ( $p=0.001$ ), germ cells ( $p=0.064$ ), sperm tail defect ( $p=0.002$ ) and E2 level ( $p=0.01$ ) and seminal zinc level, nor between the latter and varicocele. Infertile male without varicocele exhibited a significant elevation in the mean levels of IL-6 ( $21.38 \pm 6.56$ ) than those in comparison group ( $19.40 \pm 56.5$ ), with no significant variation among varicocele subgroups. Their detectable range (6.0 to 32.0) pg/ml was negatively correlated to sperm viability and positively to seminal viscosity and serum E2 ( $p<0.05$ ). Pro-inflammatory IL-6 have no role in the pathophysiology of varicocele related infertility. However, their measurement in seminal fluid seems to be not suitable for routine infertility work.

**KEY WORDS:** IL-6, Estradiol, Testosterone, seminal fluid, varicocele, male infertility

### INTRODUCTION

Varicocele is the venous dilation of the plexus pampiniformis of the spermatic cord. The prevalence of varicocele in the general population is 15%-20%, but it is notably greater (25-40%) in infertile couples with male factor infertility (French *et al.*, 2008). The most important outcome of varicocele is impaired spermatogenesis. The mechanisms of this damage have been reported as increased scrotal temperature, backflow of renal and adrenal metabolites in the testicular vein, decreased blood flow in testicular artery, and hypoxia. However, some undefined mechanisms with respect to defective spermatogenesis in varicocele remain unclear because some patients with varicocele have normal seminal parameters and furthermore, because seminal parameters do not improve in 40-50% of the cases after surgical treatment (Sigman and Jarow, 2002). Several trace elements have been shown to be essential for testicular development and spermatogenesis (Chia *et al.*, 2000). Beside sperms formation in mammals, zinc is known to play a critical role as antioxidant defense system enzymes

(Camejo *et al.*, 2011). It is secreted into the seminal plasma by the prostate gland (Kelleher *et al.*, 2011). Many investigators have reported the possible relationship between Zn and infertility (Yuyan *et al.*, 2008). However, understanding male infertility induced by zinc deficiency is complex because Zn is thought to involve in several integrated process associated with hypothalamus-pituitary-gonadal axis. Chronic prostatitis and inflammatory conditions considerably influence the secretory function of the prostate may result in an impaired turnover and a decreased secretion of Zn. (Kruse *et al.*, 2002; Wong *et al.*, 2001). Low Zn content of semen may generally affect the semen quality in different ways. Some mechanisms include reduced antioxidant capacity (Prasad *et al.*, 2004) or counteracting the effects of other trace elements (Batra *et al.*, 2001). In patients with varicocele an elevation in ROS and depression in the total antioxidant capacity levels of the semen was reported (Hendin *et al.*, 1999).

Zn is present both in spermatozoa and in seminal plasma, with a concentration considerably higher than in the other

body fluids (Marzec-Wróblewska *et al.*, 2012). It was noticed that the seminal fluid with higher percentage of motile spermatozoa contains plasma with higher Zn concentration (Wong *et al.*, 2001). Another finding by Oluyemi *et al.* (2010) stated that a low cellular zinc level in seminal plasma is a contributing factor to reduced spermatogenesis and low cellular testosterone in infertile males. Semen from infertile male with and without varicocele appeared to contain a significant low concentration of Zn in relation to fertile male (Shquirat *et al.*, 2013). However, new studies should be done to address the importance of Zn in varicocele related infertility. The exact mechanism of impaired testicular function in patients with varicocele has not yet been found. Some of the possible mechanisms include cytokines production from which interleukins (ILs) that plays a regulatory role in testicular function (Hedger and Meinhardt, 2003). The role of cytokines as a mediator of oxidative stress (OS) is well known. A compensatory over expression of interleukins can cause increased production of reactive oxygen species (ROS) in varicocele testes, causing an inflammatory response that is detrimental to testicular tissue (Agarwal *et al.*, 2009). The levels of IL-6 and ROS in the seminal fluid have been shown to correlate significantly with varicocele (Nallella *et al.*, 2004). Under normal circumstances, cytokines produced by testis, immune cells, interstitial cells, sertoli cells, and spermatogonia cells function as intracellular signals that regulate the growth and differentiation of germ cells, reproductive neuroendocrine, testicular function, and spermatogenesis. However, these regulations are mutual, and that various cells within the reproductive system can not only produce their own cytokines, but regulate the secretion of cytokines. If cytokine production is impaired, the reproductive system functions may be damaged, which leads to male infertility (Dousset *et al.*, 1997). In-vitro, cytokines affect human sperm motility (Qian *et al.*, 2011), increase the production of reactive oxygen species by human spermatozoa (Nandipati *et al.*, 2005) and reduce the ova-penetrating ability of spermatozoa. Interleukins are part of the local defense mechanism against infectious diseases, but they are also implicated as mediators of the pathology of these diseases. Interleukin-6 (IL-6) is a pleiotropic cytokine which is produced by different types of immune and non-immune cells (Tovey *et al.*, 1988). Since the seminal plasma contains IL-6 receptors (Dousset *et al.*, 1997), that form complex with IL-6 and bind to gp130 presented in sperm which result in the impairment of sperm function. Currently, little is known about IL-6 levels in seminal plasma of men characterized according to the etiological diagnosis of infertility.

