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ORIGINAL PAPER

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Relationship between seminal plasma interleukin-6 and tumor necrosis factor α levels with semen parameters in fertile and infertile men

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Abstract The levels of two proinflammatory cytokines, namely tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), were investigated in seminal plasma (SP) of proven fertile ($n=24$) and infertile ($n=55$) men to evaluate the relationship between diagnosis and semen parameters in a prospective study. Infertile men were divided into four groups as follows: (1) varicocele ($n=23$), (2) 3 months after varicocelectomy (post-varicocele, $n=14$), (3) male accessory gland infection (MAGI, $n=10$) and (4) bilateral testicular atrophy ($n=8$). IL-6 and TNF-α levels were similar in the SP of fertile and infertile men. There was a strong correlation between the levels of TNF-α and IL-6 in all groups ($P<0.001$). IL-6 levels were not correlated with seminal parameters ($P>0.05$). TNF-α levels were negatively correlated with the sperm motility and morphology ($P<0.05$), but there was no correlation with total sperm counts ($P>0.05$). The mean levels of IL-6 in the SP of the MAGI group was higher than in the other groups but did not reach statistical significance. No variation was found in the SP levels of the proinflammatory cytokines studied between the varicocele and the post-varicocele groups. Our results suggest that IL-6 and TNF-α are involved in male fertility. However, their measurement in SP seem to be unsuitable for routine infertility work, perhaps with the exception of men with inflammatory genital diseases.

Keywords Male infertility · Seminal plasma · IL-6 · TNF-α

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Introduction

A male factor is responsible for infertility in approximately 50% of infertile couples. Etiological factor(s) may involve the deficiency or excess of various factors important for spermatogenesis [27]. Although hormonal factors are essential for successful spermatogenesis, growth factors and cytokines are involved in the local control mechanisms of testicular function [16]. Cytokines, which principally regulate inflammatory and immune responses, are soluble proteins secreted by cells of the immune system [2]. They also act as growth and differentiation factors that help to orchestrate cellular interactions in normal physiological functions [12]. Human semen is composed of various cells (spermatozoa, epithelial cells and leucocytes) and plasma (SP). SP contains protein and non-protein products derived mainly from the Sertoli cells, epididymis, seminal vesicle, prostate and accessory sex glands [14]. It also contains several cytokines (tumor necrosis factor alpha, interleukins, transforming growth factor, etc.) and their soluble receptors [13, 15]. 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 functions. Cytokines appear to be produced by a wide variety of cells in the male genital tract, and act, at least in part, locally [9]. They may be produced by the testis, epididymis and/or released by immunocompetent cells that are present, even in the absence of inflammation [4, 25, 29]. The seminiferous epithelium is also a source for several proinflammatory cytokines. Interleukins (IL-1, IL-6)

and TNF-α, which may act as a spermatogonial growth factor, have been identified in Sertoli and germ cells [10, 20]. Some cytokines were shown to effect sperm motility, viability and ova penetration capacity [19]. A better understanding of these mediators in SP of normal men and patients with infertility may contribute to the management of male infertility in clinical practice.

The aims of the present study were: (1) To confirm the presence of IL-6 and TNF-α in human SP, (2) to show the relationship between IL-6, TNF-α and semen parameters in different infertility groups, and (3) to assess the clinical value of the measurement of these cytokines in SP of infertile men.

Materials and methods

A total of 273 men with a fertility problem attended the outpatient Urology Clinic of the University of Adnan Menderes between January 1999 and February 2001. The study population consisted of 79 randomly chosen men, of whom 55 were infertile and 24 fertile. They were divided into five groups according to history, physical examination and semen parameters (Table 1). When needed, measurements of serum gonadotropins, testosterone and prolactin, scrotal color Doppler ultrasonography, testicular biopsy, prostatic fluid and semen culture were also carried out. The semen samples were obtained by masturbation into a sterile container after abstinence for 3–4 days and examined within 60 min after liquefaction at 37°C. Sperm parameters were determined by standard methods using a Makler Chamber (Sefi Medical Instruments, Haifa, Israel) [31]. The criteria for a normal spermiogram were the following: (1) number of spermatozoa equal to or higher than 20 million/ml, (2) motility of 40% or more 1 h after ejaculation, and (3) more than 40% normal sperm forms. Male accessory gland infection (MAGI) was diagnosed in agreement with the WHO criteria [23]. Briefly, the presence of MAGI was considered if the patient had a thickened vas deferens, or tender prostate or epididymis on physical examination, and then the diagnosis was confirmed by the positive bacterial culture of prostatic fluid or semen.

