CYTOKINE PROFILE IN SEMINAL FLUID OF INFERTILE MEN WITH CONCOMITANT PATHOLOGIES

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Abstract

The mechanisms of formation of male infertility are increasingly becoming immune dependent. This study aims to study the role of pro-inflammatory and anti-inflammatory cytokines in seminal fluid to clarify their role in spermatogenesis. This research is carried out at rheumatology and urology departments of Danylo Halytsky Lviv National Medical University (Ukraine). 45 infertile men aged 22–48 were examined. They were divided into 2 groups: first group – 22 men with a systemic autoimmune disease – rheumatoid arthritis; second group – 23 somatically healthy patients with idiopathic infertility. The control group included 27 fertile healthy men aged 22–48 years. Student's *t*-test was used to compare the significant difference in mean values between groups. Patients with autoimmune diseases (rheumatoid arthritis) had the highest rate of leukocytospermia, indicating a possible long-term inflammatory process. The TGF-β1 level was higher in men with idiopathic infertility, and in patients with autoimmune pathology was reduced compared to the control group, however these changes were not significant. At the same time in patient of both group the level a pro-inflammatory cytokine IL-18 increased approximately by 2.5 times. In patients with idiopathic infertility and patients with autoimmune pathology, a simultaneous increase in the IFN-γ level and a decrease in the IL-1β level were found. The IL-6 level in seminal plasma of infertile men of both groups was increased. The ratio IL-10/TNF-α was decreased in seminal plasma of infertile men with rheumatoid arthritis. It was found that in autoimmune pathology, the increased level of IL-1β in blood serum compared to controls was associated with its reduced level in seminal fluid ($r=-0.51$, $p<0.05$). In idiopathic infertility, a low level of IL-1β in seminal fluid was also associated with a low concentration of this cytokine in blood serum ($r=0.62$, $p<0.05$). The functioning of the immune and reproductive systems of the male body is closely related and interdependent. Disorders of immune reactivity, which accompany the development of autoimmune pathology associated with disorders of reproductive function in men. The largest number of deviations of immune reactivity was found in infertile men with concomitant autoimmune diseases.

Keywords: *cytokines, male infertility, idiopathic infertility, rheumatoid arthritis.*

INTRODUCTION

The regulation of male reproductive function is realized through different levels, involving both the endocrine and immune systems. The mechanisms of formation of male infertility are increasingly becoming immune-dependent. Immunological isolation of the testes is provided by the anatomical blood-testis barrier and the special tolerance of the immune system to antigens expressed on male gametes. The seminal fluid ensures the microenvironment for differentiated gametes. It is a multicomponent solution and contains a range of active biological substances with immunomodulatory properties [1; 2]. In addition to hormones, cytokines TNF-α, IFN-γ, TGF-β2/β3, IL-1α/1β and IL-12 play an important role in the regulation of spermatogenesis. They regulate the penetrability of the barrier in normal physiological state and pathological conditions [3; 4]. The cytokines TGF-β2 / β3, TNF-α and IL-1α perform a leading role in the regulation of the blood-testis barrier. These cytokines in the germinal epithelium are synthesized by Sertoli cells and germ cells (specifically spermatocytes and early spermatids, since elongated spermatids produce exclusively TNF-α, receptors to which are located

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mostly on Sertoli cells). The interaction between polar proteins is regulated by cytokines. This is important both for the regulation of endocytic processes of protein transport and for the synergization of actin- and steroid-mediated effects on the blood-testis barrier [5].

The effect of cytokines on sperm in the seminal fluid is a physiological phenomenon. IL-6, IL-10 levels and TNF-α level are positively correlated with sperm concentration, motility and normal morphology [6], and IL-6 level positively correlated with the ability of sperm to penetrate egg [7]. In the seminal fluid of a healthy man, in addition to a small number of leukocytes (about 1 million/ml), cytokines TGF-α/β and IL-1β and IL-6,8 and a soluble receptor for IL-2 were detected. Some of these molecules (IL-1 and TGF-β) are synthesized in the testes, others, probably, in the appendages or other male gonads. In the seminal fluid of healthy men except a small number of leukocytes (about 1 million/ml), cytokines TGF-α/β, IL-1β and IL-6,8 and a soluble IL-2 receptor were detected. Some of these molecules (IL-1 and TGF-β) are synthesized in the testes, others, probably, in the other male gonads [8]. For a better understanding of the immunopathogenetic mechanisms of infertility, a study of the role of various immune factors is required.