This study aims to assessing the possible relation of IL-6 in seminal plasma with sperm parameters, Zn, and blood hormonal profile in the serum among infertile male with and without varicocele.

## MATERIALS & METHODS

### Study cases

One hundred and sixty two infertile male including 48(29.6%) male without varicocele (WOV) and 114(70.4%) with clinically evident varicocele (classified as varicocele grade I (VGI), II (VGII), and III (VGIII)) were enrolled in the study. Their mean ages were  $31.30 \pm 5.2$  and  $31.04 \pm 5.9$  years, respectively. Men were attending the infertility department at Medical Hussein City Hospital in Jordan during the period from May to October 2014 with complete clinical and medical history. Varicocele was diagnosed after physical examination, duplex, and Color Doppler Ultrasonography (Yamamoto *et al.*, 1996). Grading of varicocele was done using Dubin's criteria. No history of present and past illness as well as medical and surgical treatment was taken. A questionnaire survey collected data regarding patient occupation, marital status, infertility history and other data. Patients with a history of smoking, and alcoholic consumers were excluded from this study. All laboratory tests were done with due permission of the ethical committee of the Institute and informed written consent was taken from each patient. The patients with a history of hormonal treatment in the last 6 months, any scrotal pathology such as cryptorchidism, previous scrotal surgery such as orchiopexy or varicocele ligation, abnormal serum hormone levels, abnormal liver and renal function tests, any findings of obstructive azoospermia, or a systemic disease were excluded from the study.

### Seminal Fluid Samples

Semen specimens were collected through masturbation after 3 days abstinence. Each patient provided at least two samples within one month. Samples were incubated for 30 min at  $37^{\circ}\text{C}$  for liquefaction. A routine semen analysis was performed upon liquefaction according to WHO to measure volume, pH, sperm concentration, motility, viscosity, viability and morphology (WHO, 2010). The remaining semen sample was centrifuged at  $1000 \times g$  for 10 min; the seminal plasma was separated for three equal parts and stored at  $-70^{\circ}\text{C}$  until further analyses. Morphology was determined after incubation of the sample with trypsin for 10 minutes at  $25^{\circ}\text{C}$  according to the methylene blue eosin staining procedure, feathering, and fixation by flame. At least 100 cells were examined at a final magnification of 1000x. Viscosity of the liquefied sample was estimated by introducing a glass rod into the sample and observing the thread that forms on withdrawal of the rod (Comhaire and Vermeulen, 1995). Motility was expressed as a percentage of motile spermatozoa and their mean velocity. A fixed volume of semen is delivered onto a clean glass slide and covered with an mm cover slip (WHO, 2010). The preparation is then examined at a magnification of 400x. The motility of each spermatozoon encountered is graded a, b, c, or d. At least 100 spermatozoa are classified in this way. The presence of 50% or more with forward progression (categories a & b) or 25% or more with rapid progression (category a) within 60 minutes of ejaculation were considered as normal results. The results were averaged for

the two samples, and a single value was used for each parameter. Sperm motility was calculated by multiplying sperm concentration ( $\times 10^6/\text{ml}$ ) and semen volume (ml). A leukocyte (neutrophils and macrophages) in semen was assessed by a myeloperoxidase- staining test. A seminal leukocyte concentration of  $1 \times 10^6$  WBC/ml was considered normal (Park and Lee, 1979). Assessment of sperm morphology is performed by making a thin smears of a well-mixed ejaculated semen on clean slides. After air drying, the slides were stained using Diff-Quik kit (Baxter Healthcare Corporation, Inc., McGaw Park, IL) and graded on the basis of the Kruger's Strict criteria and cutoff value established by WHO guideline. A total of 100 spermatozoa were scored per slide using bright field illumination.