For cytokine analysis, the semen sample was centrifuged at 3,000 rpm for 10 min to pellet sperm. SP was separated and stored at –85°C until analysis. Before use, the samples were thawed overnight at 4°C and diluted with trizma buffer with a ratio of 1:1. Diluted samples of 500 µl were used for cytokine analysis. IL-6 and TNF-α measurements in SP were performed by Immulite auto-analyser using chemiluminescent enzyme immunometric assay kits (catalogue nos.: LKNF1-10127 for TNF-α, LK6P1-10129 for IL-6: Diagnostic Product Corporation, Los Angeles, Calif.). All samples were assessed in duplicate. The SP concentrations of cytokines were expressed as means, standard deviations and min.–max. values (pg/ml).

Statistical analysis was done by computer software (SPSS 8.0). The statistical tests used were: Mann-Whitney U-test, Kruskal-Wallis one way analysis of variance, and *t*-test. Probability values of < 0.05 were considered to be significant.

The research project was approved by the ethical committee of Adnan Menderes University.

Results

The study population was aged between 19 and 39 years (mean: 28.9 ± 4.5). The mean ages in infertile and fertile men were 29.1 ± 4.6 and 28.3 ± 4.2, respectively. There was no significant difference in ages between the two groups. Seminal plasma volume, IL-6, TNF-α and semen parameters of the five groups classified according to diagnosis are shown in Table 1. Both IL-6 and TNF-α were expressed in SP of all groups. We compared the levels of IL-6 and TNF-α in five groups using the Kruskal Wallis one way analysis of variance test. The highest values were detected in the MAGI group, but the differences were not statistically significant ($P > 0.05$). There was no statistical difference in cytokine levels between the different groups ($P > 0.05$). The men were also classified into two groups according to their normal or abnormal spermiograms (Table 2). IL-6 and TNF-α levels were not statistically different when compared for normal and abnormal semen parameters or abnormal subgroups ($P > 0.05$). Although the seminal parameters between the fertile and infertile groups were found to be different ($P < 0.001$), the differences between IL-6 and TNF-α levels between these groups were not statistically significant (Table 3). We correlated IL-6, TNF-α, the number of spermatozoa per ml, the percentage of motile

Table 1. Seminal plasma volume, IL-6, TNF-α and semen parameters in five groups classified according to diagnosis

Diagnosis		Volume (ml)	IL-6 (pg/ml)	TNF-α (pg/ml)	Number of spermatozoa ($\times 10^6$ml)	Motility first hour (%)	Normal forms (%)
Fertile (controls)	Mean	2.9	18.8	4.4	64.4	51.25	54.0
($n = 24$)	SD	0.6	11.0	3.5	24.7	12.5	10.1
	Min.–max.	1.5–4.0	5–51.8	1.2–14.3	20–120	40–70	35–70
Varicocele ($n = 23$)	Mean	2.4	21.8	5.1	23.5	21.7	33.0
	SD	0.7	13.2	3.1	24.0	14.3	18.2
	Min.–max.	1.5–4.5	7.4–51.0	1.4–13.7	0–100	0–55	0–55
Post-varicocele	Mean	2.8	17.0	4.0	49.1	37.5	47.5
($n = 14$)	SD	0.7	7.1	3.1	23.6	16.3	11.9
	Min.–max.	1.5–4.0	4.7–27.9	1.0–12.4	3–90	5–55	25–65
MAGI ($n = 10$)	Mean	2.7	42.8	11.0	19.4	30.0	31.5
	SD	1.4	50.3	17.9	11.8	14.9	14.2
	Min.–max.	1–6.3	10–152.7	2.3–61.3	0–38	0–45	0–50
Bilateral testicular	Mean	1.6	25.6	7.3	0	–	–
atrophy ($n = 8$)	SD	0.5	16.5	5.0			
	Min.–max.	1–2.5	12.4–52.8	1.7–17.1			

Table 2. Seminal plasma volume, IL-6, TNF-α and semen parameters in five groups classified according to the normal and abnormal spermiograms

