

Role for tumor necrosis factor alpha (TNF- α) and interleukin 1-beta (IL-1 β) determination in seminal plasma during infertility investigation

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Objective: To evaluate the clinical relevance of tumor necrosis factor alpha (TNF- α) and interleukin 1-beta (IL-1 β) determination in seminal plasma during infertility investigation.

Design: Prospective study.

Setting: Outpatient infertility clinic of a university-based hospital.

Patient(s): Randomly chosen asymptomatic males ($n = 148$) from subfertile couples.

Intervention(s): None.

Main Outcome Measure(s): Determination of TNF- α and IL-1 β in seminal plasma (SP) by enzyme-linked immunosorbent assay (ELISA). In aliquots of the same ejaculates: 1) evaluation of semen quality with sperm analysis and sperm function testing; 2) determination of antisperm antibodies (ASA) of the immunoglobulin (Ig) G and IgA class; 3) microbial screening; and 4) immunocytochemical round cell differentiation to determine leukocyte counts and ratios. Medical history, clinical examination, and determination of subsequent fertility (after control for female infertility factors).

Result(s): The concentrations of TNF- α and IL-1 β in SP correlated significantly ($r = 0.65$; $P < .0001$), and these parameters were significantly related to the leukocyte ratio (%LC) of the seminal round cells ($r = 0.36$; $P < .001$) and the leukocyte counts per ejaculate ($r = 0.34$; $P < .001$). There was no relationship of TNF- α and IL-1 β levels in SP with semen quality or parameters of sperm functional capacity, and there was no association with local ASA of the IgG or IgA class. The concentration of both cytokines was also not related to the outcome of the microbial screening and did not affect subsequent fertility. No correlation of TNF- α and IL-1 β levels in SP with the concentration of C-reactive protein in same-day serum samples was found.

Conclusion(s): The levels of TNF- α and IL-1 β in seminal fluid correlate significantly with leukocyte counts and ratios in the same ejaculates, as indicators of silent male genital tract infection/inflammation. However, this is not related to semen cultures in asymptomatic individuals and not associated with clinically relevant parameters of semen quality, including sperm fertilizing capacity. (Fertil Steril® 2007;87:810–23. ©2007 by American Society for Reproductive Medicine.)

Key Words: Tumor necrosis factor, interleukin-1, male silent genital tract infection, seminal plasma components, infection markers, semen quality, sperm functional capacity, sexual transmitted diseases (STD)

Cytokines mediate inflammatory responses, are important in intercellular communication, and play a multifaceted role in the reproductive physiology of men and women (1–8). These potent polypeptides are released from inflammatory cells 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 but also have systemic effects. Excessive production or actions of cytokines can lead to pathologic consequences. Administration of cytokines or of their inhibitors is a potential approach for modifying biologic responses associated with immune and inflammatory diseases.

Although it is a matter of continuing debate, there is still only a little information about relevant factors which might negatively influence semen quality. The impact of acute genital tract infections on fertility is widely accepted, but the role of silent genital inflammation in asymptomatic individuals is poorly understood. The interpretation of currently used markers which diagnose male genital tract infection or inflammation is controversial, e.g., concerning the relevance of seminal leukocytes and clinically significant thresholds (9–15). On the other hand, it could be shown that positive semen cultures in asymptomatic patients are insufficient to diagnose male genital tract infection (16–18).

Tumor necrosis factor alpha (TNF- α) and interleukin 1 (IL-1) are key mediators of acute inflammatory reactions to microbes. Owing to the occurrence of TNF- α receptors on nearly all cells, TNF- α shows a wide variety of biologic actions which might interfere with reproductive functions,

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e.g., induction of the immunocascade 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 (19, 20). 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 (21–24).

