

Cytokines and Infertility Influence of Cytokines and Local Inflammation in Women of Reproductive Age with Infertility

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Abstract

Objective: The objective of the study was to investigate concentrations of cytokines in peritoneal fluid from women with infertility and chronic infectious inflammation (CII) of reproductive system.

Methods: 65 patients were included to the study: 50 patients were diagnosed with tubo-peritoneal infertility and CII of reproductive system in remission (CII was of bacterial origin); 15 patients were almost healthy age-matched woman. The concentrations of TNF- α , IL-4, IL-6, IL-10, monocyte chemo attractant protein-1 (MCP-1), IFN- γ and sVCAM-1 were measured in peritoneal fluid using enzyme-linked immunoassay.

Results: Peritoneal fluid levels of TNF- α , IL-4, IL-6, IFN- γ , MCP-1, sVCAM-1 were significantly ($p < 0.05$) higher in patients with infertility and CII compared to control group. The most significant difference (4.52 times) was found for IFN- γ concentrations. The concentration of IL-10 was not significantly differing between the groups.

Conclusions: Local inflammation and activation of immune reactions with cytokines imbalance in patients with CII of reproductive system may play a role in the pathogenesis of tubo-peritoneal infertility.

Keywords: Chronic inflammation; Cytokines; Immune response; Infertility

Introduction

One of the most important and urgent reproductive health problems is the high rate of female infertility. Despite the development of new reproductive technologies, its prevalence is not decreasing [1-4].

Based on literature data and the results of our observations, chronic infectious and inflammatory diseases of reproductive system could be considered as important factors of infertility pathogenesis [5-8].

There are published data on the role of immune system disturbances (including changes in different cytokines concentrations and cytokines imbalance) in the pathogenesis of infectious and inflammatory diseases of reproductive system. The influence of cytokines and growth factors imbalance on synthesis of sex hormones which are critical for reproduction has also been suggested. Chronic cytokines imbalance with increasing concentrations of pro-inflammatory interleukins may lead to immunoendocrine dysregulation and infertility [1,9-14].

The major cytokine types during physiological course of pregnancy are cytokines mediating Th2-immune response. They block cellular immune response and, therefore, enable trophoblastic invasion and further development of pregnancy [11,15]. Cytokines imbalance and immunoendocrine dysregulation in patients with CII may contribute to infertility.

Although recent studies have elucidated different aspects of mechanisms of infertility in patients with CII of reproductive system,

many issues related to interactions in pro-/anti-inflammatory and regulatory cytokines network remain disputable.

For understanding the underlying mechanisms of infertility associated with chronic infectious inflammation of reproductive system it seems important to detect biologically active substances and measure their concentrations locally in peritoneal fluid which is in direct contact with the area of inflammation.

The purpose of the study was to investigate cytokines concentrations in peritoneal fluid from women with infertility and chronic infectious inflammation of reproductive system.

Materials and methods

65 women of reproductive age were examined and recruited to the study. The recruitment of the patients who met the inclusion criteria was conducted in 2013-2015. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Federal State Institution "Research Institute of Experimental and Clinical Medicine", Russian Science Academy (Protocol#7, 01.02.2013). All patients signed an informed consent form prior to participation in the study (noting that they agree to give a sample of peritoneal fluid and are aware that the study results will be used for scientific purposes).

The type of the study has features of cross-sectional study. The study group (Group I) consisted of 50 patients aged 23-36 years (mean age-28.5 years) diagnosed with tubo-peritoneal infertility associated with chronic inflammation of reproductive system. The causative agents of the inflammation were bacteria and their associations (Table 1) found by bacteriological study. Patients with other causative agents

(fungus, viruses) were not included to the study. The duration of infertility ranged from 4 to 13 years (mean duration - 9.7 years). Inclusion criteria for participation in the study were the diagnosis of tubo-peritoneal infertility and chronic infectious inflammatory diseases of reproductive system in remission stage with the remission duration not less than 30 days. The diagnoses were based on medical history, clinical, laboratory and additional methods of examination. Patients with fever, pathological changes in white blood cell count, erythrocyte sedimentation rate level and other signs of active inflammation were excluded from the study. The patients did not receive any anti-inflammatory and anti-bacterial therapy-this therapy was cancelled 3 months before peritoneal fluid collection. The control group (Group II) included 15 healthy women aged 25-37 years (mean age-29.5 years) with no history of chronic infectious and inflammatory diseases of any organ or system including reproductive system. The laparoscopy in control group was performed for diagnostic purposes; all women included to the study as control were diagnosed as healthy.

An exclusion criterion for participation in the study was any acute inflammatory disease of bacterial or viral genesis within three months prior to recruitment to the study. Patients with endocrine disorders which may affect the synthesis of sex hormones and, therefore, the functional state of the immune system, as well as patients with autoimmune and malignant diseases were also excluded from the study.

The peritoneal fluid was collected during routine laparoscopy. To avoid the contamination of peritoneal fluid with blood the aspiration

of peritoneal fluid was done at early stages of operation right after placing of laparoscope. The obtained samples were placed in a tube and centrifuged at 1500 rpm for 10 minutes. Then the top layer was collected, placed into sterile plastic tubes and frozen at -70° C. The samples were assayed within 6 months after collection.

All the laboratory analyses of peritoneal fluid were performed using commercially available enzyme immunoassay (ELISA), according to the instructions in the manuals. ELISA results were recorded on vertical photometer "Uniplan" at 450 nm wavelength.

