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SYSTEMIC CYTOKINE DYSREGULATION IN WOMEN WITH PRIMARY TUBAL ECTOPIC PREGNANCY

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Abstract. *The study evaluated the characteristics of systemic cytokine regulation in women with primary tubal ectopic pregnancy. Serum concentrations of interleukin-4 (IL-4), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) were determined in 35 women with primary ectopic pregnancy before surgical treatment and compared with the corresponding parameters of 22 women with physiologically progressing pregnancy. Blood samples were obtained before surgery and before the administration of antibacterial or anti-inflammatory therapy. Cytokine concentrations were measured by solid-phase enzyme-linked immunosorbent assay. Women with ectopic pregnancy demonstrated a statistically significant increase in all studied cytokines. The serum IL-4 concentration was 39.42±2.34 pg/ml versus 18.41±1.45 pg/ml in the control group. The TNF-α concentration reached 82.04±11.58 pg/ml versus 35.14±7.78 pg/ml, IL-1β was 27.43±1.87 pg/ml versus 12.43±1.86 pg/ml, and IL-6 was 35.78±8.10 pg/ml versus 17.85±8.10 pg/ml. All intergroup differences were statistically significant at $p<0.05$. Compared with physiological pregnancy, IL-4 increased 2.14-fold, TNF-α 2.34-fold, IL-1β 2.21-fold, and IL-6 approximately 2.00-fold. The simultaneous elevation of pro-inflammatory and regulatory cytokines indicates systemic cytokine dysregulation in women with primary tubal ectopic pregnancy. The greatest relative increase was observed for TNF-α, while the combined elevation of TNF-α, IL-1β, and IL-6 reflected pronounced activation of inflammatory mechanisms. Increased IL-4 may represent a regulatory response aimed at limiting excessive inflammatory activity.The combined assessment of IL-4, TNF-α, IL-1β, and IL-6 may provide additional information about the severity and direction of immune-inflammatory alterations associated with ectopic trophoblast implantation.*

Key words: *ectopic pregnancy, tubal pregnancy, cytokines, IL-4, TNF-α, IL-1β, IL-6, inflammation, trophoblast invasion, immune dysregulation.*

Introduction. Ectopic pregnancy remains one of the most serious problems in modern obstetrics and gynecology. It is associated with a direct risk to the life and health of women and may have adverse long-term effects on reproductive function. According to the data presented in the dissertation, ectopic pregnancy accounts for approximately 1.3–2.6% of all pregnancies worldwide and occupies a leading position in the structure of emergency gynecological conditions. In the Republic of Uzbekistan, ectopic pregnancy is diagnosed in approximately 2–4% of pregnancies, and its incidence shows a tendency to increase (1,2).

The most frequent localization of ectopic pregnancy is the fallopian tube, which accounts for 97–98% of all cases. The ampullary part of the fallopian tube is involved most frequently. The pathogenetic basis of tubal pregnancy is impaired transport of the fertilized ovum through the fallopian tube and its subsequent implantation outside the uterine cavity. Chronic inflammatory diseases of the pelvic organs may cause structural and functional changes in the fallopian tubes, including fibrosis, adhesions, deformation of the tubal lumen, damage to the ciliated epithelium, and disturbances of neuromuscular regulation (3,5).

Inflammation is not only an etiological factor contributing to tubal dysfunction but also an essential component of trophoblast implantation. Physiological implantation is accompanied by a controlled local inflammatory response. Successful implantation requires balanced production of

cytokines, participation of immune-competent cells, regulation of trophoblast invasion, vascular remodeling, apoptosis, and formation of placental tissue. Disturbance of this balance may influence the depth and localization of trophoblast implantation (4,9).

Cytokines are soluble mediators of intercellular interactions that regulate the recruitment, activation, proliferation, and differentiation of immune cells. They determine the intensity, direction, and duration of inflammatory and immune responses. Cytokines act mainly through autocrine and paracrine mechanisms; however, some mediators, including TNF-α, IL-1, and IL-6, may also produce systemic effects (6,8).

Interleukin-1 and TNF-α are considered key regulators of inflammation. IL-1 activates endothelial cells, leukocytes, fibroblasts, and other cellular components of the inflammatory response. It promotes leukocyte adhesion and migration, stimulates the production of other cytokines, influences the coagulation system, and participates in connective tissue metabolism. TNF-α activates leukocytes and vascular endothelium and contributes to the production of acute-phase proteins. Increased production of TNF-α and IL-1 has also been associated with the development and severity of peritoneal adhesions (11).