The aim of the work is to study the role of pro-inflammatory and anti-inflammatory cytokines in seminal fluid to clarify their role in spermatogenesis.

Materials & methods

Study population and semen collection

This research is carried out at rheumatology and urology departments of Danylo Halytsky Lviv National Medical University (Ukraine). Men underwent a thorough genitourinary examination to establish exclusion criteria. Individuals with normally developed urogenital organs were included in the study. The study included individuals with a diagnosis of rheumatoid arthritis without concomitant inflammatory diseases of the connective tissue, other inflammatory diseases and oncological pathology at the time of the study. The duration of the disease was from 6 to 360 months. All patients with rheumatoid arthritis were diagnosed with asthenozoospermia or leukocytospermia. The idiopathic form of infertility, characterized by an unstudied etiopathogenesis, was diagnosed by the lack of fertilization during the year of the couple's sexual life and the impossibility of finding out the cause of the disease. This form of infertility included men with oligozoospermia, oligoasthenozoospermia, asthenozoospermia, and leucospermia.

45 infertile men aged 22–48 were examined. They were divided into 2 groups: first group – 22 men with a systemic autoimmune disease – rheumatoid arthritis; second group – 23 somatically healthy patients with idiopathic infertility. The control group included 27 fertile healthy men aged 22–48 years.

Ethical approval was obtained from the Ethics Committee of Danylo Halytsky Lviv National Medical University and informed consent was obtained from all eligible, consenting participants.

Semen processing

Semen was collected, analyzed and classified according to the criteria of the WHO (2009) [9]. Semen was obtained by masturbation into sterile plastic containers following 3–5 days of abstinence. Semen analysis were performed within 30 min. of sample arrival in the laboratory. Seminal plasma was obtained by centrifugation of ejaculate samples at 3000 g for 10 min and sedimentation of spermatozoa. Seminal fluid was stored at –20°C until the beginning of the studies, mostly for two weeks.

Determination of the cytokines concentration in seminal plasma was carried out by the immunoenzymatic method. To determine the cytokines IL-1β, IL-6, IL-10, IL-18, IFN-γ, TNF-α in seminal fluid, kits from DIACLONE (France) were used, TGF-β1 – kit from DRG Diagnostics (Germany). The study was carried out according to the manufacturer's instructions. The microplayphotometer SUNRISE TECAN (Austria) was used for the analysis.

Statistical analysis

All quantitative variables were expressed as mean ± standard deviation, while qualitative data were shown in the form of number and percentage. Student's t-test was used to compare the significant difference in mean values between groups. $p<0.05$ was considered significant. SPSS 16.0 version for Windows (USA) was used for statistical analysis.

Results & discussion

Ejaculate analysis has of fundamental importance for diagnosis and determining the degree of severity of male factor in infertility. Although it is based on a quantitative change in parameters of ejaculate, functional defects are important. Spermatogenesis in humans lasts almost 3 months and the influence of exogenous factors can persist for 2–3 months.

Analysis of ejaculate was done according to WHO 5th guideline. Reduced number of normal

spermatozoa and their motility was detected in all examined groups (*Table 1*).

Patients with autoimmune diseases (rheumatoid arthritis) had the highest rate of leukocytospermia, indicating a possible long-term inflammatory process. When examining patients with autoimmune pathology, asthenozoospermia was found in 8 (36.36%) patients and leucocytospermia in 14 patients (63.64%). In patients with idiopathic infertility, oligozoospermia was diagnosed in 3 patients (13.04%), oligoasthenozoospermia in 7 patients (30.36%), asthenozoospermia in 8 patients (34.78%) and leucocytospermia in 5 patients (21.82%). In general, quantitative and qualitative changes in spermatozoa were found: in men with autoimmune diseases – in 8 patients (36.36%); in men with idiopathic infertility – in 12 patients (52.17%). The frequency of changes in spermatozoa did not differ in the examined groups. At the same time, leukocytospermia was determined much more often in patients with autoimmune pathology, compared to patients with idiopathic infertility. In infertile men with concomitant autoimmune pathology, the number of leukocytes in sperm probably exceeded the values in all other groups.

The next task was to determine the main cytokines in seminal fluid of men with infertility. To analyze the cytokine profile in seminal fluid, the levels of the following cytokines were determined: pro-inflammatory – interleukin 1β (IL-1β), interleukin 18 (IL-18), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), interferon γ (IFN-γ) and anti-inflammatory – interleukin 10 (IL-10), transforming growth factor β1 (TGF-β1) (*Table 2*). The TGF-β1 level was higher in men with idiopathic infertility and in patients with autoimmune pathology was reduced compared to the control group, however these changes were not significant. At the same time in patient of both group the level a pro-inflammatory cytokine IL-18 increased approximately by 2.5 times. In patients with idiopathic infertility and patients with autoimmune pathology, a simultaneous increase in the IFN-γ level and a decrease in the IL-1β level were found. The IL-6 level in seminal plasma of infertile men of both groups was increased.