TNF- α , IL-4, IL-6, IL-10, and interferon-gamma (IFN- γ) concentrations were measured using "Cytokine" kit (Russia). Monocyte chemotactic protein-1 (MCP-1) was measured using "Vector-Best" kit (Russia). Soluble vascular cells adhesion molecule-1 (sVCAM-1) was measured by "Bender Med Systems GmbH" kit (Austria).

All statistical analyses were performed using STATISTICA version 10 software package (Stat Soft Inc., USA). To investigate differences in variables between two study groups we used Mann-Whitney independent samples t-test. Numbers were expressed as mean \pm standard error of the mean (M \pm m). A P value of <0.05 was considered significant (95% confidence interval).

Results

Bacteria	n	%
<i>Enterococcus faecalis</i>	6	9.2
<i>Escherichia coli</i>	17	26.2
<i>Staphylococcus epidermidis</i>	9	13.9
<i>Escherichia coli</i> and <i>Staphylococcus epidermidis</i>	12	18.5
<i>Escherichia coli</i> and <i>Enterococcus faecalis</i>	7	10.8
<i>Escherichia coli</i> , <i>Staphylococcus epidermidis</i> and <i>Enterococcus faecalis</i>	14	21.5

Table 1: Bacteria associated with chronic inflammation of the reproductive system.

Variables	Group I (n=50)	Group II (n=15)
TNF- α pg/ml	23.6 \pm 0.9*	13.7 \pm 1.1
IL-4 pg/ml	23.7 \pm 1.6*	15.7 \pm 0.8
IL-6 pg/ml	68.1 \pm 10.9*	19.3 \pm 1.3
IL-10 pg/ml	23.3 \pm 3.1	18.5 \pm 2.6
IFN- γ pg/ml	94.2 \pm 8.1*	20.8 \pm 1.9
MCP-1 pg/ml	394.6 \pm 62.1*	148.2 \pm 3.1
sVCAM-1, pg/ml	352.8 \pm 47.3*	117.8 \pm 5.07

Table 2: Comparison of levels of cytokines, MCP-1 and sVCAM-1 in peritoneal fluid from women with infertility and normal women. The values are presented as M \pm m. *Differences between groups are significant at the p<0.05 level.

Table 2 shows the results of peritoneal fluid analysis. The concentration of pro-inflammatory cytokine TNF- α in peritoneal fluid of patients in the study group was 1.72 times higher than in the control group, and this difference was statistically significant (p<0.05). We suggest that these results reflect the activity of local inflammation during the remission stage in the absence of clinical signs of systemic activation of inflammation. Increased TNF- α production was described in complications of physiological pregnancy [16], and its local increase could be considered as unfavorable factor for conception and gestation.

Peritoneal fluid levels of IFN- γ were significantly (p<0.05), 4.52 times higher in patients with tubo-peritoneal infertility and chronic inflammation of reproductive system compared to control group. IFN- γ is considered an important endogenous modulator required for all stages of immune response. The increase of IFN- γ production may reflect the activation of Th1 immune response to antigen stimulation resulted from chronic inflammation. Increased concentrations of IFN- γ were also found in a number of pregnancy complications, which

make it possible to suggest that this cytokine could be involved to infertility pathogenesis.

IL-4 is an inductor of humoral immune response and its increase is observed in infectious processes of bacterial origin. The concentration of IL-4 in peritoneal fluid of patients in the study group was 1.51 times higher than in the control group ($p < 0.05$). The increased IL-4 levels in patients with chronic infectious inflammation of reproductive system could be explained by the development of humoral immune reactions and antibodies production in response to chronic antigen exposure. Overexpression of IL-4 could be associated with the development of local fibro plastic and adhesive processes [3,10,11,15].

IL-6 is a pro-inflammatory cytokine involved in inflammatory and infections responses and contributing to transformation from acute to chronic inflammation and development of autoimmune reactions. Patients from the study group had significantly (3.5 times, $p < 0.05$) increased level of IL-6 in peritoneal fluid compared to control group.

Peritoneal fluid concentrations of IL-10, an anti-inflammatory and immunosuppressive cytokine, were not significantly differing between the two groups.

MCP-1 has been shown to stimulate intensive macrophage recruitment to the sites of inflammatory-destructive process. Concentrations of MCP-1 in peritoneal fluid of patients from the study group were significantly, 2.6 times higher than in the patients from the control group ($p < 0.05$). The local raise of MCP-1 in peritoneal fluid of patients with infertility and chronic inflammation can be interpreted as the evidence of inflammatory-destructive process activity.

The concentration of sVCAM-1 was found to be approximately 3 times higher in the study group vs control ($p < 0.05$) that probably reflects the severity of inflammation and endothelial dysfunction.

Conclusion

Despite the absence of clinical signs of inflammation in woman with infertility and CII of reproductive system, the activity of local inflammation as well as pro-, anti-inflammatory and regulatory cytokines imbalance was detected. It was supported by the significant ($p < 0.05$) increase of TNF- α , IL-4, IL-6, IFN- γ and MCP-1 peritoneal fluid levels in the study group compared to control. Chronic inflammation with chronic antigen exposure presumably resulted in the increase of humoral and cellular immunity inducers (IFN- γ , IL-4) concentrations. These processes may trigger the so-called "circulus vitiosus" and lead to sustained inflammation even during clinical remission. Detected cytokines imbalance could contribute to infertility by creating unfavorable for gestation "cytokines background" or by promoting local proliferative and adhesive processes as a result of chronic inflammation. Further understanding of the role of cytokines and growth factors in infertility pathogenesis may provide new insights into its prevention and treatment in woman with chronic infectious diseases of reproductive system.

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