

INTERLEUKIN-6 EXPRESSION IN SEMINAL PLASMA OF INFERTILE MALES

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ABSTRACT

The relationship between cytokines and human reproduction has been the subject of a variety of studies because of their involvement in reproductive physiology and gonadal function.

Objective : To assess the clinical value of measurement of interleukin-6 (IL-6) in seminal plasma of infertile men and as a marker of male accessory gland infection.

Patients and Methods : Ninety men were subjected to this study (75 infertile & 15 fertile). All studied individuals were subjected to; medical history, physical examination, Doppler ultrasonography on scrotum, semen analysis, detection of antisperm antibodies (ASAbs), serum FSH and LH, measurement of IL-6 concentration in seminal plasma (SP). The studied population were classified into 6 groups; 1 (fertile), 2 (azoospermia), 3 (immuno-infertile), 4 (varicocele), 5 (oligo-astheno-terato-zoospermia, OAT) and 6 (OAT +infection).

Results : Showed that IL-6 was expressed in seminal plasma of all studied groups and its median concentration was 29 Pg/ml. IL-6 concentration showed a high statistical significant difference between all studied groups ($p < 0.001$). The highest mean value was recorded in group 6 (76.93 ± 13.07) followed by group 3 ($52.2 \pm 5/09$) and then group 4 (42 ± 6.18). No significant difference was detected between IL-6 value and different sperm parameters in studied groups ($p > 0.05$). However, pH value in group 3 and 6 showed significant difference when compared with that of fertile group ($p < 0.001$). There was no significant difference between serum FSH, LH and IL-6 concentration in seminal plasma ($p > 0.05$). High IL-6 value was noticed in cases associated with ASAbs.

In Conclusion : This study revealed that the IL-6 most probably has a role in male infertility cases which are associated with infection, immuno-infertile cases and with varicocele. Moreover, the simultaneous determination of IL-6 in SP may provide a sensitive and useful marker of silent infection / inflammation of the male genital tract.

INTRODUCTION

The human semen contains a repertoire of cytokines including transforming growth factor (TGF)⁽¹⁾, epidermal growth factor (EGF)⁽²⁾, tumor necrosis factor α (TNF α)⁽³⁾, various interleukins (ILs) and some of their soluble receptors⁽⁴⁾. Seminal concentrations of cytokines might not only provide a measure of their release but also reflect their different interactions with

spermatozoa. In vitro, cytokines affect human sperm motility⁽⁵⁾, increase the production of reactive oxygen species (ROS) by human spermatozoa⁽⁶⁾ and reduce the ova-penetrating ability of spermatozoa. However, some of these effects are still subject to debate⁽⁷⁾. Additionally, it has been suggested that an increase in cytokines expression may lead to an increased absorption onto sperm cells and a subsequent rise in regulatory activity⁽⁸⁾.

Many reports have described fluctuation in the concentration of seminal cytokines, in particular interleukins (IL1, IL2 & IL6) and /or of their soluble receptors (sRIL2 & sRIL6) in pathological conditions, focusing on their effects on sperm number and motion parameters (9).

Although male infertility is frequently the consequence of urogenital infection, it may also arise from various causes, which often are multifactorial and / or unexplained. Therefore, the seminal concentration of cytokines may provide additional information in case of male infertility (10).

The relationship between cytokines and human reproduction has been the subject of a variety of studies because of their involvement in reproductive physiology and gonadal function. Cytokines appear to be produced by a wide variety of cells in the male genital tract and act at least in part locally (11). They may be produced by the testis, epididymis and/or released by immunocompetent cells that are present, even in the absence of inflammation(12,13). Some cytokines were shown to affect sperm motility, viability and ova penetrating capacity (14).

IL-6 is a multifunctional cytokine that is involved in numerous immunological proliferative processes in human spermatozoa. It is produced by many different cells, such as fibroblasts, monocytes, endothelial cells, Leydig and Sertoli cells. The prostate seems to be the main site of origin of IL-6 in the seminal plasma (15).

It was found that IL-6 was present in a significantly higher level in seminal plasma of infertile men compared to those of fertile men, and these levels demonstrated a significant inverse correlation with the sperm number in the ejaculate, the penetration rates and with some sperm motion

parameters (16).

IL-6 has been found in significant quantities in human seminal plasma in infertile men with infection of accessory sex glands (17).

The aim of the present study

Was to quantify IL-6 concentration in the ejaculates of both fertile and infertile men, to clarify the potential relationship between IL-6 and semen parameters and to throw a light on the possible role of IL-6 as a marker of male accessory glands infection in infertile men, which may help in the management of such cases.

PATIENTS & METHODS

1. Patients

This study included 90 randomly chosen individuals (75 infertile and 15 fertile men). The age of studied population ranged from 18-48 years (mean 31 ± 6.42). The individuals attended the out-patient-clinic of Dermatology, Venereology and Andrology Department at Al-Husseini hospital, Al-Azhar University.

