Serum and seminal plasma levels of interlukin-17 in infertile patients

Abdelrahman A. Elsaied[a], Hassan M. Ibrahim[b], Eisa M. Hegazy[b], Mariam Romany Wahip[b]
[a]Deprtment of Clinical and Chemical Pathology, Faculty of Medicine, South Valley University, Qena, Egypt
[b]Deprtment of Dermatology, Andrology and Venereology, Faculty of Medicine, South Valley University, Qena, Egypt

Background: Signal transduction induced by cytokines, which stimulates cell growth, proliferation, differentiation of germ cells, spermatogenesis, reproductive and testicular function.
Objectives: The aim of this study is to evaluate seminal plasma as well as serum IL 17 levels in infertile male patients compared with fertile male with normal semen parameters.
Patients and methods: This study is across-sectional case-control study that has been conducted on 40 infertile male patients and 10 fertile males as a control group, The patients were divided into four groups; group (A) included 10 patients with azoospermia, group (B) included 10 patients with oligozoospermia, group (C) included 10 patients with asthenozoospermia and group (D) included 10 patients with oligoasthenoteratozoospermia (OAT). All the patients in the four groups and the control group (E) are subjected to routine semen analysis and seminal, serum IL-17 was measured.
Results: Seminal level of IL-17 is significantly reduced between control and patient groups . The mean is from (2.83 ± 0.6) to (11.19 ± 1.6) in azoospermia, (9.14 ± 2.3) in oligozoospermia, (9.25 ± 2.2) in asthenozoospermia and (10.88 ± 1.4) pg /ml in OAT group respectively, and (p= 0.004), The mean is from (25.31 ± 2.9) to (84.26 ± 10.0) in azoospermia, (78.51±6.6) in oligozoospermia, (65.60 ± 10.8) in asthenozoospermia and (82.43±8.5) pg/ml in AOT group respectively, and (p< 0.001). A significant negative correlation is found between seminal interleukin 17 level and the sperms count, concentration, Sperm total and progressive motility (r= -0.398, r = - 0.366, r = -0.429 and r = -0.394). And asignificant negative correlation is found between serum interleukin 17 level and the sperms count, concentration present in infertile men respectively (r= -0.338 and r = -0.341), and (p< 0.001).
Conclusion: IL-17 plays an important role in male infertility and it had a negative correlation with sperm parameter.

Keywords: Interlukin-17; Semen parameters ; Male infertility.
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***Correspondence:** mariamwahib5@gmail.com
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Introduction

Infertility is defined as failure of couples to achieve pregnancy following 12 months of unprotected sexual life. Infertility affects 13-18% of couples and male factor accounts for up to 50% of all the cases **(Havrylyuk et al., 2005).**

Infertility is divided into primary infertility and secondary infertility. Primary infertility is when the male has never impregnated a woman while secondary infertility is when the male has impregnated a woman before **(Sharma, 2017)**. Nowadays there is a marked increase in the infertility rates mainly in males **(Jungwirth et al., 2012).**

No cause can be detected for infertility in 10-15% of infertile couples **(Moraes et al., 2010).**The main causes of infertility are testicular failure, obstruction, cryptorchidism, low semen volume, erectile or ejaculatory disorders and genetic abnormalities associated with sex chromosomes (Klinefelter's syndrome and Y-chromosomal microdeletions), and also the presence of anti-sperm antibodies (ASA) **(Bayasgalan et al., 2004; Silva et al., 2012).**

Varicocele is considered as one of the main male infertility causes which is present in 2-22% of the adult males **(Al-Daghistani et al., 2010)**.

