# Psychoimmune Interactions in Women of Reproductive Age with Endometriosis

**R. F. Nasyrova, L. S. Sotnikova*, N. V. Baystrukova*, G. V. Krivoschchekova*, V. V. Novitsky*, I. E. Kupriyanova, V. Ya. Semke, I. O. Naslednikova*, and A. N. Baykov***

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Psychoimmune interactions were studied in women of reproductive age with endometriosis. Pronounced immunological shifts manifested in a shift of the T-cellular immunity, resulting in imbalanced production of pro- (IL-1β, IL-2, IFN-γ) and anti-inflammatory (IL-4) cytokines. Significant correlations between the severity of mental shifts and immunopathogenetic factors in the studied patient population demonstrated the psychoneuroimmune nature of endometriosis.

**Key Words:** *psychoneuroimmunology; mental disorders; immune disorders; endometriosis*

Endometriosis is diagnosed in 7-50% women and is one of the most incident causes of infertility. This hormone-dependent disease develops under conditions of disorders in immune homeostasis and consists in benign growth of uterine tissue, similar to the endometrium by morphological and functional characteristics, outside the uterine cavity [3]. Despite numerous studies, the etiology and pathogenesis of this disease remain an object of discussions. However, the scientists are unanimous about one thing – the disease is multifactorial.

Focused attention to evaluation of mental health of women suffering from this disease can be explained by its leading position in the structure of stubborn pain syndrome. Today endometriosis is the most incident and severe gynecological disease in women of reproductive age, which negatively tells on their mental and physical status and quality of life. Stubborn lasting painful manifestations in endometriosis are risk factors for the development of secondary mental disorders; anxiety and depression augment subjective painful sensations. The study of the relationship between pain and depression in the patients with endometriosis revealed high incidence of affective disorders in this patient population [14,16]. Chronic mental disorders become the main predictor determining the clinical and social prognosis [4,15].

Understanding of the important role of the immune system in the development of endometriosis suggested new approaches to studies of its pathogenesis and to the treatment of patients suffering from this disease. The results of many-year studies demonstrated a relationship between the mental and immunological reactions. The immune and nervous systems are components of the adaptation mechanism with a common transmitter system. Disorders in the mechanism of adaptation manifest by changes in the transmitter processes in both systems [1,10]. According to current views, disorders in immunocyte differentiation and in the cytokine profile in endometriosis are pathogenetically significant [2,16]. Cytokines modulate emotional status and activation of cognitive functions through release and exchange of transmitters. Imbalance of cytokine production, in turn, causes disorders in the mental status, motivation, and behavior. On the other

Institute of Mental Health, Siberian Division of the Russian Academy of Medical Sciences; *Siberian State Medical University, Federal Agency for Health Care and Social Development, Tomsk, Russia. ***Address for correspondence:*** nreginaf@rambler.ru. R. F. Nasyrova

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hand, the nervous and mental disorders lead to immune system dysfunction [13].

We studied the psychoimmune relationships in endometriosis in women of reproductive age.

## MATERIALS AND METHODS

The study was carried out in 50 women aged 18-45 years (mean age 35.4±1.2 years), 30 of these with internal genital (adenomyosis) and 20 with external genital endometriosis. All women signed informed consent to participation in the study. The women were observed at Regional Center for Family Planning and Reproduction (Tomsk). Patients with severe concomitant diseases were excluded from the study. The diagnosis was verified by ultrasonic examination of pelvic organs (82%), histeroscopy on days 4-7 of the cycle (56%), laparoscopy (92%), and evaluation of CA-125 oncomarker (74%). Thorough clinical and anamnestic studies were carried out in all women: the complaints were recorded and classified, case history was studied, the presence and type of extragenital disease were evaluated, and hereditary factors were analyzed.

Evaluation of the severity of pelvic organs involvement in endometriosis in accordance with the Classification of the American Society of Fertility (1999) showed stage I of the process in 22%, stage II in 42%, stage III in 24%, and stage IV in 12% patients. The majority (86%) of women had a history of reproductive function failure because of "delayed first pregnancy", 48% suffered from menstrual function disorders in the pubertal and early reproductive age (12% had menometrorrhagia, 8% oligomenorrhea, 28% algodysmenorrhea), and 64% had confirmed family history of endometriosis.

Despite the principal differences of the clinical picture of various forms of endometriosis, the leading symptom (in 96% of all examined patients) was pelvic pain resistant to courses of anti-inflammatory therapy and augmenting during coitus and menses. The clinical picture of the painful syndrome manifested with the beginning of menses (92%). This symptom often led to disability, while pain during coitus made the majority of them (74%) avoid sexual life, this largely preventing the solution of the second problem, anovulatory sterility. Control group consisted of 30 healthy women with similar characteristics.