#### **Determination of Seminal Fluid Fructose**

The method is adopted from that of Seliwanoff. The principle depends upon the presence of fructose (ketoses), which forms a pink color when heated, with resorcinol in the presence of hydrochloric acid (ARCOMEX, Fructose.S.F). The intensity of the red complex is proportional to the fructose concentration and measured photometrically at 490 nm (Mant, 1948). Normal fructose level in the seminal plasma is 120-500 g/dl.

#### **Determination of Zinc in Seminal plasma**

The level of seminal Zn, was determined using Atomic absorption spectrophotometer (AA 6650 Shimadzu). Frozen semen samples were liquefied at room temperature and digested in covered beakers in a fume cupboard with a 1:1 solution of ultrapure  $\text{HN}_3$  under moderate heating conditions ( $-85^\circ\text{C}$ ). All lab ware used was previously treated with 10% nitric acid for 48 hours and copiously rinsed with distilled-deionized water to eliminate possible traces of heavy metals. Semen samples were diluted 1:50. The concentrations were determined by comparison with standard curves covering different concentration ranges. Aqueous standards for plotting calibration graphs were obtained by serial dilution of stock solutions containing 1000 pg/mL of the analyte as nitrates. Blanks were prepared in a similar fashion as samples.

#### **Estimation of testosterone, estradiol, and prolactin concentration**

Serum levels of testosterone, estradiol, and prolactin were estimated by a competitive chemoluminescent enzyme immunoassay (IMMULITE 2000, Bio DPC, Los Angeles, CA, USA) which utilized specific antibody-coated polystyrene beads as a solid phase (Vankrieken, 2000; Abraham, 1977). After the sample was incubated with alkaline phosphatase-labeled reagent, the bound label was then quantified using a specific chemoluminescent substrate and light emission was detected by photomultiplier tube, and the results were calculated for each sample. A standard curve is constructed by plotting absorbance values against concentrations of standards, and the concentrations of unknown samples are determined using this standard curve. The normal ranges for testosterone is 262–1593 ng/dl, estradiol <60 pg/ml, and Prolactin 2-18 ng/ml.

#### **Interlukin-6 determination**

Before assay, seminal plasma samples were vortexed gently and centrifuged for 10 minutes at  $300 \times g$  and then filtered with a 0.22- $\mu\text{m}$  sterile Millex filter (Millipore, Billerica, MA). The concentration of IL-6 in seminal plasma was determined with commercially available ELISA kit, which employed the quantitative sandwich enzyme immunoassay technique. This assay included an amplification system in which the alkaline phosphatase reaction provided a cofactor that activated a redox cycle, leading to the formation of a colored product. The resulting color reaction was proportional to the amount of IL-6 bound in the initial step. The concentrations were read on a standard curve. Testing was performed strictly according to the manufacturer's instructions, and included negative and positive controls in each of the test series. The mean of duplicate tests was taken for analysis. Seminal plasma concentrations of 10, 15, 30, 50 and 100 pg/ml were used as thresholds. Intra- and inter-assay variations were <10%. The minimum detectable concentration was 0.01 pg/ml (product information's).

#### **Statistical analysis**

Statistical Package for Social Sciences (SPSS) version 19 was used. Study variables were described using frequency and percentage. The continuous variables within the database were converted to four rank cases. Ranking was used as procedure to make it possible to test two discrete variables using non-parametric statistics. Kruskal-Wallis test was used to test the effect of independent variables.

