Study of Interleukin-6 (IL-6) Profile in the Seminal Fluid of Oligozoospermia Men

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Abstract

Background:

Oligozoospermia is one of male infertility type which related with sperm lowering count in seminal fluid under limited reference. There are many factors leads to oligozoospermia like, congenital defects, endocrinal disorders, genetic abnormalities, immunological factors , life style , or in some cases unknown reasons. Immune and some non-immune cells produce immuno-chemical messengers proteins called (Interleukins) work as alerts proteins and contribute in immune body defend. These mediators found in all body fluids, also found in seminal fluid,and it's presence of certain concentrations reflects the state of health of the body.

Objective:Measure the level of interleukin-6 in fertile (normospermia) and infertile (Oligozoospermia).

Materials and Methods:

Microscopically and macroscopically ejaculates analysis according to WHO (2010) criteria for eighty -seven ejaculate from normospermia and oligozoospermia patients with age rang (21-47)yrs. , in duration of (Nov- 2013 to Mar- 2014), study divide in to three groups according to the concentration ranges in to (normospermia > 15X 10^6 cells/ml), (Mild to Moderate Oligozoospermia 15-5 X10^6cells/ml) , (Severe Oligozoospermia< 5X10^6). Then store all samples in(-20℃) , IL-6 was detected through the (ELISA) specific way and kit .

Results:

Interleukin-6level is less in normospermia group than Oligozoospermia groups at ($P<0.05$), IL-6 level not correlated with sperm count, except weak correlation in severe group at ($P\leq 0.05$) and ($t\ \alpha=0.05$).

Conclusions:

There are no correlation between IL-6 level and sperm cells concentration but the variation of interleukin-6 level among the groups indicate that some sperms parameters influence on interleukin-6 levels among the groups like sperm motility or morphology and also that the causative factors which lead to oligozoospermia also effect on IL-6 level and effect on spermatogenesis .

Key words: oligozoospermia, interleukin-6, normospermia

Introduction:

Male infertility term related with male partner sexual problems known as which is associated with many factors or defects which affects on male reproduction ability. These factors are genetic disorder or congenital defects or endocrinal disorders or immunological factors, or infectious or inflammatory factors or life style behavior or environmental factors which lead to form low sperm count or poor sperm quality or both [1] [2] .Semen analysis reports results rely to detect the male infertility through the macroscopic analysis and microscopic examination. This analysis gives full profile about the seminal fluid health situation and detection the semen quality.

Oligozoospermia one of the male infertility problems which mean decreasing the sperm concentration under the limit reference [3].

The inflammations and infections are considered the most important influences which are cause later male infertility, immune system responseby humoral and cellular mechanisms to protect the body from any harm and damage resulted from microorganisms or any antigens particles[4].As a kind of immune response , immune cells like T-lymphocyte , macrophage , and some kind of non-immune cells like endothelial cells , fibroblast cells[5] , and testis cells like (sertoli cells, Leydig cells) secret immuno-chemical communicator proteins called Interleukins (ILs) [6].

Actually, IL-6 protein with (26-KD) consider one of the most important pro-inflammatory and cytokine and anti-inflammatory .with multi-function like fever inducer in inflammation and also regulation roles of immunity, hematopoiesis, inflammation and oncogenesis [7].

IL-6 Produced by Sertoli may be also needed to regulate local lymphocyte functions important for immunological protection of the tissue[8] .

Materials and Methods:

Sample Collection:

The ejaculates were obtained from eighty-seven patients , which attended to the Clinic of the High Institute of Infertility Diagnosis and Assisted Reproductive belong to (Al-Nahrain University) .for four months the samples collected in (Semen analysis lab) . all samples were collected from the patients after 5 days of sexual abstinence in private room by masturbation way. After that, all samples received and marked with patients details such as patient name, time of sample fetch and lab number. After record details the samples incubate in 37°C until liquefied and then immediately examination

Sperm Microscopically Concentration:

The sperm concentration detection through the count the spermatozoa cells on slide by count 10 random fields then calculate the average of these felids ,then multiple the sperm counts value with (10^6 factor) . That because each spermatozoa in fields represent the count 1 million cells / ml. The study divide in to three groups according to the concentration ranges in to (normospermia > 15X 10^6 cells/ml), (Mild to Moderate Oligozoospermia 15-5 X10^6) ,(Severe Oligozoospermia< 5X10^6).

