

Increased Tumor Necrosis Factor Alpha and Interleukin-18 Levels in Seminal Plasma of Infertile Males

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Abstract: Objective: To evaluate the role of tumor necrosis factor alpha (TNF α) and interleukin-18 (IL-18) in male infertility. Setting: Andrology Clinic – Dr. Ahmad Fikry Medical Centre, Abu Dhabi, United Arab Emirates. Intervention: Seminal plasma levels of TNF α and IL-18 were measured in different groups of infertile males as well as in control subjects. Results: TNF α and IL-18 seminal levels were statistically significantly elevated in infertile groups compared to normal control subjects. Conclusions: Cytokines and especially TNF α and IL-18 may play a role in pathogenesis of male factor infertility and may be a part of infertility workup in near future.

Key words: Tumor necrosis factor alpha, interleukin 18, seminal plasma, male infertility.

INTRODUCTION

Male infertility is a serious diagnostic problem and in many cases the exact cause of failure to reproduce remains unknown. New diagnostic methods are being evaluated in search of more precise diagnosis and possibility of causal treatment. Measuring the levels of cytokines, in seminal plasma, does not only expand the diagnostic option, but also, through the growing knowledge of immune process, can give rise to new therapeutic methods of improving the quality of semen and increasing the chance to reproduce^[1]. Cytokines, important intra-cellular communicators, are involved in numerous physiological and pathological processes, which include mediation of inflammatory responses, reproductive physiology and regulation of gonadal steroid production and release^[2]. Human sperm contains a wide spectrum of cytokines such as tumor necrosis factor alpha (TNF α) and interleukin 18 (IL-18)^[3]. There is clear evidence indicating the effects of cytokines on spermatozoal functions. It has been found that TNF α decreases the sperm motility^[4] and stimulate sperm membrane lipid peroxidation by increasing reactive oxygen species generation^[5].

The aim of the present study is to evaluate the levels and significance of TNF α and IL-18 in the seminal plasma of infertile males.

MATERIAL AND METHODS

Thirty five males with average age of 31.2 ys. were included in the study, they were selected from patients attending the Andrology Clinic, Ahmad Fikry Medical Center, Abu Dhabi, United Arab Emirates during the period from November 2007 - August 2008.

They were divided into 3 groups: Group^[1], including 15 oligoastheno-zoospermic males, Group^[2], including 8 azoospermic males and group^[3], comprising 12 fertile healthy subjects as controls. All were subjected to thorough medical and sexual history as well as precise andrological examination. Each subject gave a written consent for participation.

Patients having pyospermia were excluded to avoid any possible contamination with interferon originating from seminal leucocytes. Semen samples were obtained by masturbation following 3 days of abstinence. Seminal plasma was obtained by centrifugation of samples at 4000 rpm for 15 minutes at 4°C. Seminal plasma were transferred in liquid nitrogen through airport to Egypt and stored at -80°C till assay. TNF α and IL-18 were assayed by ELISA immunoassay using commercial kits provided by (Quantikine R & D system, USA and Medical & Biological Laboratories Co. JAPAN) respectively.

All data were statistically analysed using windows SPSS version 10 employing analysis of variance (ANOVA) as well as Pearson correlation coefficient.

RESULTS AND DISCUSSION

Results: As shown in Table (1), there is highly and significantly elevations in TNF α and IL-18 levels in both oligo-asthenozoospermic and azoospermic groups compared to control group. Also, a significant positive correlation was detected between TNF α and IL-18 levels only in the normal group compared to the other two groups as shown in Fig. (1) where $r = 0.81$ and $P < 0.001$.

Table 1: showing the mean levels \pm SD of TNF α (pg/ml) and IL-18 (pg/ml) in seminal plasma of all studied groups.

	Normo-zoospermic	Oligoastheno-zoospermic	Azoospermic
No. of cases	12	15	8
TNF α	2.7 \pm 0.9	9.1 \pm 2.6*	57.2 \pm 18.5*
IL-18	309.1 \pm 37.3	448.1 \pm 57.4**	645.4 \pm 54.2**

F test for TNF α = 104.14; F test for IL-18 = 105.9; P<0.001 (highly significant)***.