Other cause of infertility is acute and chronic infections of the genitourinary (GU) tract which present in up to 15% of males. GU tract infections facilitate the increase in seminal leukocytes (SL). The role of seminal leukocytes is to scavenge the faulty sperm **(Domes et al., 2012].**

Cytokines are small peptides which have different immune activities. They combined to receptors of target cells and enhances a signal transduction, Signal transduction induced by cytokines, which stimulates cell growth, proliferation, differentiation of germ cells, spermatogenesis, reproductive and testicular function **(Hayder et al., 2018; Oku and Shimizu, 2008).**

In the male gonad, somatic testicular cells (Sertoli, Leydig, peri tubularcells) in physiological conditions produce excessive amounts of cytokines, such as interleukin 1 (IL-1), IL-6 and IL-17, which take part in spermatogenesis, semen maturation and participates in regulation of its normal function. So cytokines considered as natural components of seminal plasma **(Pacey, 2012; Fraczek et al., 2012).**

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 disturbed, the reproductive system functions may be damaged, which leads to male infertility **(Hayder et al., 2018; Qian et al., 2012)**.

T lymphocytes, T helper (Th) 17 cells is a lineage of CD4 +T cells that is characterized by the production of interleukin (IL)-17A, IL-17F, IL-21 and IL-22. Th17 cells play an essential role in infectious diseases, autoimmune conditions, adaptive immune response and mucosal immunity **(Jacobo et al., 2011; Hossam et al., 2015)**.

IL-17 is considered as a pro-inflammatory cytokine which is recently discovered. IL-17 is a glycol-protein with 155 amino acid residues and an N-terminal signal peptide. Binding to specific receptors, it promotes inflammation, participates in immune responses, hematopoiesis and other functions. It has a potent inflammatory inducing activity, which promotes local production of chemokines such as IL-8, monocyte chemo attractant protein-1 and growth regulatory factor α, which leads to rapid increase of monocytes and neutrophils. It stimulates the production of IL-6 and PGE2, enhancing local inflammation **(Ihsan, 2014)**

The aims of the present study were: to detect the levels of IL-17 in serum and seminal plasma in the control group of healthy men and in different subgroups of infertility. In order to study the roles of IL-17 in male infertility.

Patients and methods

This study is a cross-sectional case-control study; where patients randomly selected from the outpatient clinic of Dermatology,

Venereology and Andrology department in Qena university hospitals, Faculty of Medicine, South Valley University, Egypt. In the period between Novemper 2017 to Novemper 2018. An informed consent was taken from all patients. The study was approved by the Scientific and Ethical Committees at Faculty of Medicine, South Valley University.

The study includes 40 patients and 10 males as a control group. They were selected according to the following criteria: Age between 20-60 years, The average age of patients was (35.03 ± 6.4) with impaired Semen quality, no clinical or laboratory evidence of genetic disorders e.g. Klinefelter syndrome, no history of systemic diseases e.g. Diabetes, hypertension, liver or kidney diseases, and no history of undescended testis. They were divided into five groups: 10 azoospermic patients (A), 10 oligozoospermic patients(B),10 asthenozoospermic patients (C), 10 oligo-astheno-teratozoospermic patients (OAT) (D) and 10 fertile males were used as acontrol group (E).

Methodology

Semen and serum samples were collected from all patients and controls (10 samples from each group) were assessed by Semen analysis according to WHO criteria 2010 and detection of IL-17 in serum and seminal plasma using a 96 special kit (Human IL-17, ELISA E0063h) with ELISA assay. All specimens and reagents must be at room temperature before use. All reagents are mixed softly without foaming.

Sample size calculation

Sample size calculation was carried out using G*Power 3 software. A calculated minimum sample of 50 participants was needed (40 infertile males and 10 controls) to detect an effect size of 0.5 in the main sperm parameters, with an error probability of 0.05 and 80% power on a one-tailed test **(Politch et al., 2007).**

Statistical analysis

Data were verified and analyzed using IBM-SPSS 21 (IBM-SPSS Inc., Chicago, IL, USA) *. Descriptive statistics: Means, standard deviations, medians, ranges and percentages were calculated. Test of significances: chi-square test was used to compare the difference in distribution of frequencies among different groups. For continuous variables; independent t-test analysis was carried out to compare the means of dichotomous data. The clinical and demographic factors with proven statistical significance from the univariate analyses were further included in the multivariate logistic regression models. Correlation analysis was used to test the association between variables (Pearson's rank correlation). Kruskal-Wallis was used to compare the median difference between groups of more than two categories that don't follow normal distribution; post-hoc test was calculated using Bonferroni corrections. A significant p-value was considered when it is equal or less than 0.05.