Primary infertility was found in 26%, and secondary infertility in 74%. The median duration of infertility was 4.8 years (rang 2 -10 years). The ninety studied men were classified into 6 groups on the basis of their medical history, physical examination, spermiograms, biochemical analysis of the seminal plasma, culture and cytological examination of the prostatic fluid; measurement of serum gonadotrophins (FSH, LH) levels, scrotal Doppler ultrasonography and testicular biopsy served as additional criteria of classification.

2. Methods

The ejaculates were obtained by masturbation after a period of abstinence for 3-4 days and were

examined within 60 minutes after ejaculation and liquefaction in air at room temperature (37°C).

After evaluation of the liquefaction and measurement of the volume and viscosity, an aliquot of the well mixed semen sample was used for spermogram and the remainder was centrifuged at 3000 rpm for 10 minutes, the seminal plasma having been separated and stored at -20°C until the time of biochemical assay of IL-6. Sperm parameters were determined by CASA⁽¹⁸⁾.

The criteria for a normal spermogram were as the followings: (1) number of spermatozoa equal to or higher than 20 million/ml. (2) motility of 50% or more after 1 hour of ejaculation. (3) normal sperm forms more than 60% (19).

Oligo-terato-astheno-zoospermia (OTA) was considered when semen sample with sperm concentration below 20×10^6 /ml, motile sperm cells < 40% and abnormal morphology > 40%.

2.1. Prostatic and semen culture

Genital tract infection was diagnosed with the presence of tender prostate or epididymis on physical examination, positive bacterial growth of semen or prostatic culture (growth of one or more colonies of aerobic or anaerobic bacteria or $>1 \times 10^6$ leukocytes/ml/semen⁽²⁰⁾).

2.2 Detection of antisperm antibodies

Antisperm antibodies (ASAbs) were estimated in seminal fluid samples by Latex agglutination techniques⁽²¹⁾.

2.3 Measurement of IL-6 in seminal plasma

IL-6 was measured in the stored seminal plasma by enzyme-linked immunosorbent assay (ELISA) technique using commercial kit from Accucyte Inc. USA by fully automated microplate reader ETISTAR Diasorin⁽²¹⁾.

2.4 Serum samples

The serum FSH and LH were estimated by Radio Immuno Assay (RIA).

2.5 Doppler ultrasonography

Presence of varicocele was detected by palpation of the spermatic cord and was confirmed by Doppler ultrasonography on scrotum.

From the above different methodology we could classify the studied populations into 6 groups: group (1) (fertile men, n=15), group (2) (infertile men with azoospermia, Azoo, n=15), group (3) (infertile men with ASAbs, immuno infertile, n=10), group (4) (infertile men with varicocele, n=20), group (5) (infertile men with OTA, n= 15) and group (6) (infertile men with OTA & infection, n=15).

Statistical analysis:

The results were tabulated and statistically analyzed by using the student t test. The correlation coefficient were calculated by the method of Pearson. All findings were expressed as mean \pm SD and probability values (p) of < 0.05 were considered to be significant.

RESULTS

This study enrolled 90 men (75 infertile and 15 fertile); their age ranged between 18-48 years (mean; 31 ± 6.41). The values of mean age in infertile and fertile men were; 31.07 ± 6.41 and 35.33 ± 4.69 years, respectively. There was no statistically significant difference in age between the two groups (p > 0.05).

Seminal plasma volume, IL-6 and semen parameters of all studied groups are illustrated in table (I). Interleukin-6 was expressed in seminal plasma of all groups and its levels in the 6 groups

were compared by a Nova "F test" (Table II).

The median concentration of IL-6 in seminal plasma was 29 pg/ml (range 9-100 pg/ml). Seminal plasma concentrations of > 50 pg/ml were defined as "high" IL-6 (based on the 75% percentile) and such findings were noticed in 19/90 samples (21.11%) that were mostly observed in group 6 (OAT + Infection). Meanwhile, IL-6 concentration < 18 pg/ml was defined as a "low" IL-6 (based on 25% percentile) and it was found in 22/90 samples (24.44%) this observation was mostly found in group 5 (OAT).

IL-6 concentration showed a higher statistical significant difference between all studied groups ($p < 0.0001$). Group 6 (OAT + Infection) recorded the highest mean value (76.93 ± 13.07), followed by group 3 (immuno-infertile; mean = 52.2 ± 5.09) then group 4 (varicocele; mean = 42 ± 6.18) (Table II).

Furthermore, we observed a significant statistical difference between the three infertile groups (6, 3 & 4) and the fertile group ($p < 0.0001$). However, there was no statistical significant difference between group 2 (Azoo group) and group 5 (OAT) as compared with the fertile group ($p > 0.05$).

