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Evaluation of tissue factor antigen level in human seminal plasma

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Abstract We measured the seminal plasma levels of tissue factor (TF) and interleukin-6 (IL-6) in men and examined their relationship with sperm concentration and motility. The study comprised 71 patients in three groups: an infertile group with ($n = 11$) and without ($n = 50$) leukocytospermia and a fertile group ($n = 10$). The seminal plasma levels of TF were significantly higher in the infertile patients than in the fertile ones. The seminal plasma levels of both TF and IL-6 were significantly higher in the infertile patients with leukocytospermia than in those without leukocytospermia. In 54 nonazoopermic cases the seminal plasma levels of TF were significantly correlated with the sperm concentration and sperm motility. Further studies are necessary to clarify the role of TF in human fertilization.

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Introduction

Tissue factor (TF) is a membrane-bound glycoprotein that serves as the nonenzymatic cofactor for factor VII for the initiation of blood coagulation activation [2]. TF has been also implicated in the cellular immune response and in the pathogenesis of some infectious diseases. Thus the expression of TF by cells of the monocytic lineage is associated with the activation of the inflammatory and cellular immune responses. In monocytes TF may be induced by cytokines or by cell to cell interaction. The presence of TF and factor VII [21] has been previously reported in human semen, and there is evidence that seminal TF derives from the prostate, and that it is associated with prostasome, a vesicular product secreted by acinar cells of the prostate gland [7]. The interaction of prostasomes with neutrophils and monocytes inhibits the ability of the cells to phagocytose latex particles. Thus prostasomes in semen could play an important role in conjunction with prostaglandins in the defense of spermatozoa against cellular immune attack in the female reproductive tract. Recently it was suggested that TF prevents bleeding and the subsequent vascular access of semen-borne agents during intercourse-associated tissue damage, and that TF may contribute to the anti-inflammatory properties attributed to prostasomes [7, 21].

Elevation in the plasma interleukin-6 (IL-6) levels has been reported in patients with autoimmune diseases such as rheumatoid arthritis [8] and acute and severe infections [24]. IL-6 is thought to promote inflammatory protein synthesis, and is a marker of immune system activation [10]. Furthermore, IL-6 has been suggested to play an important role in the cross-talk between the cytokine and coagulation cascades. The blood levels of both TF and IL-6 are increased in disseminated intravascular coagulation [22].

In this study we measured the seminal plasma levels of TF antigen and IL-6 in infertile patients and examined their relationship with the number and motility of spermatozoa.

Materials and methods

Human semen samples were collected after masturbation from 71 patients requiring semen analysis at the Toyama Medical and Pharmaceutical University. There were no abnormal physical findings in any of these men that could be associated with their infertility. The semen samples were categorized into two clinical groups, those from men who were infertile ($n = 61$) and those from men who were fertile ($n = 10$). In the infertile group there were 17 azoospermic confirmed men, 9 of whom had nonobstructive or obstructive azoospermia confirmed by hormonal analysis, spermatography, and testicular biopsy. Four patients had obstructive azoospermia and five patients nonobstructive azoospermia. The fertile group was composed of 10 pregnancy-confirmed men: 5 with varicocele and 5 without etiological findings that could be associated with their infertility, but who wished to obtain a semen analysis. The semen specimens from the patients in the fertile group were allowed to liquefy at room temperature. The concentration and motility of the specimens were assessed approximately 1 h after collection. The mean age of the patients was 30.9 ± 4.37 years and ranged from 25 to 40 years old. Semen analysis was performed in accordance with the World Health Organization guidelines (1999). After liquefaction the semen samples were centrifuged (2000 *g*) at room temperature for 10 min, and the seminal plasma was collected and stored at –80°C until use.

The seminal plasma levels of TF antigen were measured by one-step sandwich enzyme-linked immunosorbent assay using a commercial kit (Chemo-Sero-Therapeutic Research Institute, Kumamoto City, Japan) [25]. In brief, 50 µl horseradish peroxidase conjugated anti-TF monoclonal antibody (K-180) was added to wells of polyvinyl chloride microtiter plate coated with anti-TF monoclonal antibody (K-14). Then 100 µl of a seminal plasma sample was added to the wells and incubated at 37°C for 2 h. After washing 200 µl substrate solution containing 3,3,5,5-tetramethylybenzidine and H_2O_2 were added to the wells and incubated for 30 minutes at 37°C. The enzyme reaction was stopped by adding 100 µl stop solution (1 N sulfuric acid). The absorbance was measured at an optical density of 450 nm.