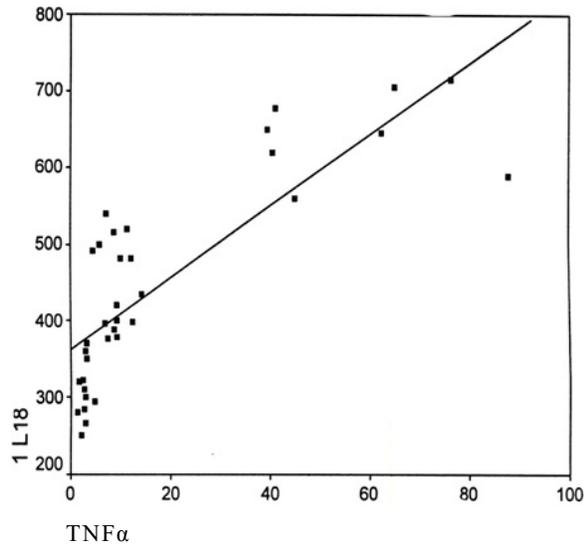


Fig. 1: Correlation between TNF α and IL-18 levels in the normal group compared to the other two groups.

Discussion: Male infertility caused by impaired semen quality is an enormous problem for the infertile couple and the andrologist. Therapeutic options are rare, because the causes are largely unknown. Subclinical infection may be one factor, with negative influence on spermatogenesis and sperm function^[6].

Male factor infertility reflects a variety of pathogenic factors, predominantly defective sperm production, sperm dysfunction and impaired transport. Infection of seminal duct can lead to infertility by various mechanisms either direct damage by microorganisms and/or their secretory products, while secondary inflammation may further increase the number of activated leukocytes and an increased secretion of cytokines^[7].

The present study focused, in parallel, on two cytokines which are key proteins in inflammatory reactions, TNF α and IL-18 to evaluate their potential significance in male infertility investigations.

Cytokines are potent poly-peptides that are released in response to a wide variety of signals, frequently initiated by infection or injury and usually act in a network of other cytokines locally in an autocrine or

paracrine fashion. Cytokines may interact, antagonize each other's action, and may also act in a synergistic effect^[8]. but also they have systemic effects, excessive production can lead to pathological consequences.

Owing to the occurrence of TNF α receptors on nearly all cells, TNF α show a wide variety of biological actions which may interfere with reproductive functions, e.g., induction of immune cascade and chemotactic activity on neutrophils, cytolytic and cytostatic effects on tumor cells, induction of fibroblastic growth, stimulation of collagenase and prostaglandin synthesis and potential influence on sperm motility and functional capacity^[9]. Furthermore, testicular macrophages can exert cytokine-guided paracrine regulatory influence on Leydig cell function as an example of immune-endocrine interactions in the male reproductive system^[10].

However, the pathophysiological significance of cytokines in sperm function is still controversial. The seminal plasma contains significant levels of several cytokines which are normally present in the male genital tract. It has been proposed that they are released by germ cells, Leydig cells, Sertoli cells, epididymis and prostate, their expression is modulated during the seminiferous cycle^[11].

The principal physiological function of TNF α is to stimulate the recruitment of neutrophils and monocytes to sites of infection and activate these cells to eradicate microbes^[12]. On the other hand, a significant effect of soluble products of activated immune cells and TNF α in high concentrations on sperm motility was detected in in-vitro studies^[9].

The significant elevation of TNF α in infertile males in our study compared to normal subjects are in accordance with other studies that showed similar results and suggested that TNF α might influence sperm motility and thereby affecting its cervical mucus penetration properties with subsequent reduced male fertility^[13]. On the other hand, other studies reported no statistically significant difference detected among fertile and infertile groups regarding TNF α levels^[14,15].

Interleukin-18 (IL-18), previously known as interferon (IFN)- δ inducing factor, is an immunoregulatory cytokine that is produced predominantly by activated macrophages^[16]. IL-18 shares functional properties with interleukin-12 and has structural similarities with IL-1 protein family but exert its effects independently of both (17). IL-18, further exerts pro-inflammatory properties by inducing the production of IL-16, IL-6, IL-8 and TNF α ^[18].

A highly significant elevation of seminal plasma levels of IL-18 was detected in this study in both oligo-asthenozoospermic and azoospermic males

compared to controls. Such finding is supported by other studies that found out significant elevation of IL-18 levels in infertile patients^[19].

This study is the first to report existence of IL-18 in seminal plasma, they also reported an inverse correlation of IL-18 levels and number of spermatozoa but the reason for such finding is not directly evident. Up to our knowledge no other studies about IL-18 levels in seminal plasma are available in literature. Moreover, a significant positive correlation was detected between TNF α and IL-18 in normozoospermic versus oligoasthenozoospermic and azoo-spermic males. This could point out that TNF α stimulates secretion of IL-18 as an example of cascade of cytokines, which have similar or complementary biological activities.

Conclusion: Cytokines are abundant in the seminal plasma and have diverse physiological and pathological actions. It is possible that further intense study of their role in some cases of male infertility especially idiopathic ones may help to open new frontier in the management of patients with poor semen quality that have limited therapeutic options nowadays.

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