IL-6 is involved in the systemic inflammatory response and reflects activation of immune-inflammatory mechanisms. Its production may increase under the influence of infection, tissue damage, and activation of monocyte-macrophage cells. Excessive or prolonged production of IL-6 may indicate an imbalance in the mechanisms controlling inflammation (1,7).

IL-4 has regulatory and anti-inflammatory properties. The induction of IL-4 may suppress excessive production of pro-inflammatory cytokines and limit damage to healthy cells and tissues. Therefore, an increase in IL-4 together with pro-inflammatory mediators should be interpreted not as an isolated anti-inflammatory effect but as a component of a complex regulatory response to pronounced immune activation (5,10).

The scientific literature analyzed in the dissertation contains conflicting data regarding cytokine changes in ectopic pregnancy. Some studies describe increased concentrations of pro-inflammatory mediators and reduced IL-4, whereas the results of the present investigation demonstrated simultaneous elevation of both pro-inflammatory and regulatory cytokines. This emphasizes the complexity of immune regulation in ectopic trophoblast implantation and the necessity of studying cytokines as an interconnected system rather than as isolated laboratory parameters (4,8).

The assessment of serum IL-4, TNF-α, IL-1β, and IL-6 may therefore improve the understanding of the immune-inflammatory mechanisms associated with primary tubal ectopic pregnancy (2).

Purpose of the study. To evaluate serum concentrations of IL-4, TNF-α, IL-1β, and IL-6 and determine the characteristics of systemic cytokine regulation in women with primary tubal ectopic pregnancy.

Materials and methods of research. The study was conducted among women who were examined and treated for ectopic pregnancy in the gynecological department of the Bukhara branch of the Republican Scientific Center for Emergency Medical Care.

At the initial stage of the immunological investigation, the serum cytokine profile was evaluated in 35 women with primary ectopic pregnancy. The comparison group included 22 women with physiologically progressing pregnancy.

The diagnosis of ectopic pregnancy was based on the results of clinical examination, gynecological evaluation, laboratory investigations, serum β-human chorionic gonadotropin determination, pelvic ultrasonography, and operative examination. The present article analyzes only the preoperative immunological results.

Peripheral venous blood was obtained from women with ectopic pregnancy before surgical intervention and before antibacterial or anti-inflammatory therapy was initiated. Serum concentrations of the following cytokines were determined:

interleukin-4;
tumor necrosis factor-alpha;
interleukin-1 beta;
interleukin-6.

The cytokine study was performed by solid-phase enzyme-linked immunosorbent assay using reagent kits manufactured by Vector-Best, Novosibirsk, Russian Federation, in accordance with the methodological recommendations of Cytokine LLC, Saint Petersburg.

Laboratory investigations were performed at the Bukhara branch of the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan.

Statistical processing was performed using Statistica 6.0 software. Microsoft Excel was used for data coding. Quantitative indicators were expressed as the mean and its error in the format M±m. The nonparametric Mann–Whitney U test was used to compare independent groups. Differences were considered statistically significant at $p<0.05$.

In addition to the values presented in the dissertation, the absolute difference, fold change, and percentage increase relative to the control group were calculated from the original mean values.

Research results. The analysis demonstrated pronounced changes in the serum cytokine profile of women with primary tubal ectopic pregnancy. Before surgery, concentrations of all four studied cytokines were statistically significantly higher than the corresponding concentrations in women with physiologically progressing pregnancy.

Table 1.

Serum cytokine concentrations in women with primary ectopic pregnancy before surgical treatment, M±m

Indicator, pg/ml	Physiological pregnancy, n=22	Ectopic pregnancy, n=35	Statistical significance
IL-4	18.41±1.45	39.42±2.34	p<0.05
TNF-α	35.14±7.78	82.04±11.58	p<0.05
IL-1β	12.43±1.86	27.43±1.87	p<0.05
IL-6	17.85±8.10	35.78±8.10	p<0.05

Note: p represents the statistical significance of differences compared with women with physiologically progressing pregnancy.

The IL-4 concentration in women with primary ectopic pregnancy was 39.42±2.34 pg/ml, whereas in women with physiological pregnancy it was 18.41±1.45 pg/ml. The absolute intergroup difference was 21.01 pg/ml. Thus, the concentration of IL-4 in ectopic pregnancy was 2.14 times higher than the control value.

The observed IL-4 elevation indicates activation of regulatory mechanisms together with the inflammatory response. IL-4 may suppress excessive production of pro-inflammatory mediators. Therefore, its increase may reflect an attempt by the immune system to limit tissue damage associated with abnormal trophoblast implantation and the underlying inflammatory process.