Results

The clinical study was conducted on 40 infertile males and 10 fertile males as a control group. The mean of demographic variables are reported in **(Table.1).**

Table 1. Comparison between the study groups regarding sociodemographic data and smoking.

Variables	Cases (n=40)	Controls (n=10)	P-value*
Age/years	35.03 ± 6.4	35.60 ± 8.3	0.812*
Occupation			
Unskilled	23(57.5%)	4(40%)	0.261**
Skilled	17(42.5%)	6(60%)	
Smoking			
Non smoker	7 (17.5%)	6(60%)	0.012**
Smoker	33(82.5%	4(40%)	

Independent t-test test was used to compare the mean difference between groups; **Chi-square test was used to compare proportions between groups

There were no statistically significant differences as regarding age and occupation among patients and controls. There was statistically significant differences regarding the special happits (smoking) between cases and controls p- value (= 0.012).

There was statistically significant difference between control and patient groups as regard the mean of seminal level of IL-17, It is from (2.83± 0.6) to (11.19±1.6) in azoospermia, (9.14±2.3) in oligozoospermia,(9.25 ± 2.2) in asthenozoospermia and (10.88±1.4) pg/ml in OAT group respectively and (p= 0.004) **(Table. 2 & Fig.1)**.

Table 2. Comparison of seminal and serum IL-17 levels (pg/ml) between patients and controls

Variables	Mean±SD					P value
	Controls	Azoo	Oligo	Astheno	OAT	
Semen IL-17	2.83 ± 0.6	11.19 ± 1.6	9.14± 2.3	9.25±2.2	10.88±1.4	0..004
Serum IL-17	25.31± 2.9	84.26 ± 10.0	78.51 ±6.6	65.60±10.8	82.43±8.5	<0.001

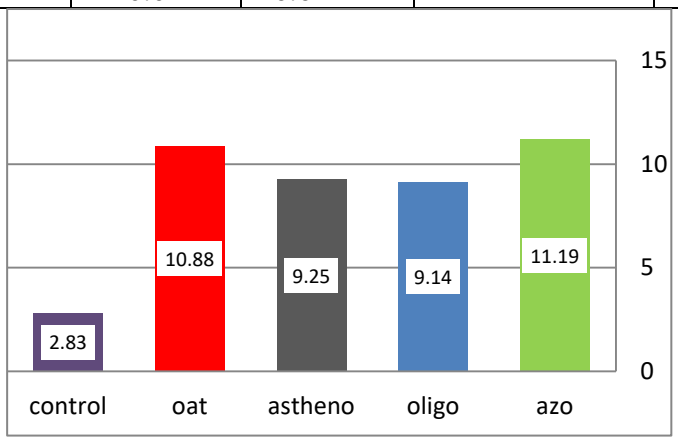

Fig .1. Comparison of seminal IL-17 level between control, azoospermia, oligospermia, asthenozoospermia and OAT Groups.

In comparison of serum IL17 level between controls and patients groups the mean was (25.31±2.9) to (84.26±10.0) in azoospermia, (78.51±6.6) in oligo-zoospermia, (65.60±10.8) in astheno-zoospermia and (82.43±8.5) pg/ml in OAT group respectively. There was statistically significant difference between cases and control groups as (p< 0.001) **(Table 2) and (Fig.2).**

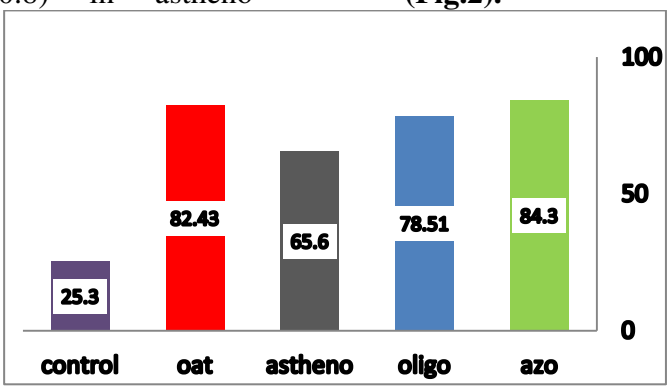

Fig. 2. The comparison of Serum IL-17 level between Control, Azoospermia, Oligospermia, Asthenozoospermia and OAT Groups