## **RESULTS**

#### **Seminal fluid parameters**

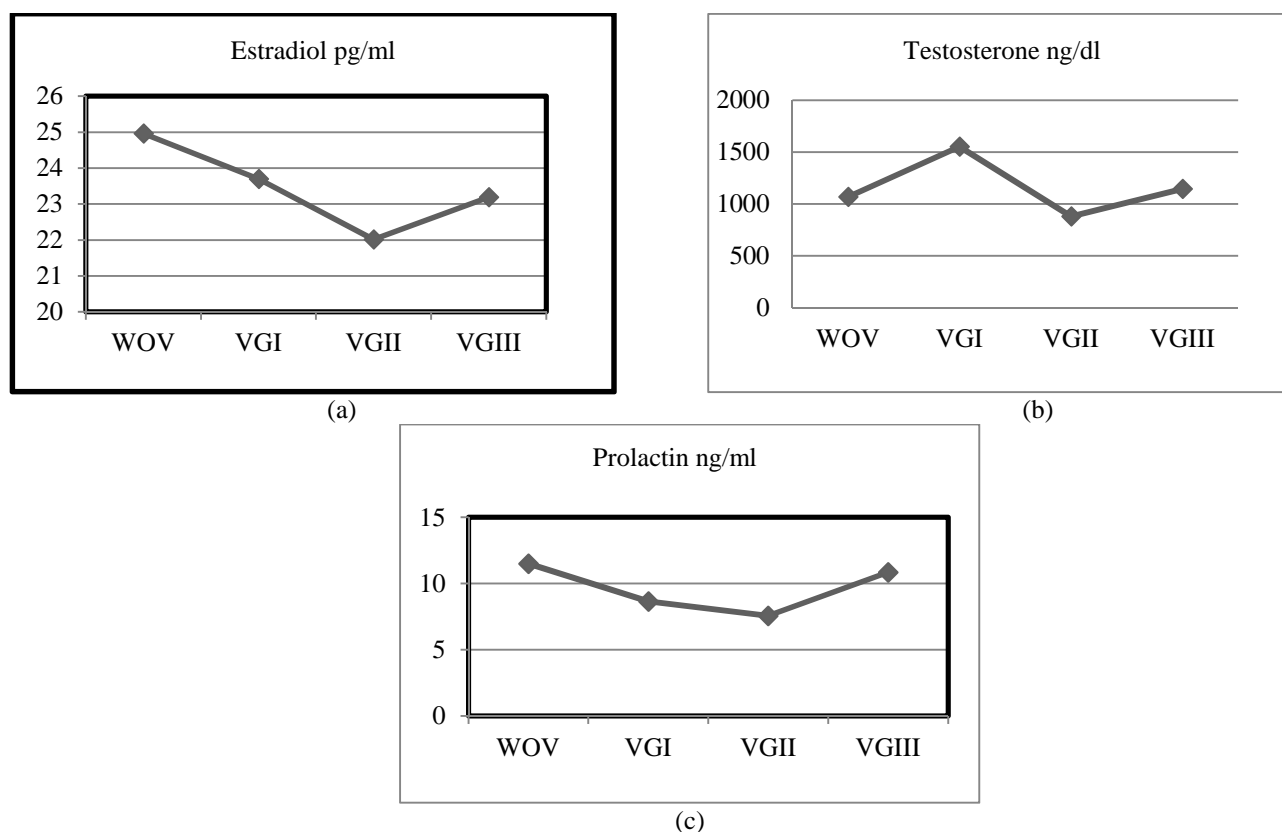
The mean ages of infertile males was ranged between 22–50 years thus covering the entire span of the reproductive years, and the mean duration of infertility was  $3.11 \pm 2.58$  years. Seminal fluid characteristics in relation to infertility were shown in table 1. The mean seminal fluid volume was significantly higher in male with varicocele ( $p=0.006$ ). In addition, the mean non progressive motility(c), abnormal sperm morphology (mainly head and middle neck deformity) was significantly higher in male with varicocele ( $p=0.05$ ,  $p=0.024$ , respectively). No significant differences was found between infertility groups or varicocele subgroups defined on the basis of seminal fluid pH, count, progressive motility (a+b), viability, viscosity, germ cells, leukocytes, and fructose concentration.