Post Microscopic Examination:

In the end of microscopic examination and detected all samples ,all samples collected and transferred to the plastic disposable Eppendorff tube 2ml and sealed then put in cooler Eppendorff centrifuge , and in 14000 rpm within 15 duration with the 24°C in the end of centrifugation the tubes separation into two layer supernatant seminal plasma and residual (cellular component + spermatozoa).Micropipette used to separate the supernatant and leave the residual and transported to clean sterile plan tube and labeled by the number and patient name , then storage to the deep lower freezer in degree range (-20 °C to -40°C) until used as pro- inflammatory marker (Interleukin 6) measurement by specific kits.

Seminal Plasma Interleukin-6 Assay:

Interleukins -6 seminal plasma was detected by) ELISA technique) Interleukin – 6 Human bio assay ™ ELISA kit (US biological, USA).

Statistical Analysis:

All Data presented by the Mean ± Standard Deviation (S.D) .

Use the (t- test) to detection the significant data in each group .

Pearson correlation was done and the(r-value) correlation coefficient was calculated with its P-value.

The P- value at (0.05) in comparisons and (t- value α0.05) at P-value (≤ 0.05) in correlations .

The software used were Microsoft excel 2010 and Statistical Package for Social Sciences (SPSS) , and according to Duncan principle [9].

Results:

The study included three groups of patients samples. The patients age range (21-47) years and (mean ±SD) is (30.796 ± 7.43) .

Table (1) refer to the interleukins levels among the three study groups, interlukin-6 level more in the severe oligozoospermia group than other groups.

Table (1) : Sperms Concentration and Interleukin-6 level in three groups:

No.	S.c.1	Lev	S.c.2	Lev	S.c.3	Lev	No.	S.c.1	Lev	S.c.2	Lev	S.c.3	Lev
1	44	1.6	١٥	٤.٩	١	١٠.٢	16	45	3.3	١٠	٢.٧	١	٣
2	42	3	٦	٢.٨	٤	٨.٤	17	44	4	١٤	١.٢٦	١	١٠
3	78	8.2	١٤	٧.٦	٣	٢.٣٣	18	35	3.3	١٦	٣.٢٦	١	١١.٠٢
4	45	6	٧	٦.٨	٠.٢٥	٣.٢٦	19	42	3.8	٧	٥.٣٤	٣	٥.٣
5	32	2	٩	٦.٢٤	٤	١٥	20	74	2	٦	٧.٨	٢	٣.٦
6	21	5.2	٦	٦.٧	٠.٥	٣.٢	21	40	4.2	١٥	٦.٧	٣	٢.٢
7	72	5.3	١٤	٤.٨٢	١	٥.٦	22	46	2.2	١٣	٢.٨	٤	٢.٨٧
8	40	50.4	٨	٢.٦	٠.٥	٣.٠٢	23	40	1.8	١٢	٤.٨٨	٢	١.٠١
9	23	2.3	١٤	٣.٨٦	٠.٥	٥.٠١	24	33	1.5	٦	٣.٢٨	٠.٦	٤
10	46	3.2	٧	٢.٤٢	٢	٤.١	25	46	2.4	١٢	٣.٨	٢	٥.٠٢
11	40	2.2	٦	٨.٧	٣	٥	26	40	3.2	٨	٥.٨	٤	٥.١
12	52	1.5	٦	٦.٨	٤	٢.٤	27	50	2.5	١٣	٣.٢٤	٣	١٠
13	42	3.02	١٣	٤.٨	٠.٢	٢.٤٤	28	40	1.5	٦	٣.١٨	٢	٨.٢
14	38	3.2	١٥	٣.٦	١	٣	29	38	2.1	١٠	١٠.٥	٠.٣	٣.٨٤
15	76	2.5	٧	٧.٦	٣	٤.٣							

Sperm concentration = number of cells X10^6 cells /ml.

S.c.1= Sperms concentration (normospermia) >15X10^6 .

S.c.2 =Sperms concentration (Mild to Moderate Oligozoospermia) 15-5X10^6

S.c.3 = Sperms concentration (Severe Oligozoospermia)< 5X10^6 .

Lev = Level of interleukin -6 concentration in pg./ml

Groups Data Description Analysis and Comparisons:

The study statistical analysis designs to clarifies the effects the IL-6 variable among the groups according to analysis of variance table (Anova) and least significance difference (L.S.D) under level of significant 0.05.

Interleukin-6 variation among the groups .

Table (2) refer to that the groups comparisons of IL-6 variable among the group, this table not appeared the difference with significant semantics .that indicate the IL-6 not effect on groups, at (P<0.05) .