Spermiogram		Volume (ml)	IL-6 (pg/ml)	TNF-α (pg/ml)	Number of spermatozoa ($\times 10^6$/ml)	Motility first hour (%)	Normal forms (%)
Normal	Mean	2.8	18.5	4.1	58.9	49.0	52.0
Normospermia	SD	0.6	9.6	3.0	24.1	11.4	9.9
($n=39$)	Min.–max.	1.5–4.8	5.0–51.8	1.0–14.3	20–120	45–70	30–70
Abnormal	Mean	1.6	32.9	10.9	0	–	–
Azoospermia	SD	0.5	29.2	15.7			
($n=13$)	Min.–max.	1.0–2.5	12.4–117.0	1.7–61.3			
	Mean	2.8	21.5	4.1	43.9	21.7	43.9
Asthenozoopermia	SD	0.8	12.8	2.1	23.4	9.4	11.1
($n=9$)	Min.–Max.	2.0–4.5	10.0–44.2	2.0–8.0	21–100	0–30	30–55
Oligoasthenozoopermia	Mean	2.4	19.1	5.8	12.4	22.3	34.2
($n=13$)	SD	0.7	12.8	4.1	8.7	8.1	9.5
	Min.–max.	1.5–3.5	4.7–51.0	1.4–13.7	3–19	10–35	20–50
Pyospermia	Mean	3.3	49.2	6.5	22.8	32.0	34
($n=5$)	SD	1.8	59.2	2.6	9.5	9.6	11.4
	Min.–max.	2.0–6.3	10.0–152.7	4.0–10.0	15–38	25–45	20–50

Table 3. Comparisons for means of IL-6, TNF, the number of spermatozoa per ml, the percentage of motile spermatozoa and the percentage of normal sperm forms in fertile and infertile men. NS indicates not significant

		Mean	*t*-value	*P*
IL-6	Fertile	20.3	0.759	NS
	Infertile	24.3		
TNF	Fertile	4.5	0.979	NS
	Infertile	6.2		
Concentration ($\times 10^6$/ml)	Fertile	63.5	–5.967	0.001
	Infertile	26.2		
Motility first hour (%)	Fertile	49.4	–6.346	0.001
	Infertile	24.9		
Normal forms (%)	Fertile	54.3	–6.508	0.001
	Infertile	31.6		

Table 4. Correlation of the IL-6, TNF-α, the number of spermatozoa per ml, the percentage of motile spermatozoa and the percentage of normal sperm forms. NS indicates not significant

Association between	Pearson correlation coefficient (*r*)	*P*
IL-6 – TNF-α	0.563	0.001
IL-6 – number of spermatozoa	–0.178	NS
IL-6 – motility	–0.181	NS
IL-6 – morphology	–0.181	NS
TNF-α – number of spermatozoa	–0.214	NS
TNF-α – motility	–0.229	0.042
TNF-α – morphology	–0.286	0.010
Number of spermatozoa – motility	0.750	0.001
Number of spermatozoa – morphology	0.745	0.001
Motility – morphology	0.838	0.001

spermatozoa and the percentage of normal sperm forms. A significant positive correlation was found between IL-6 and TNF-α. There was no significant correlation between IL-6 and semen parameters, or between TNF-α and the number of spermatozoa, whereas the correlation between TNF-α and motility and between TNF-α and morphology was significant (Table 4).

Discussion

Cytokines are released by various cells in the male urogenital tract and have an effect on sperm function and fertility [18]. Their production occurs in response to foreign antigens, pathogens (infection challenge), and chronic inflammation (immunologic activation) [13, 21]. The defence strategies of the immune system against bacterial infections include the release of proinflammatory cytokines, especially IL-1, IL-6, and TNF-α as primary or secondary signals [17]. IL-6 is a multifactorial cytokine produced by fibroblasts, monocytes/macrophages and endothelial cells [28]. 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 [17]. It is also produced by Sertoli cells during spermatogenesis in a stage dependent manner. TNF-α is a key cytokine in the initiation and orchestration of the inflammatory response against invading microorganisms [12]. De et al. have shown that pachytene spermatocytes and round spermatids express TNF-α mRNA [7].

In this study, we examined the levels of IL-6 and TNF-α in SP of fertile and infertile men with bilateral testicular atrophy, MAGI, varicocele and 3 months after varicocele repair. No differences in IL-6 or TNF-α were shown in SP between the infertile groups and the controls, which is in accordance with recently published results [9, 13]. Previous reports indicated the presence and fluctuations of the same cytokines in infertile patients and in certain andrological diseases [6, 15, 22]. Urogenital infections have been considered to be the cause of approximately 15% of male infertility cases due to the decreasing number, density, and motility of the spermatozoa [12]. MAGI may lead to an increased release of proinflammatory cytokines, most probably by

immunocompetent cells of lymphocyte/macrophage origin [8, 12]. Some reports indicated a significant association between the presence of MAGI and elevated levels of IL-6 in semen [6, 17]. We also detected higher IL-6 and TNF-α values in the MAGI group than in the other groups, but these findings were not statistically significant. This may be due to a wide range of values for the proinflammatory cytokines in the MAGI group.