The TNF-α concentration showed the most pronounced relative and absolute increase among the studied cytokines. In women with ectopic pregnancy, it was 82.04±11.58 pg/ml, compared with 35.14±7.78 pg/ml in women with physiological pregnancy. The absolute increase was 46.90 pg/ml, while the ectopic pregnancy value was 2.34 times higher than the control value.

TNF-α is an important effector and regulatory mediator of inflammation. An increase in its serum concentration may reflect activation of monocytes, macrophages, endothelial cells, and other components of the immune response. In the context of tubal pregnancy, increased TNF-α may be

associated with inflammatory alterations of the fallopian tube, abnormal trophoblast invasion, endothelial activation, and tissue damage.

The serum IL-1β concentration in the ectopic pregnancy group was 27.43±1.87 pg/ml, whereas in the physiological pregnancy group it was 12.43±1.86 pg/ml. The absolute difference was 15.00 pg/ml, corresponding to a 2.21-fold increase.

IL-1β is one of the principal mediators initiating and maintaining the inflammatory response. It stimulates endothelial activation, leukocyte migration, production of other cytokines, and metabolic activity of connective tissue cells. Its elevation in women with ectopic pregnancy may reflect both the chronic inflammatory background of tubal damage and the local tissue response to ectopic implantation.

IL-6 was also significantly elevated. Its serum concentration was 35.78±8.10 pg/ml in women with ectopic pregnancy and 17.85±8.10 pg/ml in the control group. The absolute increase was 17.93 pg/ml. Thus, the IL-6 concentration was approximately twice the control level.

The increase in IL-6 supports the presence of systemic inflammatory activation. Since IL-6 participates in the regulation of the acute-phase response and immune cell activity, its elevation may indicate that abnormal trophoblast implantation is accompanied not only by local changes in the fallopian tube but also by measurable systemic immune alterations.

Table 2.

Magnitude of cytokine elevation in primary ectopic pregnancy relative to physiological pregnancy

Indicator	Absolute increase, pg/ml	Fold change	Increase relative to control
IL-4	+21.01	2.14-fold	+114.1%
TNF-α	+46.90	2.34-fold	+133.5%
IL-1β	+15.00	2.21-fold	+120.7%
IL-6	+17.93	2.00-fold	+100.4%

The comparative assessment showed that TNF-α had the largest absolute and relative increase. Its concentration exceeded the control value by 133.5%. IL-1β increased by 120.7%, IL-4 by 114.1%, and IL-6 by 100.4%.

Although all cytokines were significantly elevated, their biological roles were not identical. TNF-α, IL-1β, and IL-6 primarily reflected activation of the pro-inflammatory component of the immune response. IL-4 represented the regulatory component, which may become activated in response to excessive pro-inflammatory stimulation.

The simultaneous elevation of these mediators suggests that ectopic pregnancy is not characterized by a simple unidirectional shift toward inflammation. Instead, it is accompanied by dysregulation of the cytokine network, with simultaneous activation of damaging and compensatory mechanisms.

The pro-inflammatory cytokines in the ectopic pregnancy group increased approximately 2.00–2.34 times. Such a coordinated elevation supports the presence of systemic cytokinemia and indicates that the inflammatory process associated with ectopic implantation involves several interconnected immune pathways.

TNF-α and IL-1β may reinforce each other's biological effects. Both cytokines activate vascular endothelial cells, increase expression of adhesion molecules, promote leukocyte recruitment, and stimulate other inflammatory mediators. IL-6 may contribute to the systemic component of this response. The concurrent increase in all three mediators therefore indicates a broad activation of the inflammatory cascade.

From a pathogenetic perspective, this cytokine profile may be associated with the high frequency of chronic inflammatory diseases of the pelvic organs in women with ectopic pregnancy. Inflammation may damage the ciliated epithelium, alter tubal peristalsis, promote fibrosis and adhesion formation, and create conditions for retention and ectopic implantation of the fertilized ovum.

The findings also have potential relevance to postoperative complications. According to the pathogenetic concepts presented in the dissertation, increased production of TNF-α and IL-1 is associated with activation of fibroblasts, connective tissue remodeling, and peritoneal adhesion formation. Therefore, the cytokine changes detected before surgery may characterize not only the inflammatory background of ectopic pregnancy but also the biological conditions contributing to subsequent fibrotic and adhesive processes.

At the same time, the increased concentration of IL-4 may be interpreted as a compensatory immune-regulatory response. IL-4 is capable of limiting excessive production of pro-inflammatory cytokines. However, the persistence of markedly elevated TNF-α, IL-1β, and IL-6 indicates that this regulatory response is insufficient to restore normal cytokine balance.

Table 3.