Table (2): The Variation of (IL-6) among the groups

Comparisons	*Diff.B.M*	*> or <*	*L.S.D.0.05*	*Significant*
Severe Vs Control	0.514482	<	2.943839	N.S
Severe Vs Moderate	0.263448	<	2.943839	N.S
Moderate Vs Control	0.251034	<	2.943839	N.S

L.S.D =Least Significant Difference, S = Significant, N.S =Non- Significant, Diff.B.M= Different Between the Means .Vs =Versus.

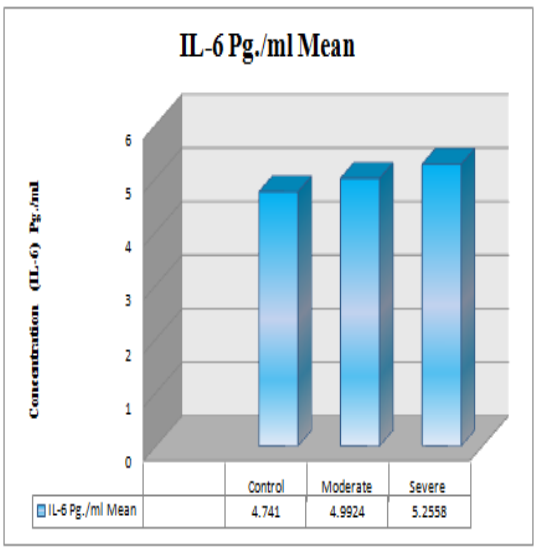

Figure (1): Interleukin-6 Concentration variation among the groups .

1-Correlation between Interleukin-6 with Sperm concentration in groups:

This analysis Show the interaction and statistic correlation between interleukin-6 and sperm concentration in each groups and if the interleukin-6 effect on sperm concentration decreasing. Table (3) ,clarifies that IL-6 not show the significance in the three groups , the (r-value) of these relations are less , and when (r- value) used to extract (t-calculate) which be less than (t-value) , so there are no correlation between interleukin -6 and sperms concentration in $P \leq 0.05$.

Table (3): Correlations between interleukin -6 and sperm cells concentration among the groups.

Correlation Between	r -value	t-value α=0.05	t-calculate	Significant P ≤ 0.05
Sperm& IL-6 (normospermia)	-0.01556	-2.052	-0.08086	N.S
Sperm& IL-6 (Mild toModerate-Oligozoospermia)	-0.24841	-2.052	-1.33253	N.S
Sperm& IL-6 (Severe-Oligozoospermia)	0.188477	2.052	0.997226	N.S

S = Significant, N.S =Non- Significant

Discussion:

The results of these study show that the normospermia group samples show less level of interleukin-6 measurement than the infertile groups regardless of the reasons of infertility or type of the semen abnormal parameter which lead to infertility , and whenever the sperm parameters change the interleukin-6 are inverse change [10],[11] [12].Also this study results show that Interleukin-6 concentration not significant difference among the groups that agreement with some studies [10],[13].

Nevertheless there found the variation between normospermia and oligozoospermia (Moderate and severe) and graduated increasing in ($P< 0.05$) , As a (figure .1) , that mean interleukin-6 related with causative factors of oligozoospermia and also there are other sperms parameter effect of interleukin-6 levels .

Oligozoospermia patient previous diagnose with a long time without any benefit of the treatment that mean that the problem resulted from genetic due to Karyotype abnormalities and Y chromosome micro deletions were present in (2–16) % and (1–55) % of subjects with azoospermia or severe oligozoospermia, respectively[14] , and these abnormality effect on the spermatogenesis and effect on level of these interleukin-6 .

Endocrinal disorder like hormones which regulate the spermatogenesis like (FSH, LH, Testosterone) which contributed with the (ILs) like (interleukin-1 interleukin-6) to begin spermatogenesis leads to change these ILs levels , the lower level of LH and Testosterone combined with increased level of FSH which control to sertoli cells secretion of interleukin-6 during spermatogenesis , and this causes infertility[15]. In infection of male genital tract or increase the oxidative stress lead to IL-6 increase level [16].

In addition to inflammation of any part of reproduction specially prostatitis[17].so that the natural of interleukin-6 function in spermatogenesis regulation and pro-inflammation effected by the failure of the sperm production process [18] and immune response .

(Tables(3)) clarify that interleukin-6 not correlated with the sperm concentration at (t-value $\alpha = 0.05$) and in ($P \leq 0.05$) . the non-significant correlation indicate ,that no essential relation between the sperm concentration reduction and interleukin-6 ,but although that ,interleukin-6 have the

role spermatogenesis and any defects in sperm production results from hormonal disorder (LH,FSH and Testosterone (T)) ,since the IL-6 co-work with (FSH) in control of spermatogenesis and sertoli cells function [18],so there are other factor in spermatogenesis effect on interleukin-6 level and in addition to oligozoospermia causatives factor related with interleukin-6 level and also lead to decreasing sperms concentration [19].