Interleukin 1 also is an important mediator of immunologic and pathologic responses to stress, infection, and antigenic challenge. It acts synergistically with other factors in the activation and differentiation of B- cells to immunoglobulin-secreting cells, and it stimulates the activation and differentiation of natural killer (NK) cells, fibroblasts, and thymocytes. It acts antiproliferatively, increases the tumor cytotoxicity of macrophages, and induces tumor regression. In synergy with TNF- α , IL-1 plays an important role in bone metabolism. It has a variety of effects in the brain, such as induction of fever as an endogenous pyrogen, alteration of slow-wave sleep, and an important role in modulating reproductive functions through stimulation of corticotropin-releasing factor and ACTH secretion and further influence on the hypothalamic-pituitary-gonadal axis (25–29).

Little is known about the interference of TNF- α and IL-1 β as infection or inflammation markers in seminal fluid and the potential association with microorganisms in the same ejaculates of patients without clinical symptoms of infection. Therefore, in this prospective study the concentration of both TNF- α and IL-1 β were determined in seminal fluid of an unselected group of males from subfertile couples. All patients were asymptomatic with regard to genital tract infections.

The results of TNF- α and IL-1 β determination were analyzed for their relationship with seminal white blood cells (WBC) as an established inflammation marker and with the concentration of C- reactive protein (CRP) in same-day serum as a routine parameter of infection. A potential association with the outcome of a broad microbial screening, including mycoplasmas and *Chlamydia trachomatis*, was evaluated. Because of the sexually transmitted nature of many genital microorganisms, the vaginal microbial pattern of patients' female partners was also considered.

Cytokine levels were related to multiple determinants of semen quality, such as count and motility and the total motile sperm count, local antisperm antibodies (ASA), and sperm ability to migrate into cervical mucus (CM) of their female partners in vitro as a global indicator of sperm functional capacity using the in vitro sperm-CM penetration test (SCMPT) as biologic model. Furthermore, couples were followed for subsequent sperm fertilizing capacity.

MATERIALS AND METHODS

Patients

This prospective study included 148 men from subfertile couples without symptoms of genital tract infections. Men with azoospermia were excluded; otherwise couples were not selected with regard to infertility factors. The medium duration of infertility was 3 (range 1–16) years. The median age of the male patients was 35 (range 22 to 57) years, and the median age of the female partners was 32 (range 20–43) years. Couples presented for primary infertility in 65% and for secondary infertility in 35% of the cases. Cytokine determination in seminal plasma (SP) was part of a comprehensive clinical routine infertility follow-up. Informed consent was obtained from all patients.

Basic Infertility Investigation

A detailed medical history was taken, and physical examinations were performed on both partners. Investigation for female infertility factors was done as previously described (30). Ejaculates were obtained in the hospital (early morning) after a recommended 5-day period of sexual abstinence. All samples were examined immediately after liquefaction. All variables of semen or seminal fluid quality evaluated in this investigation were determined in aliquots of the same ejaculates.

Semen Analysis

Standard semen analysis according to World Health Organization (WHO) criteria (31) included determination of sperm count, progressive motility after liquefaction, after 2 and 4 hours, pH, morphology, and viability (eosin testing). If necessary, seminal plasma was kept frozen at -80°C until further use.

Semen samples were also used to screen for ASA by means of the mixed antiglobulin reaction (MAR) test (32, 33). It was performed in parallel with IgG- and IgA-coated erythrocytes and specific antiserum. Reading was done in triplicate, and the mean was taken. A level of $\geq 30\%$ of motile spermatozoa involved in the mixed agglutinates was considered MAR positive. Further analyses were performed with MAR $\geq 10\%$ as an additional cut-off point.

Examination of Cytokines

For the determination of TNF- α in SP a commercial ELISA kit was used (Milenia Biotech, Nauheim, Germany) according to the manufacturer's instructions. Briefly, the test kit is a solid-phase ELISA on a microplate coated with a monoclonal antibody specific for TNF- α . After addition of a second polyclonal horseradish peroxidase-labeled antibody directed against another epitope of the TNF- α molecule, a sandwich complex consisting of the two antibodies and TNF- α is formed. As chromogenic substrate, 3,3',5,5'-tetramethyl benzidine (TMB) is used, and the optical density of the colored end product is measured with a microplate reader at 450 nm.

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