Various proinflammatory cytokines are present in SP, but their effect on sperm cell motility, viability and morphology is unclear. Increased levels of IL-6 and inverse correlation with total sperm number and sperm motility was demonstrated in SP of infertile men [18]. In contrast, other in vivo studies did not show a reduction of sperm motility by TNF-α or IL-6 [6, 15]. We also did not find a statistically significant relationship between IL-6 and semen parameters, or between TNF-α and the number of spermatozoa, whereas the relationship between TNF-α and motility and morphology was statistically significant. The adverse effect of TNF-α on sperm motility previously demonstrated in vitro [11] was confirmed. Buch et al. reported that interleukin 1-α and TNF-α can induce lipid peroxidation by reactive oxygen species generated within spermatozoa. This might be a potentially important pathologic mechanism of sperm damage by cytokines [5].

Varicocele is found in approximately 30% of infertile males [27]. The majority of infertile males with varicocele have an abnormal spermiogram and pregnancy rates increase as sperm quality improves after the surgical repair [26]. In our study, we operated on 14 patients in the varicocele group, four of whom were able to provide their spouses with successful pregnancies in 1 year follow-up. Although the seminal parameters were improved in the post-varicocele group compared with the varicocele group, the differences in the cytokine levels in SP were not statistically significant. This result suggests that the mechanism of action of varicocele on seminal parameters may not be modulated by IL-6 or TNF-α and that varicocelectomy does not effect SP levels. Nitric oxide (NO) is a short-lived free radical involved in both pathologic and physiologic processes in sperm function in a concentration depended manner [30, 32]. Cytokines have been reported to upregulate the expression of inducible nitric oxide synthetase in Sertoli cells resulting in high levels of NO [3]. Recently Aksoy et al. indicated that NO production might influence sperm production, motility and morphology in patients with varicocele [1]. Additional studies are needed to determine whether there is any association between cytokines and NO activity. At present we have an ongoing study to investigate the relationship between proinflammatory cytokine levels and NO levels in SP of patients with varicocele, and in patients who underwent varicocele repair.

In conclusion, the presence of similar levels of IL-6 and TNF-α in SP of fertile and infertile men and the lack of correlation to whole sperm parameters indicate that both IL-6 and TNF-α may be involved in the physiological functioning of the male genital tract. The origin of these cytokines in SP could be the male genital tract, accessory sex glands and/or leucocytes. In addition, cytokine levels in SP may also be affected by inadequate sample collection and handling (liquefaction, centrifugation) as well as by their molecular heterogeneity and instability [24]. Hence, IL-6 and TNF-α in SP do not appear to play an essential role in male infertility, with perhaps an exception for men with MAGI. More research is needed on the role of cytokines in men with inflammatory genital diseases.

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References

1. Aksoy H, Aksoy Y, Ozbey I, Altuntas I, Akcay F (2000) The relationship between varicocele and semen nitric oxide concentrations. Urol Res 28: 357
2. Alexander RB. Ponniah S, Hasday J, Hebel JR (1998) Elevated levels of proinflammatory cytokines in the semen of patients with chronic prostatitis/chronic pelvic pain syndrome. Urology 52: 744
3. Bauche F, Stephan JP, Touzalin AM, Jegou B (1998) In vitro regulation of an inducible-type NO synthase in the rat seminiferous tubule cells. Biol Reprod 58: 431
4. Boockfor FR, Schwartz LK (1991) Effects of interleukin 6, interleukin 2 and tumor necrosis factor α on transferrine release from Sertoli cells in culture. Endocrinology 58: 1093
5. Buch JP, Kolon TF, Maulik N, Kreutzer DL, Das DK (1994) Cytokines stimulate lipid membrane peroxidation of human sperm. Fertil Steril 62: 186
6. Comhaire F, Bosmans E, Ombelet W, Punjabi U, Schoonjans F (1994) Cytokines in semen of normal men and of patients with andrological diseases. Am J Reprod Immunol 31: 99
7. De SK, Chen HL, Pace JL, Hunt JS, Terranova PF, Enders GC (1993) Expression of tumor necrosis factor-alpha in mouse spermatogenic cells. Endocrinology 133: 389
8. Depudydt CE, Bosmans E, Zalata A, Schoonjans F, Comhaire FH (1996) The relation between reactive oxygen species and cytokines in andrological patients with or without male accesory gland infection. J Androl 17: 699
9. Dousset B, Hussenet F, Dardin M, Bujan L, Foliguet B, Nabet P (1997) Seminal cytokine concentrations (IL-1β, IL-2, IL-6, sR IL-2, sR IL-6), semen parameters and blood hormonal status in male infertility Hum Reprod 12: 1476
10. Dugast I, Jegou B (1994) Cytokines and Sertoli cell and germ cell interactions. Contracept Fertil Sex 22: 631
11. Eisermann J, Register KB, Strickler RC, Collins JL (1989) The effect of tumor necrosis factor on human sperm motility in vitro. J Androl 10: 270
12. Hales DB, Diemer T, Hales KH (1999) Role of cytokines in testicular function. Endocrine 10: 201
13. Huleihel M, Lunenfeld E, Levy A, Potashnik G, Glezerman M (1996) Distinct expression levels of cytokines and soluble cytokine receptors in seminal plasma of fertile and infertile men. Fertil Steril 66: 135
14. Huleihel M, Lunenfeld E, Horowitz S, Levy A, Potashnik G, Mazor M, Glezerman M (1999) Expression of IL-12, IL-10, PGE2, sIL-2R and sIL-6R in seminal plasma of fertile and infertile men. Andrologia 31: 283
15. Hussenet F, Dousset B, Cordonnier JL, Jacob C, Foliguet B, Grignon G Nabet P (1993) Tumor necrosis factor-α and interleukin 2 in normal and infected human seminal fluid. Hum Reprod 8: 409