Since male ejaculate is both an inducer of Th2 and an inhibitor of Th1 response, the Th2/Th1 balance coefficients in the seminal fluid of the examined groups were calculated (*Table 3*). The ratio IL-10/TNF-α was decreased in seminal plasma of infertile men with rheumatoid arthritis.

Table 1. Evaluation of the spermogram of infertile men with various accompanying pathologies

Parameters	Control group	Idiopathic infertility	Rheumatoid arthritis
Number of sperm in 1 ml (million)	58.27±7.51	38.42±4.73	49.37±6.82
Number of sperm in ejaculate (million)	195.69±24.32	107.9±12.64*	121.87±13.56
Sperm motility (%)	56.71±7.51	31.36±4.20*	42.16±5.42*
Morphologically normal sperm count (%)	68.83±8.95	38.11±5.23*	39.17±5.36*
Leukocytes (10^6/ml)	0.28±0.06	0.46±0.08	0.34±0.07

Note: * – *p* value between parameters is significant if $p<0.05$.

Table 2. The level of the cytokines in seminal plasma of infertile men

Parameters, pg/ml	Control group	Idiopathic infertility	Rheumatoid arthritis
TGF-β1	116.28±17.57	151.18±13.28	95.70±12.47
TNF-α	11.39±4.40	20.02±4.42	16.22±4.27
IFN-γ	59.76±7.10	79.40±6.48*	68.63±6.58
IL-1β	75.13±8.11	28.99±6.48*	44.34±9.79*
IL-6	27.49±4.76	41.33±5.08*	57.22±7.82*
IL-10	11.78±3.69	16.21±2.50	12.29±2.71
IL-18	9.62±1.61	23.54±3.07*	24.25±2.94*

Note: * – *p* value between parameters is significant if $p<0.05$.

Table 3. The ratio IL-10/TNF-α and IL-10/IFN-γ in seminal plasma of infertile men

Parameters, pg/ml	Control group	Idiopathic infertility	Rheumatoid arthritis
IL-10/TNF-α	1.05±0.12	0.86±0.19	0.78±0.10*
IL-10/IFN-γ	0.20±0.05	0.21±0.08	0.19±0.03

Note: * – *p* value between parameters is significant if $p<0.05$.

It was important to determine whether changes in cytokines in blood serum were correlated with corresponding changes in seminal fluid. It was found that in autoimmune pathology, the increased level of IL-1β in blood serum compared to controls was associated with its reduced level in seminal fluid ($r=-0.51$, $p<0.05$). In idiopathic infertility, a low level of IL-1β in seminal fluid was also associated with a low concentration of this cytokine in blood serum ($r=0.62$, $p<0.05$). Such a result can explain the reduced number sperm in the ejaculate of this group, since IL-1β in physiological concentrations contributes to the maturation of spermatozoa.

The interaction of cells of the immune system with each other depends on their production many biologically active substances, in particular cytokines, which can have both pro-inflammatory and anti-inflammatory effect. A change in the cytokine profile is one of the immunopathogenetic mechanisms of many diseases, in particular the reproductive system [10]. Elevated serum levels of proinflammatory cytokines are a characteristic feature of impaired immune reactivity for autoimmune pathology [11].

The immune system functions as a single integrated mechanism, and under physiological conditions, the cytokines synthesis with multidirectional effects is usually interdependent. Therefore, the correlations between pro- and anti-inflammatory cytokines are important. In some cases, antagonistic cytokines inhibit each other's synthesis. In another case, the over synthesis of cytokines of one effect causes a homeostatic increase in the cytokines synthesis of the opposite effect [12; 13]. As a rule, various pathological conditions are accompanied by changes in the number and nature of correlation relationships between different parameters of immune reactivity, including serum levels of cytokines of different effects in comparison with correlations in healthy persons.

In patients with autoimmune diseases, there was no correlation between cytokines of different effects, which may indicate an imbalance in the adequate immune response. Negative correlations were observed in these groups of patients, especially in chronic inflammatory diseases. Men with idiopathic infertility showed three significant correlates different from those found in healthy fertile men. Systemic disruption of cytokine balance is closely related to shifts in local cytokine balance in individual compartments, including the reproductive tract. Taking into account the above we analyzed the cytokine profile of seminal fluid. Seminal fluid contains a wide range of cytokines. The level and properties of cytokines determines the final stages of post-testicular maturation of spermatozoa and the effectiveness of fertilization under physiological conditions.