Clinical psychopathological method and methodological instruments Hamilton anxiety scale (HAS), Hamilton depression rating scale (HDRS), Aleksandrovich clinical questionnaire (a variant of B. D. Karvasarsky questionnaire [6]), and Holmes–Rahe social adaptation scale were used in the study.

Immunophenotyping of peripheral blood lymphocytes by CD markers (CD3, CD4, CD8, CD16, CD25, CD72, and CD95) was carried out by the immunocytochemical method. The results were expressed in percent and absolute values. The concentrations of immunocytokines (IL-1β, IL-2, IL-4, IFN-γ) in culture fluids were measured. Mononuclear cells were isolated in Ficoll-Paque density gradient (Pharmacia) and cultured ($2\times10^6$ cell/ml) in RPMI-1640 with 10% inactivated FCS, 0.3 mg/ml L-glutamine, 10 mM HEPES (Flow), and 100 μg/ml gentamicin at 5% $CO_2$ for 24 h. Cytokine production was stimulated by adding phytohemagglutinin (PHA) to the medium. EIA was carried out according to instructions for commercial kits (Procon). Optical densities of solutions were recorded on a Multiskan EX microplate photometer (ThermoLabSystems). Cytokine concentrations were calculated by calibration curves.

The results were processed using standard Statistica 8.0 software. The normality of data distribution was verified by the Kolmogorov–Smirnov test. The equality of selected means was tested by the Student *t* test and Mann–Whitney *U* test. Analysis of correlations of the psychometric and immunological values was carried out using Pirson's coefficient of correlations (*r*).

## RESULTS

Evaluation of the mental status of patients with endometriosis detected depressive symptoms in 78% (HDRS score 11.8±1.2). In accordance with this objective clinical rating, the majority of women (74%) had been surviving a petit mal depression during the study, 22% had a depressive episode of medium severity, and 4% had a severe depressive episode. It is noteworthy that the women noted a relationship between aggravation of depressive moods and emergence of painful symptoms in the reproductive system.

The degree of stress loading in patients with endometriosis was 223.4±34.8 points according to T. H. Holmes score and corresponded to the critical level of stress resistance. As generally stress is closely related to speculations related to potential threat, and hence anxiety is the predominating and obligate element of stress and an important component of the pathogenesis of somatic disease. The mean group value indicated predominating moderate level of anxiety in the patients (HAS score 21.2±1.7). The mental component of anxiety was the most incident and intense (HAS score 12.5±1.3 points) in the structure of mental disorders, while somatic manifestations were less pronounced (8.7±1.1 points). According to published data, one of the main mechanisms of combined anxiety and depression symptoms phenomenon in this patient population was changed activity of neurotransmitter activities in anxiety. This imbalance led to the development of an

opposite status by the reduced regulation mechanism, that is, anxiety transformed into depression [7].

Anxiety and depression were as a rule co-morbid in examined patients. Analysis of correlations showed positive correlations between anxiety level and intensity of depressive symptoms ($r$=0.41 at $p$<0.001). The anxiety symptoms predominated over depression symptoms in the clinical picture of mental disorders in the studied patient population. It is noteworthy that combination of anxious and depressive disorders camouflaged the depression symptoms, thus determining the deterioration of the prognosis for these disorders. A total of 72% patients complained of asthenic manifestations (reduced working capacity, rapid fatigue, disorders in concentration of attention and memory). Half (52%) of the patients complained of sleep disorders, 46% of them suffering from presomnic insomnia, 28% had superficial sleep with frequent awakening episodes, and 36% patients complained of having no proper sleep at night and felt tired in the morning.

Evaluation of the subpopulation composition of the peripheral blood lymphocytes demonstrated polarization of the immune response towards the Th1-route. This phenomenon manifested by a significant reduction of activity of cytotoxic T-lymphocytes, carrying surface CD8 molecules, in comparison with the parameters in healthy women (Table 1). The percentage of $CD4^+$ cells with helper activity increased significantly, and hence, the immunoregulatory index increased in comparison with the control.

On the other hand, the absolute counts of cells with cytotoxic potential ($CD16^+$) were significantly lower in the studied patients in comparison with donors (Table 1). Study of the B-component showed a statistically significant ($p$<0.01) elevation of the absolute count of $CD72^+$ lymphocytes in examined patients in comparison with the controls (Table 1). The count of T-lymphocyte subpopulation expressing CD25 activation marker, for which IL-2 served as the ligand, was significantly lower in the studied patient population than in healthy controls (Table 1). The study of lymphocyte sensitivity to proapoptogenic signals showed significant changes in this parameter in women with endometriosis, which was shown by reduction of the absolute count and percentage of cells carrying $CD95^+$ receptor on their surface (Table 1).