There was no significant correlation between IL-6 values and different sperm parameters in the studied groups. However, PH values showed a high statistically significant difference in both group 3 (immuno-infertile) and group 6 (OAT + infection) when compared with that of the fertile group ($p < 0.001$) (Table III).

There was no significant difference between serum FSH, LH and seminal plasma IL-6 concentrations in the studied population ($p > 0.05$) (Table IV).

Ig-G class anti-sperm antibodies (ASAbs) were detected in group 3 (10/90; 11.11%), and high IL-6 concentration was noticed in this group (Table II).

DISCUSSION

Cytokines are released by various cells in the male urogenital tract and have an effect on sperm functions and fertility (9). Their production occurs in response to foreign antigens, pathogens (infection challenge) and chronic inflammation (immunologic activation) (4,22). The defense strategies of the immune system against bacterial infections include the release of proinflammatory cytokines, specially IL-1, IL-6 and TNF- α as primary or secondary signals (23).

IL-6 is a multifactorial cytokine produced by fibroblasts, monocytes/macrophages and endothelial cells (15). IL-6 also serves as an autocrine and paracrine growth factor in a variety of tissues and cell lines. The prostate appears to be the main site of origin of IL-6 in the seminal plasma (SP) (23). It is also produced by Sertoli cells during spermatogenesis in a stage dependent manner (15).

In the present study, IL-6 was detectable with different levels in all SP of all 6 studied groups (fertile and infertile men). Naz and Kaplan (16), demonstrated increased levels of IL-6 and its inverse correlation with total sperm number and sperm motility in SP of infertile men. In contrast, other *in vivo* studies did not show a reduction of sperm motility in comparison to IL-6 concentration (24). Our study, also, did not find a statistically significant relationship between IL-6 and different semen parameters, which was in agreement with the finding of Eggert-Kruse et al., (25) and Kocak et al., (26) who found no correlation between IL-6 concentration and seminal parameters.

Cytokines probably play a physiological part as local mediators of the action of sex hormones and are involved in the paracrine regulation of spermatogenesis. It was found that FSH augments Sertoli cell IL-6 secretion in a dose-dependent manner, indicating that IL-6 secretion may be regulated by a complex interplay of various hormonal factors (12). Thus hormonal imbalance (specially FSH) may also be contributing to defective levels of IL-6 in some of these infertile men, because increased IL-6 levels correlated inversely with the sperm number in the ejaculate, and FSH has been shown to have a predominant role in regulation of spermatogenesis(16). However, our result revealed that IL-6 concentration was independent / not correlated with blood hormonal profile of LH and FSH which was also observed in other studies (27, 10).

Urogenital infections have been considered to be the cause of approximately 15% of male infertility due to the decreasing number, density and motility of the spermatozoa (28). Male accessory gland infection (MAGI) may lead to an increased release of proinflammatory cytokines, most probably by immunocompetent cells of lymphocyte/macrophage origin (29). Some reports indicated a significant association between the presence of MAGI and elevated levels of IL-6 in semen (23, 24). The present study, also, detected a significant higher IL-6 values in "OTA + infection" group than in the other groups ($p < 0.001$). This observation denoting the presence of strong correlation between "OTA + infection group" and the elevated expression of IL-6 suggesting that urogenital infection may lead to elevated levels of IL-6 in SP.

The sperm cell is foreign to the immune system repertory and should be protected in the male and

female genital tract. Also, immunosuppressive properties of seminal plasma are one of the mechanisms proposed to avoid immune response to sperm; decrease of the immunosuppressive capacity could affect sperm cell quality. It was observed that an inverse relationship existed between sperm autoimmunity and T-cell suppressing activity in seminal plasma. The immunosuppressive effects of seminal plasma might safe-guard normal reproductive functions and thus represent an important factor in normal fertility (30). Increase in proinflammatory cytokines such as IL-6 and decreasing in immunosuppressive one such as IL-10 could change the tolerance to sperm cell in male and female genital tract and reduce the favorable conditions to reach the fecundation and implantation (31).

In the present study, "immuno-infertile" men (group 3) showed that IL-6 level was more than 52.2 Pg/ml with a maximum level of approximately 60 pg/ml. Additionally, we found significantly higher levels of IL-6 in seminal plasma of immuno-infertile men ($p < 0.0001$) as compared to fertile and infertile groups; OAT and azoospermic, that correlated inversely with the sperm number and motility characteristics. This finding was in agreement with Naz and Kalpan (16). The higher levels of IL-6 in "immuno-infertile" men (group 3) could be attributed to an increased number of leucocytes (secreting IL-6) in the semen of these patients (32).

Varicocele is found in approximately 30% of infertile males (33). The majority of infertile male with varicocele have abnormal sperm parameters which was also confirmed in our study in association with increased level of IL-6 ($p < 0.05$).