Clinical and pathogenetic interpretation of the studied cytokine profile

Cytokine	Change in ectopic pregnancy	Principal interpretation
IL-4	Increased 2.14-fold	Activation of regulatory and compensatory anti-inflammatory mechanisms
TNF-α	Increased 2.34-fold	Pronounced activation of systemic inflammation, endothelial and immune-cell responses
IL-1β	Increased 2.21-fold	Activation and maintenance of inflammation, leukocyte recruitment, connective tissue response
IL-6	Increased approximately 2.00-fold	Systemic immune-inflammatory activation and acute-phase regulation
Combined profile	Simultaneous elevation of all four cytokines	Cytokine dysregulation with concurrent pro-inflammatory and compensatory regulatory activation

The combined cytokine profile provides more pathogenetic information than the assessment of a single mediator. An isolated increase in TNF-α or IL-1β would indicate inflammatory activation, but the simultaneous elevation of TNF-α, IL-1β, IL-6, and IL-4 demonstrates that both pro-inflammatory and regulatory mechanisms are involved.

The detected cytokine pattern may be described as a state of systemic cytokine dysregulation. It reflects the inability of regulatory mechanisms to fully control the inflammatory response associated with tubal pathology and ectopic trophoblast implantation.

The findings also indicate that the immune response in primary ectopic pregnancy is quantitatively pronounced. None of the studied mediators showed only a minor increase. Each cytokine was approximately two or more times higher than in physiological pregnancy, and all differences reached statistical significance.

A pathogenetic sequence may therefore be proposed on the basis of the results obtained. Chronic inflammatory changes in the fallopian tube contribute to structural and functional impairment. Abnormal implantation of the trophoblast into the altered tubal wall activates local immune cells and inflammatory mediators. Increased production of TNF-α, IL-1β, and IL-6 contributes to the systemic inflammatory response. Increased IL-4 reflects activation of compensatory regulation, but this response does not prevent the formation of a pronounced cytokine imbalance.

Table 4.

Integrated assessment of systemic cytokine changes in primary tubal ectopic pregnancy

Component of cytokine regulation	Studied markers	Main study finding	Biological significance
Pro-inflammatory activation	TNF-α, IL-1β, IL-6	All markers were significantly elevated	Evidence of pronounced systemic inflammatory activity
Regulatory response	IL-4	Significant 2.14-fold elevation	Compensatory limitation of excessive inflammation
Cytokine balance	All four markers	Simultaneous multidirectional activation	Systemic cytokine dysregulation
Potential clinical application	Combined assessment	Differences from physiological pregnancy were significant	Additional characterization of immune-inflammatory alterations

The present results do not support the use of any single cytokine as an independent diagnostic test for ectopic pregnancy. The diagnosis of ectopic pregnancy must continue to be based on clinical manifestations, serum β-human chorionic gonadotropin assessment, ultrasound findings, and operative verification when indicated.

However, the combined determination of the studied cytokines may complement conventional examinations by characterizing the immune-inflammatory state of the patient. Such information may be particularly important when planning postoperative observation and pathogenetically oriented rehabilitation aimed at reducing persistent inflammation, excessive connective tissue formation, and the risk of reproductive complications.

Thus, women with primary tubal ectopic pregnancy demonstrated significant and coordinated activation of the cytokine system before surgical treatment. The increase in TNF-α, IL-1β, and IL-6 indicated pronounced inflammatory activation, while the increase in IL-4 reflected a compensatory regulatory response. The simultaneous elevation of all four markers is a characteristic manifestation of cytokine dysfunction in primary ectopic pregnancy.

Conclusions. Women with primary tubal ectopic pregnancy had statistically significantly higher serum concentrations of IL-4, TNF-α, IL-1β, and IL-6 than women with physiologically progressing pregnancy.

The IL-4 concentration increased from 18.41±1.45 to 39.42±2.34 pg/ml, TNF-α from 35.14±7.78 to 82.04±11.58 pg/ml, IL-1β from 12.43±1.86 to 27.43±1.87 pg/ml, and IL-6 from 17.85±8.10 to 35.78±8.10 pg/ml.

The greatest relative increase was observed for TNF-α, which was 2.34 times higher than the control value. IL-1β increased 2.21-fold, IL-4 2.14-fold, and IL-6 approximately 2.00-fold.

The simultaneous elevation of pro-inflammatory cytokines and IL-4 indicates systemic cytokine dysregulation, combining pronounced inflammatory activation with an insufficient compensatory regulatory response.

The combined assessment of IL-4, TNF-α, IL-1β, and IL-6 may be used as an additional method for characterizing immune-inflammatory alterations in women with primary tubal ectopic pregnancy, but it should not replace established clinical, laboratory, and instrumental diagnostic methods.

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