According to modern concepts, stimulation of Th1-lymphocytes, conjugated with the production of the key cytokines IL-1β, IL-2, and IFN-γ, stimulates cellular immunity, and the immune response determi-

**TABLE 1.** Subpopulation Composition of the Peripheral Blood Lymphocytes in Healthy Women and Patients Suffering from Endometriosis ($X$±$m$)

| Parameter | | Group | |
|---|---|---|---|
| | | healthy women of reproductive age | women of reproductive age suffering from endometriosis |
| $CD3^+$ | % | 67.53±4.60 | 52.87±3.87 |
| | $\times10^9$/л | 1.31±0.12 | 1.28±0.09 |
| $CD4^+$ | % | 38.51±3.07 | 67.43±4.09* |
| | $\times10^9$/л | 0.49±0.05 | 0.78±0.06* |
| $CD8^+$ | % | 32.13±2.76 | 21.97±1.92* |
| | $\times10^9$/л | 0.57±0.04 | 0.32±0.02* |
| Immunoregulatory index ($CD4^+/CD8^+$) | | 1.23±0.08 | 1.95±0.14* |
| $CD72^+$ | % | 16.14±1.27 | 15.74±1.36 |
| | $\times10^9$/liter | 0.29±0.02 | 0.46±0.04* |
| $CD16^+$ | % | 12.64±1.03 | 7.04±0.59* |
| | $\times10^9$/л | 0.21±0.02 | 0.14±0.01* |
| $CD95^+$ | % | 8.01±0.75 | 3.89±0.04* |
| | $\times10^9$/л | 0.23±0.02 | 0.13±0.09** |
| $CD25^+$ | % | 17.09±1.36 | 8.11±0.76* |
| | $\times10^9$/л | 0.27±0.02 | 0.46±0.03** |

**Note.** Here and in Table 2: *$p$<0.05, **$p$<0.01, ***$p$<0.001 in comparison with the corresponding parameters in healthy women.

nation by the humoral type is realized under conditions of predominant effect of Th2-cytokines, of which IL-4 is the discriminant one [8]. Study of the cytokine-producing function of peripheral blood mononuclears in endometriosis patients showed shifts in the studied parameters. Study of IFN-γ production by mononuclears showed a significant increase of its spontaneous and PHA-stimulated secretion in comparison with the levels in donors (Table 2). Previous studies showed that elevation of IFN-γ level was clinically paralleled by disorders in the psychoemotional sphere, memory, cognitive and mnestic activities, by reduction of attention concentration, sleep disorders, and a significant reduction of performance abilities [5].

It was found that disorders in IL-1β level also led to psychoneurological disturbances. Increase in the level of this IL in infectious diseases was associated with anorexia, increase of slow-wave sleep, and reduction of motor activity. Anti-inflammatory IL-10 (IL-1β receptor antagonist) presumably limited activities of proinflammatory cytokines [5]. Our studies showed than the constitutional production of IL-1β by immunocompetent cells increased, while induced synthesis of this cytokine reduced in comparison with its values in the control group (Table 2).

Another key Th1-cytokine, IL-2, also contributed to modulation of mental activity in endometriosis. Intracentral injection of IL-2 caused a reduction of the electrical activity of the hypothalamic supraoptical and paraventricular nuclei. Microphoretic delivery of IL-2 to the hippocampal sensorimotor cortical neurons modified the neuronal activity pattern. On the other hand, the cytokine effect depended on the CNS reactivity. The dual nature of the cytokine effect manifested by its capacity to stimulation of the neuronal pulsed activity and to inhibition of the neuronal discharges, which was determined by individual reactions to stress factors [5]. Immunological studies revealed a statistically significant ($p<0.01$) elevation of the basal production of IL-2, discriminant for realization of the Th1-mediated immune response in the studied patient population, the level of induced synthesis of this cytokine remaining comparable to that in healthy women (Table 2).

Recent studies demonstrated the impact of chronic stress, present in the studied patient population, on the status of the immune system and psychoemotional sphere, which were characterized by changes in IL-4 synthesis by peripheral blood mononuclear cells [8]. The constitutional and PHA-stimulated mononuclear production of IL-4 stimulating T-helper polarization towards Th2 was significantly decreased in patients compared to controls (Table 2). These data indicated pronounced immunological shifts in endometriosis patients, manifesting in deficiency of T-cellular immunity presumably resulting in the detected imbalance of pro- (IL-1β, IL-2, IFN-γ) to anti-inflammatory (IL-4) cytokine production.