Nitric oxide (NO) is a short-lived free radical

involved in the pathological and physiological processes in sperm function in a concentration dependent manner (34). Cytokines have been reported to upregulate the expression of inducible NO synthetase in Sertoli cells resulting in high levels of NO (35). Aksoy et al., (36), stated that NO production might influence sperm production, motility and morphology in patients with varicocele. Moreover, it was reported that an increased production of cytokines (e.g IL-6) can induce an increased reactive oxygen species (ROS) production in the male genital tract (37). Some evidence suggests that the varicocele is associated with excessive ROS production in semen (38). ROS have been clearly shown to impair multiple aspects of sperm function, including fertilizing capacity and sperm DNA integrity (39).

Transferrin is of pivotal importance in the transportation of iron through the blood-testis-barrier to the dividing spermatocytes and the spermatid. Under the effect of varicocele, Sertoli cells produce excess amount of IL-6 which inhibits the production and secretion of Sertoli cell-transferrin resulting in decreased cell division (40)

CONCLUSION

The results of this study detected that the IL-6 most probably has a role in male infertility that is associated with infection, immuno- infertility and varicocele. Moreover, the simultaneous determination of IL-6 in SP may provide a sensitive and useful marker of silent infection / inflammation of the male genital tract. A better understanding of these mediators in seminal plasma of normal men and patients with infertility may contribute to the management of male infertility in clinical practice.

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Table I : Comparison between the means of different parameters of the studied groups

Parameters	Group 1 Fertile (15)	Group 2 Azoospermia (15)	Group 3 Immunoinfertile (10)	Group 4 Varicocele (20)	Group 5 OAT (15)	Group 6 OAT + infection (15)
Age/year range (Mean/SD)	26 - 42 35.33±4.69	20 - 48 33.27±8.62	28 - 40 33.8±4.68	24 - 40 30.4±4.68	20 - 43 31.8±6.81	18 - 38 27.2±5.79
IL-6 (pg/ml) (Mean/SD)	17±4.19	16.67±4.03	52.2±5.09	42±6.18	15.8±5.09	76.93±13.07
Volume (ml) (Mean/SD)	3.58±0.75	2.65±1.09	3.6±0.88	3.2±0.97	2.01±0.68	2.25±0.56
pH (Mean/SD)	7.44±0.22	7.36±0.19	7.44±0.12	7.47±0.19	7.38±0.17	7.65±0.12
Sperm count (mill/ml) (Mean/SD)	90.46±44.7	--	69.66±26.18	68.69±43.7	13.44±5.5	17.55±10.12
Sperm motility (Mean/SD)	42.33±14.2	--	17.5±6.13	16.5±13.45	8.13±7.16	7.4±6.69
Abnormal morpholog (%) (Mean/SD)	54.8±17.7	--	69.8±11.01	79.15±9.59	88.33±7.7	81.8±7.29

Table II : Comparison between the concentrations of IL-6 in the different studied groups

Group		Number of patients	Range	Mean/SD
1	Fertile	15	9 - 22	17±4.19
2	Azoospermia	15	10 - 22	16.67±4.03
3	Immuno-infertile	10	46 - 60	52.2±5.09
4	Varicocele	20	30 - 52	42±6.18
5	OAT	15	9 - 28	15.8±5.09
6	OAT + infection	15	50 - 100	76.93±13.07

* F 83.06

* P < 0.0001

* There was a high significant difference in IL-6 between the studied groups

Table III : Correlation between the concentration of IL-6 and sperm parameters in the different studied groups

Group	Volume	Concentration	Motility %	Ahnormal morphology	pH
1 (Fertile)	0.18	-0.02	- 0.34	0.25	0.15
2 (Azoospermia)	- 0.04	--	--	--	0.1
3 (Immunoinfertile)	0.01	0.14	0.22	0.02	0.81*
4 (Varicocele)	0.01	0.12	0.22	-0.18	0.05
5 (OAT)	0.15	0.13	0.03	-0.19	-0.47
6 (OAT + infection)	- 0.08	-0.01	-0.05	-0.17	0.7*

- * Significant correlation between IL-6 and pH in immuno-infertile (group 3) and OAT + infection (group 6) only.
- $p > 0.001$

Table IV : Correlation between the concentration of IL-6 and serum FSH, LH in different studied groups

Group	LH	FSH
1 (Fertile)	0.08	0.07
2 (Azoospermia)	0.25	-0.07
3 (Immunoinfertile)	-0.36	-0.03
4 (Varicocele)	-0.36	-0.16
5 (OAT)	-0.08	-0.08
6 (OAT + infection)	-0.07	0.14

- * No significant difference between the concentration of IL-6 and serum FSH, LH
- * ($p > 0.05$)