#### **Determination of testosterone, estradiol, and prolactin hormone in the serum**

No significant difference was found between infertile male with or without varicocele or between varicocele subgroups concerning estradiol and prolactin concentration. All hormonal values were within their normal ranges. Testosterone appeared insignificantly lower in cases without varicocele. However, significant differences appeared between varicocele subgroups. Varicocele grade I showed the highest mean concentration of testosterone  $1551 \pm 978$  (mean $\pm$ SD) in comparison to  $878 \pm 483$ , and  $1144 \pm 907$  ng/dl for varicocele GII and GIII, respectively (Figure 1a, 1b, 1c).

**TABLE 1.** Variation in seminal fluid parameters among infertile male with and without varicocele

Seminal fluid parameters	Male Without Varicocele (No=48)	Male With Varicocele (No.114)			p-value
		Grade I (No=18)	Grade II (No=48)	Grade III (No=48)	
Seminal fluid volume (ml)	3.166±1.869	3.177±2.195	3.437±1.376	4.787±3.688	0.006
PH	7.925±0.442	7.966±0.216	7.966±0.1667	8.004±0.185	NS
WBCs x10(6)/ml	1.416±3.03	0.333±0.485	1.383±2.4712	1.791±2.440	NS
Germ cells	2.250±3.342	3.777±4.893	2.666±3.309	4.166±5.248	NS
Sperm count x10(6)	22.92±32.01	20.84±19.9	26.85±36.80	25.47±36.37	NS
Progressive motility (a+b) (%)	21.91±18.81	24.44±19.05	28.50±22.03	30.54±19.65	NS
Non progressive (c) (%)	26.04±17.28	18.88±13.79	20.166±12.50	25.87±13.18	0.05
Immotile (d) (%)	39.166±24.14	46.00±27.81	47.45±26.38	44.125±20.1	NS
Normal sperm morphology (%)	5.583±5.892	4.222±4.570	4.33±3.899	4.791±4.104	NS
Abnormal sperm forms (%)	73.58±38.49	73.55±40.64	74.00±38.62	90.62±19.63	0.046
Head deformity (%)	63.45±33.31	64.33±36.22	63.83±34.02	80.08±18.91	0.024
Tail deformity (%)	6.12±6.80	6.77±7.04	6.80±5.45	7.41±5.34	NS
Middle neck deformity (%)	2.83±4.56	2.44±4.20	4.91±5.48	2.70±3.31	0.05
Viability (%)	55.54±23.02	60.44±24.77	60.16±16.72	63.58±12.01	NS
Fructose g/dl	327.2±72.1	312.4±74.5	317.3±72.10	315.5±71.3	NS

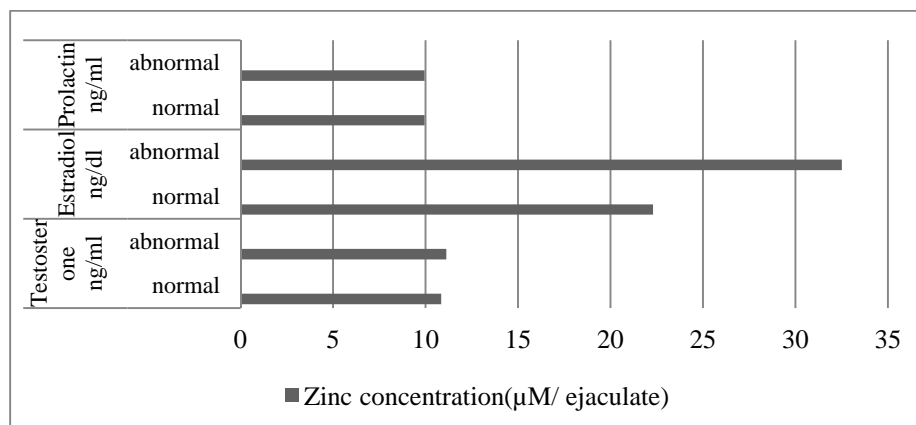
**FIGURE 1.** Concentration of estradiol (a), testosterone(b), and Prolactin (c) hormone in the serum of infertile male with and without varicocele**Zinc and seminal fluid parameters**

The detectable mean concentration of Zn in male without varicocele was 2.03  $\mu$ mole in comparison to 2.28  $\mu$ mole for male with varicocele which does not reach a significant value (table 2). Varicocele grade III showed the highest level (2.892±3.592). Seminal zinc concentration in male with and

without varicocele correlated with seminal fluid volume ( $r=0.322$ ,  $p=0.001$ ), leukocytes ( $r=0.273$ ,  $p=0.001$ ), germ cells ( $r=0.146$ ,  $p=0.064$ ), and sperm tail defect ( $r=0.241$ ,  $p=0.002$ ). A correlation between zinc concentration and plasma estradiol level was observed ( $r=0.209$ ,  $p=0.008$ ) among the study groups (figure 2).