The seminal plasma levels of IL-6 antigen were measured by a sandwich enzyme-linked immunosorbent assay using a commercial kit (R&D systems, Minneapolis, Minn., USA). In brief, after adding 100 µl assay diluent to each well 100 µl seminal plasma was added and incubated at room temperature for 2 h. After washing four times 200 µl polyclonal antibody against IL-6 conjugated to horseradish peroxidase was added to each well and incubated at room temperature for 2 h. After washing appropriately 200 µl substrate solution (tetramethylbenzidine with hydrogen peroxide) was added and incubated at room temperature for 20 min. The enzyme reaction was stopped by adding of 100 µl stop solution (2 N sulfuric acid). The absorbance at 450 nm was measured using a microplate reader. To assess the linearity of the assay four seminal plasma samples were spiked with high concentrations of TF and IL-6 in various matrices and diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

Data were expressed as the mean ± standard deviation. The statistical differences between the two groups was assessed by Welch's or Student's *t* test. The correlation between the two groups was analyzed by linear regression analysis. *P* values less than 0.05 were considered statistically significant.

Results

Eleven patients (men with obstructive azoospermic) of the infertile group presented leukocytospermia ($\geq 1 \times 10^6$ white blood cells/ml). All of them had suffered from an inflammatory disease of the accessory sex glands. Three of the 11 patients had a past history of prostatitis, and the other eight had a history of epididymitis. Fifty patients with normal urological and hormonal findings had normal nonleukocytospermia ($< 1 \times 10^6$ white blood cells/ml). The partners of our patients in the infertile group were found to have no abnormal gynecological findings. The seminal plasma levels of TF antigen were significantly higher in infertile patients without leukocytospermia (78.9 ± 33.5 ng/ml) than those in the fertile group (53.2 ± 45.5 ng/ml, $P < 0.05$; Table 1). The seminal plasma levels of TF antigen were significantly higher in patients with obstructive azoospermic (278 ± 113 ng/ml) than those in the fertile group (53.2 ± 45.5 ng/ml, $P < 0.01$) and those in infertile group without leukocytespermia (78.9 ± 33.5 ng/ml, $P < 0.05$; Table 1).

The levels of IL-6 were significantly higher in the group of patients with leukocytospermia (67.2 ± 60.1 pg/ml) than those in the group without leukocytospermia (26.3 ± 23.9 pg/ml, $P < 0.05$; Table 1). The seminal plasma levels of IL-6 were significantly different between the infertile patients with (67.2 ± 60.1 pg/ml) and those without leukocytospermia (26.3 ± 23.9 pg/ml, $P < 0.05$; Table 1). The seminal plasma levels of IL-6 antigen were

Table 1. Seminal plasma levels of TF and IL-6 in fertile, infertile, and azoospermia patients

	TF antigen (ng/ml)	IL-6 antigen (pg/ml)
Fertile group ($n = 10$)	53.2 ± 45.5	17.2 ± 10.3
Infertile group		
With leukocytospermia ($n = 11$)	208 ± 189*,***	67.2 ± 60.1*,***
Without leukocytospermia ($n = 50$)	78.9 ± 33.5**	26.3 ± 23.9**
Azoospermia group		
Obstructive ($n = 4$)	278 ± 113*,***	83.1 ± 45.2
Nonobstructive ($n = 5$)	112 ± 128	32.1 ± 15.8*

*$P < 0.01$ vs. fertile group, **$P < 0.05$ vs. fertile group, ***$P < 0.005$ vs. infertile group without leukocytospermia

significantly higher in the nonobstructive azoospermic patients (32.1 ± 15.8 pg/ml) than those in the fertile group (17.2 ± 10.3 pg/ml, $P < 0.05$) and those in the infertile group without leukocytespermia (78.9 ± 33.5 ng/ml, $P < 0.05$; Table 1). The sperm concentration was negatively correlated with TF antigen levels ($r = -0.256$, $P = 0.052$). The percentage of motility in sperm was correlated with the TF antigen levels ($r = -0.341$, $P = 0.011$). In 54 nonazoopermic cases the sperm concentration was correlated with the TF antigen levels ($r = -0.468$, $P = 0.023$). The percentage of motility in sperm was correlated with the TF antigen levels ($r = -0.449$, $P = 0.037$).