In correlation between seminal and serum IL-17 level between patients groups the (r) value was (0.572), and (p< 0.001) which is significant **(Table.3 & Fig.3)**. A negative significant difference was found between seminal interleukin 17 level and the sperms count, concentration, Sperm total and Progressive motility % respectively (r= -0.398, r = **-**0.366, r = -0.429, r = -0.394) and a negative non-significant difference was found between seminal interleukin 17 level and the sperms normal forms (r = -0.142) **(Table. 3& Fig.4).**

Table 3. Correlation between seminal IL-17 and Infertility Correlates among Patients

Parameters	Seminal IL 17	
	r*	p-value
Serum IL-17	0.572	**< 0.001**
Sperm Concentration	-0.398	**0.006**
Sperm Count	0.366	**0.010**
Total Motility %	-0.429	**0.003**
PR%	-0.394	**0.006**
Normal Form %	-0.142	**0.215**

*Pearson's Correlation Coefficient

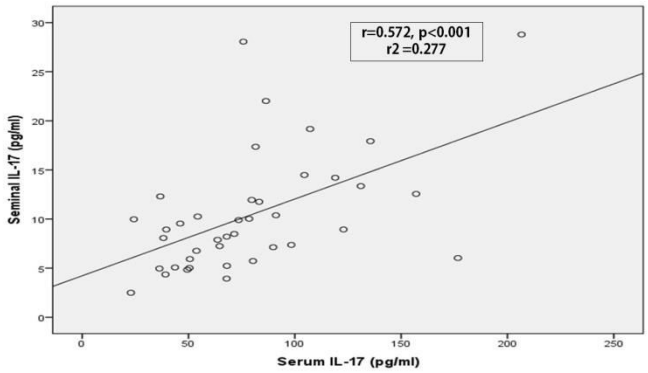

Fig. 3. Correlation between seminal and serum IL-17 in infertile male

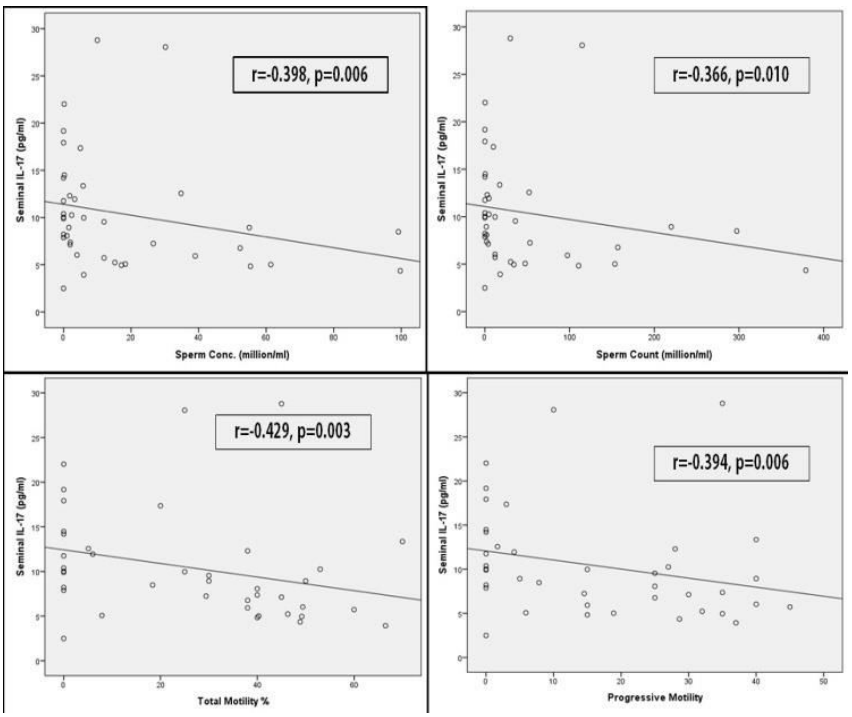

Fig. 4. Correlation between seminal IL-17 and semen parameters in infertile male

A negative significant difference was found between serum interleukin 17 level and the sperms count and concentration respectively (r = -0.338, r = -0.341) and a negative non-significant difference was found between serum interleukin 17 level and the sperms total, progressive motility and sperm normal forms respectively (r = -0.111, r = -0.058, r = -0.159) **(Table 4 & Fig.5).**