**TABLE 2.** The mean concentration of seminal zinc among infertile males with and without varicocele

Infertile male	Mean $\pm$ SD $\mu$ mole/ejaculate	Range
Without varicocele	2.031 $\pm$ 1.543	1.11- 9.10
Varicocele Grade I	2.062 $\pm$ 0.555	1.05- 3.05
Varicocele Grade II	1.753 $\pm$ 0.396	1.29-3.05
Varicocele Grade III	2.892 $\pm$ 3.592	1.29-17.70
Total	2.207 $\pm$ 2.180	1.05-17.70

**FIGURE 2.** Means Zn concentration in the seminal fluids of infertile men in relation to testosterone, estradiol, and prolactin**Seminal plasma Interlukin-6**

The concentrations of IL-6 were determined in the seminal plasma of infertile males with and without varicocele. Threshold value for IL- 6 was 24.25 pg/ml. A statistically significant increase ( $p=0.01$ ) in the means level of IL-6 among infertile male without varicocele (mean $\pm$  SD, median) (21.37 $\pm$ 6.56, 22.0) was appeared in comparison to male with VGI, VGII, and VGIII (16.22 $\pm$  3.45, 15.0; 20.50 $\pm$ 5.84, 19.5; and 19.50 $\pm$ 5.79, 20.5) pg/ml, respectively (table 3, figure 3). The mean concentration of IL-6 was

correlated to multiple determinants of semen quality (table 4). There was a significant negative correlation between seminal plasma IL-6 and sperm viability ( $r= -0.172$ ;  $p=0.02$ ), and germ cells ( $r= -0.243$ ;  $p= 0.002$ ), whereas a positive correlation was appeared with seminal viscosity ( $r= 0.149$ ;  $p= 0.05$ ). Correlation analysis showed the absence of a significant relation between serum testosterone and prolactin hormone and seminal IL-6 level in comparison to the significant positive effect (0.01) that has been observed with estradiol hormone.

**TABLE 3.** Concentration of seminal Interlukin-6 among infertile males with and without varicocele

Cases	Interukin-6 pg/ml Mean $\pm$ SD	Median
Without varicocele	21.38 $\pm$ 6.56	22.0
Varicocele Grade I	16.22 $\pm$ 3.45	15.0
Varicocele Grade II	20.50 $\pm$ 5.84	19.5
Varicocele Grade III	19.50 $\pm$ 5.79	20.5
Total	19.99 $\pm$ 5.99	19.0

**FIGURE 3.** Mean levels of IL-6 pg/ml concentration among infertile male with and without Varicocele

**TABLE 4.** Correlation between interleukin-6 concentration and sperm parameters among infertile male with and without varicocele

Study parameters	Interukin-6 pg/ml	p-value
Seminal fluid volume	-0.055	NS
Viscosity	0.147	0.060
Leukocytes	-0.109	NS
Germ cells	-0.243	0.002
Sperm count	0.044	NS
Progressive motility(a+b)	-0.017	NS
Non progressive motility(c )	0.014	NS
Non motile sperm	-0.074	NS
Normal morphology	0.054	NS
Abnormal morphology	-0.052	NS
Head deformity	-0.030	NS
Tail deformity	-0.011	NS
Med neck deformity	0.065	NS
Viability	-0.172	0.029
Fructose	-0.043	NS