Discussion

Semen coagulates immediately after ejaculation, and its liquefaction takes 5–20 min. The high molecular weight of seminal vesicle protein is degraded by proteases of the prostatic fluid when the clot liquefies. It has been suggested that semen coagulation is important for fertility. A lower osmolality and buffering capacity and a higher pH may cause poor semen coagulation. Many proteins may play roles in the coagulation-liquefaction system of semen. For example, the activated protein C inhibitor has been reported to be the principal inhibitor of prostate-specific antigen [5]. Although altered ovarian follicular function has been reported in factor XI deficient Holstein cows, the presence of blood-coagulation enzymes in semen remains speculative in the absence of data showing that they affect the physiological activity of seminal plasma, sperm, or the global fertility. In the present study the seminal plasma levels of TF antigen were about 1,000-fold higher than the peripheral circulating levels of TF. Recently it was reported that TF is present in over 90% of seminal plasma [21].

In the current study leukocytospermia was observed in 11 of 61 infertile patients, and the seminal plasma levels of IL-6 were higher in the leukocytospermia group than in the nonleukocytospermia group. The seminal plasma levels of TF antigen were also higher in infertile patients with leukocytospermia than in those without leukocytospermia. The expression of TF is stimulated by several inflammatory mediators such as lipopolysaccharide [16], IL-1 [19], tumor necrosis factor [1], and IL-6 [23]. However, none of our 50 infertile patients had leukocytospermia, indicating that infertile patients have no severe infections. The seminal plasma levels of TF antigen were significantly higher in infertile patients with or without leukocytospermia than in fertile men. However, the TF antigen levels in seminal plasma were negatively correlated with the IL-6 antigen levels. Increased TF antigen levels in seminal plasma may be caused by inflammatory reactions, but also by fibrinolysis-coagulation processes occurring in semen. In our study the seminal plasma levels of IL-6 were significantly higher in the group with leukocytospermia than in those without leukocytospermia, but they were not significantly different between the infertile patients without leukocytospermia and the fertile patients. It has been suggested that IL-6 could serve as an accurate marker of accessory sex gland inflammation [26]. In our present study the lack of correlation between sperm count and IL-6 levels and the absence of significant difference in IL-6 levels between the patients with normal sperm concentration and those with abnormal sperm concentration due to idiopathic testicular lesions suggests that the testes do not contribute to the high IL-6 levels in seminal plasma [12]. Furthermore, it was reported that IL-6 is not relevant for the diagnosis of immunological disease [4]. It was revealed that the seminal plasma levels of TF antigen found in obstructive azoospermic patients were not significantly higher than in infertile patients with leukocytospermia (Table 1). Obstructive azoospermia is induced by accessory sex gland inflammation [26]. This is the same pathoclinical situation.

The total sperm concentration and the percentage of sperm motility were significantly correlated with TF antigen levels in the group of nonazoospermic cases. The correlation between the TF antigen level and sperm concentration is still not clearly understood. A relationship between TF activity and sperm count or sperm motility has been reported in a small group [3]. It was previously reported that there is no significant correlation between the seminal plasma levels of TF and sperm concentration, although there was a tendency suggesting that high TF levels are associated with a low sperm concentration.

It is possible that TF plays a role in the fertilization potential of semen, but this remains to be investigated in future experiments. Sperm capacitiation and the acrosome release reaction are particularly interesting in this regard since both involve changes in the sperm plasma membrane that should increase the binding of coagulation factors [3]. The function of TF is not only to enhance procoagulant activity but also to act as a direct receptor for FVIIa/FVII [13]. Although there is no evidence of the role that cytokines play in the regulation of prostasome activity, some cytokines may induce TF expression on the prostasome surface [11, 14, 25]. Further studies are needed to clarify the role of TF in human fertilization.

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