16. Kretser DM, Loveland KL, Meinhardt A, Simorangkir D, Wreford N (1998) Spermatogenesis. Hum Reprod 13: 1
17. Matalliotakis I, Kiriakou D, Fragouli I, Sifakis S, Eliopoulos G, Koumantakis E (1998). Interleukin-6 in seminal plasma of fertile and infertile men. Arch Androl 41: 43
18. Naz RK, Kaplan P (1994) Increased levels of Interleukin-6 in seminal plasma of infertile men. J Androl 15: 220
19. Naz RK, Chaturvedi MM, Aggarwal BB (1994) Role of cytokines and proto-oncogenes in sperm cell function: relevance to immunologic infertility. Am J Reprod Immunol 32: 6
20. Niederberger CS, Shubhada S, Kim SJ, Lamb DJ (1993) Paracrine factors and the regulation of spermatogenesis. World J Urol 11: 120
21. Prescott LM, Harley JP, Klein DA (1999) The fundamentals of immunology. The immune response: chemical mediators, B and T cell biology, and immune disorders. Microbiology, 4th edn, part VIII. McGraw-Hill, Boston, p 627
22. Rajasekaran M, Hellstrom WJG, Naz RK, Sikka SC (1995) Oxidative stress and interleukins in seminal plasma during leukocytospermia. Fertil Steril 64: 166
23. Rowe PJ, Comhaire FH, Hargreave TB, Mellows H (1993) WHO manual for the standardized investigation and diagnosis of the infertile couple. Cambridge University Press, Cambridge
24. Ruiz-Argüelles GJ (1995) Laboratory measurement of human cytokines. J Int Fed Clin Chem 7: 12
25. Saez JM (1994) Leydig cells: endocrine, paracrine, and autocrine regulation. Endocr Rev 15: 574
26. Schlesinger MH, Wilets IF, Nagler HM (1994) Treatment outcome after varicocelectomy: a critical analysis. Urol Clin North Am 21: 517
27. Sigman M, Howards SS (1998). Male infertility. In: Walsh PC, Retik BA, Vaughan ED, Wein AJ (eds), Campbell's urology, 7th edn, vol II. Saunders, Philadelphia, p 1287
28. Syed V, Stephan JP, Gerard N, Legrand A, Parvinen M, Bardin CW, Jegou B (1995) Residual bodies activate Sertoli cell interleukin-1α (IL-1α) release, which triggers IL-6 production by an autocrine mechanism, through the lipoxygenase pathway. Endocrinology 136: 3070
29. Twillie DA, Eisenberger MA, Carducci MA Hseih WS, Kim WY, Simons JW (1995) Interleukin-6: candidate mediator of human prostate cancer morbidity. Urology 45: 542
30. Weinberg JB, Doty E, Bonaventura J, Haney AF (1995) Nitric oxide inhibition of human sperm motility. Fertil Steril 64: 408
31. World Health Organization (1992) Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd edn. Cambridge University Press, Cambridge
32. Zini A, O'Brayn MK, Magid M, Schlegel PN (1996) The immunohistochemical localization of endothelial nitric oxide synthase in testis, epididymis and vas suggests a possible role for nitric oxide in spermatogenesis, sperm maturation and programmed cell death. Biol Reprod 55: 935