## DISCUSSION

Although the pathogenesis of varicocele remains enigmatic, varicocele often results in a generalized impairment of sperm production characterized by abnormal semen quality. This study confirms the potential effects of clinically significant varicoceles on semen parameters in groups of infertile males. A statistically significant differences in seminal parameters between infertile male with and without varicocele were observed regarding seminal fluid volume ( $p=0.006$ ), non progressive motility ( $p=0.05$ ), and abnormal morphology ( $p=0.046$ ) including head and middle neck deformity ( $p=0.024$  and  $p=0.05$ , respectively). These results came in accordance with others (Fabio *et al.*, 2005). On the other hand, varicocele grade III showed a significant increase in sperm deformity, and non progressive motility compared to grade I, II and infertile male without varicocele. It has been found that the quality of semen is inversely associated with the grade of varicocele (Al- Dujaily *et al.*, 2014). The grade of forward progressive movement, and the percentage of morphologically normal sperm was reported to be significantly lower in the semen of grade III group than that of grade I and grade II groups (Allamaneni *et al.*, 2004). However, Grades II and III varicocele cause a decrease in testicular volume and patients with varicocele grade III presented lower values for sperm parameters than those with grade II (Mori *et al.*, 2008). This may be as a result of high production of free radicals or the imbalance between reactive oxygen species (ROS) production and seminal antioxidants in the semen leading to spermatozoa damage and male infertility (Hendin *et al.*, 2015). Despite the importance of testosterone in fertility, there is a paucity of publications on the impact of varicoceles on the testosterone production. Abnormal spermatogenesis is often associated with altered serum gonadotropins and testosterone. In the current study, we confirms that men without varicoceles have insignificant lower testosterone levels in relation to a comparison group without across age categories. In addition, varicocele grade I revealed

significant increase in testosterone level as compared to other subgroups of varicocele. It is well established that age

is associated with testosterone levels and serum testosterone decrease in older men (Wu *et al.*, 2008). Although there were no statistically significant differences between the mean ages, in all cases, varicocele subgroups have a lower mean age than that of those in the comparison group which give consideration of these separate finding. Since testosterone variation always has severe indirect effects, it is suggested that in order to encounter the type of infertility, special efforts should be made to pinpoint the disturbance that actually is the cause of elevation in this hormone for the particular patient. Understanding male infertility induced by trace elements deficiency is complex because they are involved in several integrated processes associated with reproduction. However, it is not clear with certainty, as to how much the level of these elements in seminal plasma affects the sperm function. The prostate gland secretes a solution which contributes to sperm motility and viability (Ng *et al.*, 2004). Besides their effects on semen quality and infertility, they are required for normal functioning of hypothalamic-pituitary-gonadal axis (Khaki *et al.*, 2009). As shown by Colagar *et al.* (2009), fertile subjects displayed significantly higher levels of Zn in their seminal plasma than the infertile groups. Previous data from infertile men with varicocele showed a detectable decrease in antioxidant which was related to the decrease Zn seminal plasma levels (Agarwal *et al.*, 2006). Similar to the finding of Canale *et al.* (1986), the mean concentration of Zn/ejaculate among infertile male with varicocele grade II showed the lowest value in comparison to grade I and III, and also in patients without varicocele. In addition, a significant relation was established between Zn and leukocytes, germ cells, and sperm morphology. This is true since a related research has pointed out that zinc is necessary to stabilize chromatin and membrane in sperm and enhances mechanical properties of sperm such as normal flagella, midpiece formation and