Male seminal fluid is both an inducer of Th2 and an inhibitor of Th1 response. It induces and potentiates a cascade of events, the result of which is an increase in the endogenous expression/production of cytokines (LIF, GM-CSF and other growth factors) with embryotrophic properties. Factors of seminal fluid play a positive role in the preimplantation development and implantation of the embryo. Cytokines play a leading role in the inflammatory process of the urogenital tract. They also potentiate the pro-inflammatory effects of other inflammatory mediators. Under normal conditions, IL-1β contributes to the maturation of spermatozoa, under pathological conditions it facilitates the development of an inflammatory reaction, not only acting chemotactically on neutrophils and monocytes, but also promoting the release of histamine, which accelerates the formation of the entire inflammation cascade. IL-6 is synthesized by monocytes and macrophages under the influence of IL-1 and exhibits pro-inflammatory properties.

In seminal fluid and sperm membranes, the elevated level of IL-6 positively correlates with the level of lipid peroxidation and negatively with sperm motility and their number, as it inhibits DNA synthesis during meiosis of spermatogenic cells [6]. The anti-inflammatory cytokine IL-10 is found in high concentrations in the seminal fluid of healthy men, since it maintains immunological balance at the local level and protects spermatozoa from damage [14].

The level of TNF-α in seminal fluid probably correlates with the number of leukocytes and the proportions of their subpopulations in the ejaculate.

Therefore it can serve as an indicator of the presence of infection or inflammation. Increased level of TNF-α does not affect the ability to fertilize [15], however has a negative effect on motility and the degree of DNA damage in spermatozoa [16] and on all spermatogenesis, since it inhibits the synthesis of testosterone by Leydig cells [17].

The cytokine TGF-β1 in seminal fluid has an immunoregulatory effect on the cellular elements of the female genital tract to support sperm survival and preservation of fertilizing capacity [18]. The cytokine IFN-γ is a pro-inflammatory cytokine, the increased level of which in seminal fluid reflects the presence of an infection or an autoimmune disease. In autoimmune orchitis, IFN-γ works in synergy with IL-12 [19], which, in turn, interacts with IL-18 in the implementation of many of its functions. IL-18 belongs to the IL-1 family and is also produced by activated macrophages. Like IL-1 it interacts with TLRs by signaling through the MyD88 gene, which activates TGF-receptor-associated factor and NF-κB. An important function of IL-18 is the regulation of functionally different subpopulations of T-helpers necessary for cell-mediated immune response. It enhances the synthesis of FasL, which mediates the cytotoxic effect of NK cells and together with IL-12 affects the functions of NK cells (induction of IFN-gamma synthesis, increased cytotoxicity and proliferation) [20].

An increased level of IL-18 in the seminal fluid correlates with a decrease in sperm motility. IL-18 inhibits the harmful effects of infection/inflammation on spermatogenesis by enhancing the proliferation of germ cells, therefore it can be called a paracrine protective factor in the male gonads [21].

Cytokine IL-1β has pleiotropic properties, it is not only a mediator of inflammation, but also a modulator of innate and acquired immunity. IL-1β is able to influence regulatory T-lymphocytes, which leads to the masking of autoreactive effector cells from the control of suppressor cells. IL-1β is able to be included in the negative modulation of the immune response. This can be explained by the fact that due to a decrease in the IL-1β level the immune system will be more ready to synthesize antibodies, especially to autoantigens [22]. TGF-β1 in normal concentrations also inhibits the function of T cells with regulatory properties, therefore its reduced concentration promotes the synthesis of autoantibodies.

It was demonstrated that the functioning of the immune and reproductive systems of the male body is closely related and interdependent. Immune reactivity disorders accompanying the development of autoimmune pathology are associated with reproductive function disorders in men.

Conclusion

The functioning of the immune and reproductive systems of the male body is closely related and interdependent. Disorders of immune reactivity, which accompany the development of autoimmune pathology associated with disorders of reproductive function in men. The largest number of deviations of immune reactivity was found in infertile men with concomitant autoimmune diseases.

DECLARATIONS:

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

Data Transparency

The data can be requested from the authors.

Statement of Ethics

The authors have no ethical conflicts to disclosure.

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Consent for publication

All authors give their consent to publication.

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