Conclusions:

Pro-inflammation mediator interleukin-6 levels in normospermia seminal plasma samples less than the oligozoospermia seminal plasma groups samples. but the variation found in interleukin-6 level among the groups indicate that the interleukin-6 affected by the oligozoospermia causatives factors and also affected by other sperms parameters .

References :

1.Jungwirth ,T ;Diemer ,G *et al* .Guidelines on Male Infertility, European Association of Urology . 2012.

2.Shefi ,S and Turek ,P,J. Definition and current evaluation of subfertile men. International.Bra J Urol. 2006;32:385-397.

3. World Health Organization. Laboratory Manual For Examination and Processing of Human Semen 5th ed. Geneva, Switzerland.2010 .

4.Kumar , V ;Abbas ,A, K ;Aster, J,C. Robbin's Basic Pathology 9th ed .Elsevier Saunders : Philadelphia , USA.2013 .

5.Abbas ,A ,K ;Andrew ,H, L . Basic Immunology (Function and Disorders of Immune system) 3rd ed,Saunders Elsevier ,Philadelphia, USA . 2011.

6.Hedger M, Pand Hales, D, B.Immuno-physiology of the Male Reproductive Tract In: Jimmy D. Neill .Knobil and Neill's physiology of reproduction 3rd ed. vol (1) Elsevier Inc. USA.2006 pp 1196- 1286.

7.Kishimoto ,T. Interleukin -6 From Basic Science to Medical – 40 years in Immunology. Annu Rev Immunol.2005 ; 23:1-21 .

8.Boockfor,F,R and Shwarz,L,K. Effects of interleukin-6, interleukin-2, and tumor necrosis factor a on transferrin release from Sertoli cells in culture. Endocrinology.1991 ; 129: 256-62.

9.Duncan,D.B.Multiple range and multiple F tests. Biometrics .1955;11:1-42.

10.Egger-Kuruse,W ;Ruxandra,B ;*et al.*Relationship of Seminal Plasma Interleukin-8 and Interleukin -6 with Semen Quality ,Human Reproduction .2001;16(3) : 517-528 .

11.Dziadecki ,W; Anna, C ; Stanislaw, F ; Leszek, B and Ewa B.Interleukin-6, and Interleukin- 8 Concentration in Seminal Plasma of Male with Seminogram Abnormalities, CentrEur J Immunol.2010; 35 (3) :162-167.

12.AL-saimary ,I.E.A. Evaluation of serum levels of pro-inflammatory cytokines (interleukins 2,6,8) in fertile and infertile men. Donn.J.Micro.Biotech.Res. 2014; 1(2) :23-34.

13.Huleihel M, Lunenfeld, E; Levy, A. *et al*. Distinct expression levels of cytokines and soluble cytokine receptors in seminal plasma of fertile and infertile men. Fertil Steril.1996; 66: 135–139.

14.Martinez G ,S,G; Gallegos, R ,M,C; Vargas, M, M; Rubio,R ,J,M; De Los ,M,R,M,E et al..Genetic screening in infertile Mexican men: chromosomal abnormalities, Y-chromosome deletions, and androgen receptor CAG repeat length. J Androl.2008; 29: 654–660.

15.Kumanov ,P ; Kalyana ,N; Analia ,T and Ashok ,A.. Inhibin- B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility ,Fertility and Sterility.2006 ; 86(2):332-338.

16.Fraczek, M and Maciej, K.Inflammatory mediators exert toxic effects of oxidative stress on human

spermatozoa , Journal of Andrology.2007; 28(2) :325-333 .
17.Alshahrani ,S ; John, M and Ashok, A. Prostatitis and male, infertility Journal of Reproductive Immunology.2013 ; 100 (1) :30-36.
18.Mahmoud, Huleiheland Eitan, Lunenfeld .Regulation of spermatogenesis by paracrine/autocrine testicular factors .Asian J Androl.2004 .;6: 259-268.
19.Dousset, B; Franc ,O,H; Myriam, D; Louis, B and Bernard , F .Seminal cytokine concentrations (IL-1b, IL-2, IL-6, sRIL-2, sR IL-6), semen parameters and blood hormonal status in male infertility , Human Reproduction .1997; 12 (7) : 1476-1479 .