sperm motility (Hidioglou and Knipfel, 1984). Several factors are associated with seminal zinc deficiency. Inflammatory conditions considerably influence the secretory functions of the prostate which may results in an impaired turnover and decrease secretion of zinc (Dissanayake *et al.*, 2010). This fact is clearly proved by the significant correlation of Zn and leukocytes present in our current study. In addition, frequent ejaculation is another possible factor that can reduce the seminal plasma Zn levels. No Correlation was found between sperm count or progressive motility, viscosity, fructose, T and PRL hormones and the seminal Zinc level, nor between the latter and varicocele sub groups. However, our findings are compatible with other studies (Canale *et al.*, 1986). On the other hand, our results revealed that Zn levels in the seminal plasma are significantly correlated with E2 which means that the secretory functions of human testis are dependent on Zn level. The Leydig cell, under LH control, is the source of most of the E2 secreted by the adult human tests. Zn are critical nutrients in the male reproductive system for proper hormone metabolism (Ng *et al.*, 2004). Ghareeb and Sarhan, 2014 reported that zinc deficiency is associated with decreased testosterone levels and have a direct effect on sperm count. Likewise, high zinc concentration in the culture medium of spermatozoa from fertile men in an *in vitro* study results in both sperm motility and sperm penetration dysfunction of ZP-free hamster oocytes (Liu *et al.*, 2009). A diverse conclusion was expressed on whether high seminal plasma zinc concentration affects sperm quality positively, negatively or shows no effect at all. It seems that no significant relationship between Zn and the clinical classification of male infertility was noted. Although the association of varicocele and infertility has long been recognized, the underlying pathophysiology has yet to be clearly elucidated. Several theories have been proposed. According to one theory, varicocele-related infertility is associated with cytokines. However, there is little information's about IL-6 interference with sperm production and semen quality, and whether it increases in parallel to hormonal deficiency in cases of varicocele. Our current study results corroborate previous reports demonstrating that male without varicocele have a significant higher level of IL-6 than their level in the comparison group having normal or abnormal semen parameters. However other studies presented significantly higher IL-6 level in seminal plasma of varicocele-related infertility (Mancini *et al.*, 2012). Varicocele grade I showed the lowest level of the cytokine in comparison to grade II, and III. These results are in accordance with other previous studies (Moretti *et al.*, 2009). An association of varicocele with the stress sperm pattern in the form of increased number of abnormal forms, decreased progressive motility and decreased sperm density was documented (Fabio *et al.*, 2005; and Al- Daghistani *et al.*, 2010). In addition, IL-6 is known to be associated with worse spermatozoal parameters in seminal analysis of patients with grade II and III varicocele (Ahmadi *et al.*, 2012). In contrast, other investigators reported no significant

correlation between IL-6 levels with seminal parameters in infertile males with varicocele (Koçak, 2002).

Measuring the level of cytokines, both in seminal plasma and serum, does not only expand the diagnostic options, but also, through the growing knowledge of immune processes, can give rise to new therapeutic methods of improving the quality of semen and increasing the chance to reproduce. Earlier studies have shown that seminal fluid contains proinflammatory mediators including IL-6 and other cytokines ( Politch *et al.*, 2007; Takaya *et al.*, 2002). These cytokines play direct and indirect biological roles in sperm functions and spermatogenesis, which affect male fertility (Pasqualotto *et al.*, 2015). The higher levels of IL-6 in infertile male without varicocele seems to have clinical significant because it is negatively related to sperm viability and positively to seminal viscosity, but not to sperm density, morphology, and motility. Some authors reported similar results, suggesting that IL-6 in seminal plasma did not correlate with sperm motility (Kocak *et al.*, 2002; Friebe *et al.*, 2003). This might be explained by the presence of various factors in the seminal plasma that helps in maintaining sperm motility and regulates their motion in the seminal fluid (Gurbaz *et al.*, 2003). Lack of significant correlation between the IL-6 and other sperm parameters may be due to small sample size of the study. However, our study did not address whether elevated seminal IL-6 levels in male with varicocele are the cause of spermatozoal dysfunction.

Seminal hyperviscosity is associated with increased oxidative stress in infertile men and increased proinflammatory interleukins in patients with male accessory gland infection, and more when the infection spreads to the seminal vesicles (Castiglione *et al.*, 2014). The importance of semen viscosity lies in the fact that the spermatozoa are tangled in the mucoid mass in the semen and prevented from migrating into the cervical tract to ascend to the site of fertilization. Seminal viscopathy was shown to be associated with male infertility (Gopalkrishnan, 2000). Several conditions, such as concentrations of prostate-specific antigen, zinc level, and activity of neutral  $\alpha$ -glucosidase in seminal plasma, were found to be correlated with changed semen viscosity (Mendeluk *et al.*, 2000; Andrade-Rocha, 2005)

Some cytokines affect the hypothalamic-pituitary gonad axis and testis. The current results revealed a significant positive correlation between IL-6 and estradiol hormone. However, regulation of cytokines in the seminal fluid have not been yet elucidated. They may be produced by the testis and integrated into the intrinsic local system which regulates germ cell proliferation (Poñlanen *et al.*, 1989), as well as Leydig-cell differentiation and steroidogenic responsiveness (Saez, 1994). This may occur at least in part through cytokine interactions with the pituitary hormones (Ben-Rafael and Orvieto, 1992). Based on our findings, the measurement of IL-6 in seminal fluid appears not to be relevant to the routine clinical management of varicocele-related infertility.

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