Table 4. Correlation between serum IL-17 and infertility correlates among patients

Parameter	Serum IL-17	
	r*	P value
Sperm Concentration	-0.338	0.016
Sperm Count	-0.341	0.016
Total Motility %	-0.111	0.249
PR%	-0.058	0.361
Normal Form %	-0.159	0.164

*Pearson's correlation coefficient

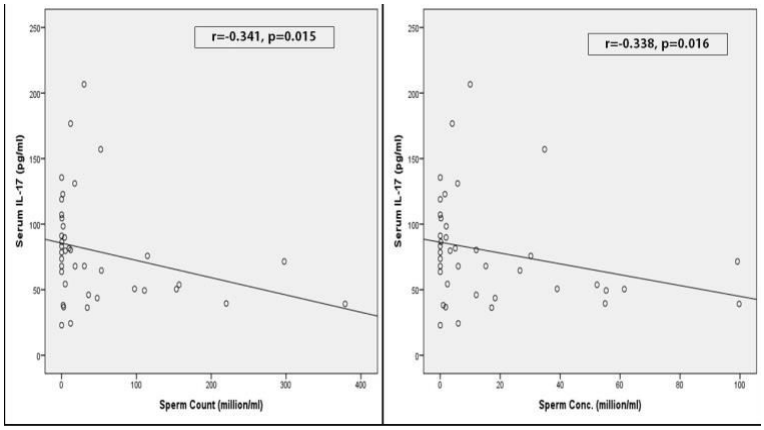

Fig. 5.Correlation between serum IL-17, sperm conc. & count in infertile male

Discussion

Seminal fluid contains IL17 and other cytokines. These cytokines play direct and indirect biological roles in sperm functions and spermatogenesis, which affect male fertility **(Politch et al., 2007).** Our study shows that the levels of seminal and serum IL-17 was significant increase in infertile males than normal fertile males. This finding coincides with the work of Qian et al **(Qian et al., 2012).** Also Hossam et al were in correlation with our results as they found a base line expression of IL-17 in normozoospermic subjects, suggesting that IL- 17 is involved in the regulation of testicular immune privilege and spermatogenesis, so any local or systemic changes of these factors caused by inflammation or infection can negatively affect testicular function and as a result affect male fertility **(Hossam et al., 2015)**. Hayder et al disagreed with our results as he found that interleukin 17 was significant decrease in unexplained infertility, Asthenozoospermia and Oligozoospermia compare with Normospermia fertile men may be due to increase evidence that sperm necrosis can adversely affect spermatogenesis **(Hayder et al., 2018)**. Our results also showed a significant negative correlation between serum and seminal level of IL-17, with sperms concentration, Sperm count sperm total and Progressive motility in infertile men, these result agree with Hayder et al study that shows negative correlation was found between cytokine level, with

sperms concentration, Sperm Progressive motility and sperm normal morphology **(Hayder et al., 2018)** Hossam et al agreed with our results and concluded that increased levels of IL-17 that occurs during inflammation help in inducing azoospermia **(Hossam et al., 2015).** Also Blackwell and Zaneveld agree with our results as the study shows negative correlation was found between cytokine level, with sperms concentration, Sperm Progressive motility and sperm normal morphology **(Blackwell and Zaneveld, 1992)**. Qian et al disagreed with our results as they found that IL-17 level is not related to sperms concentration, Sperm count and sperm normal morphology **(Qian et al., 2012)**.

Conclusion

IL-17 plays an important role in male infertility and it had a negative correlation with sperm parameter.

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