Modern studies in psychoneuroimmunology demonstrated that the immunoregulatory processes were an obligatory component of intricate network of adaptive reactions. Our study of psychoimmune interrelationships detected significant correlations between the immune factors involved in the pathogenesis of the endometrioid disease and the psychopathological status of patients. Depression and anxiety were among the basal mental reactions. Positive correlations between the severity of depressive symptoms and spontaneous production of IL-2 ($r=0.46$, $p<0.01$) and induced synthesis of proinflammatory cytokines IL-1β ($r=0.38$,

**TABLE 2.** Spontaneous and PHA-Induced Production of Immunocytokines by Peripheral Blood Mononuclear Cells (pg/ml) in Healthy Women and Patients Suffering from Endometriosis ($X\pm m$)

| Parameter | | Group | |
|---|---|---|---|
| | | healthy women of reproductive age | women of reproductive age suffering from endometriosis |
| IL-1β | spontaneous | 50.91±3.09 | 121.28±9.62** |
| | PHA-stimulated | 132.87±8.97 | 95.46±9.32* |
| IL-2 | spontaneous | 42.70±3.65 | 53.25±10.15 |
| | PHA-stimulated | 196.30±18.42 | 105.63±15.74** |
| IL-4 | spontaneous | 58.89±9.34 | 116.33±11.79*** |
| | PHA-stimulated | 146.05±18.04 | 236.31±13.19** |
| IFN-γ | spontaneous | 106.01±12.78 | 146.82±18.15** |
| | PHA-stimulated | 276.32±22.09 | 146.82±18.15** |

$p<0.05$) and IFN-γ ($r=0.43$, $p<0.05$) were detected. Inverse relationships between depression symptoms and the counts of the following lymphocyte subpopulations were found: T-lymphocytes, identified as $CD3^+$ cells ($r=-0.39$, $p<0.05$), cytotoxic lymphocytes ($r=-0.42$, $p<0.05$), NK cells ($r=-0.53$, $p<0.01$), and stimulated T-lymphocytes carrying surface $CD25^+$ molecules ($r=-0.36$, $p<0.05$). These associations confirmed the hypothesis on the involvement of the cytokine network functioning in the pathogenesis of affective disorders.

Some associations of anxiety level are worthy of note: positive correlations between anxiety level and activity of lymphocytes carrying the CD16 cell differentiation marker ($r=0.38$, $p<0.05$) and between anxiety level and levels of spontaneous production of proinflammatory IL-1β ($r=0.46$, $p<0.01$) and IL-2 cytokines ($r=0.55$, $p<0.01$) and inverse correlations between anxiety level and total counts of T-helper $CD4^+$ ($r=-0.33$, $p<0.05$) and $CD8^+$ lymphocytes ($r=0.36$, $p<0.05$). It was previously hypothesized [9] that immune dysfunction associated with depression was caused by changes in the cytokine pattern and contributed to emergence of sleep disorders and asthenic symptoms. Basic studies in neurosciences proved that proinflammatory cytokines modulated the CNS activity and, presumably, were involved in the emergence of insomnia and asthenia symptoms [11].

Study of the relationship between the intensity (classified by Alexandrovich' score) of manifestations of sleep disorders and asthenia (frequent comorbid symptoms of affective disorders) and immune parameters also revealed some significant correlations. Insomnia formed positive relationships with the basal level of Th1-cytokine IL-1β ($r=0.37$, $p<0.05$) and Th2-cytokine IL-4 ($r=0.41$, $p<0.05$) and with NK cell count in the peripheral blood ($r=0.34$, $p<0.05$). Analysis of correlations showed positive relationships between the intensity of asthenic manifestations and spontaneous production of IL-2 ($r=0.38$, $p<0.05$) and level of B-lymphocyte population in the peripheral blood ($r=0.35$, $p<0.05$); opposite trends were found for the count of cytotoxic T-lymphocytes ($r=-0.39$, $p<0.05$). These data confirmed the theory of genesis of asthenia as a multifactorial disorder of neuroimmune interactions, leading to immune imbalance and CNS dysregulation.

Hence, the results of the study, demonstrating close psychoimmune interactions in endometriosis in women of reproductive age suggest regarding the pathogenesis of endometrioid disease from the standpoint of a close relationship of the mental status and immune and reproductive systems functioning. The elements of the universal functional neuroimmunoendocrine system are related to each other by mutual regulation for performance of a common function – homeostasis maintenance. This fact explains the formation of a combined disease of the component systems, irrespective of the initial disorder. Further comprehensive studies of the relationships between the pathological processes in the female reproductive system and mental status will make it possible to create highly effective integrative methods for prevention, therapy, and rehabilitation of women suffering from endometriosis.

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