Cytokine investigations in organs and tissues of experimental animals under prenatal hypoxic conditions of varying severity

The paper presents the research results of varying severity experimental hypoxia influences on the content of cytokines (IGF-1, TNF-α, IL-1β, IL-4, IL-6, IFN-γ) in organs, tissues and cells (blood, liver, brain, uterus, adrenal glands, placenta, placental lymphocytes and macrophages) of pregnant rats. The results showed the increase of pro-inflammatory cytokines synthesis on the local and system levels during experimental hypoxia augmentation. There was found the difference between placental lymphocytes and macrophages secretion. These changes of cytokine profile leads to local deficiency of anti-inflammatory factors and indicate on development of pregnancy complications and “mother-placenta-fetus” system dysfunction.

Zhanar Utesheva,
Natalya Kravtsova,
Ludmila Dzoz,
Talgat Kudaibergenov,
Tatyana Kravtsova

Scientific Center of Obstetrics
Kazakhstan

Keywords: Experimental hypoxia, insulin-like growth factor (IGF-1), tumor necrosis factor (TNF-α), interleukin (IL-1β, IL-4, IL-6), interferon-γ (IFN-γ).

UDC: 618.32:612.273.2]-003.96-072.7:577.121

Introduction

The prediction, early diagnostics and treatment of hypoxic states of mother and fetus are an important demographic issue today. The hypoxic states are holding one of the leading positions in the structure of prenatal mortality rate (up to 12.8%); the incidence among infants who underwent intrauterine hypoxia reaches 116.1-162 per 1000. The literature analysis shows that among all the problems of this aspect, a special place is taken by fetoplacental insufficiency which develops during pregnancy as a result of extra genital or obstetric pathology and leads to disorders of the fetal oxygen supply.

The placenta, being an interconnecting link between the organisms of mother and fetus, is one of the most complex tissues of higher mammals and humans. The metabolic processes that underlie its functional activity are very flexible, and the regulating mechanisms have increasing possibilities of differential control. The failure of adaptive-homeostatic reactions of the organ, its inability to maintain adequate interchange between the organisms of mother and fetus often lead to miscarriage and is accompanied by chronic hypoxia and/or hypotrophy of the fetus and is a major cause of prenatal morbidity and mortality (Orlov et al., 2003). In this case, various disorders of placental metabolism and its regulating mechanisms were observed (Krukier, 2009). An important role which predetermines future development of the placenta is played by a number of immunological factors including cytokines, growth factors, etc.

The study considers effects of hypoxia of varying severity on the content of cytokines in organs, tissues and cells of experimental animals.

Material and methods

The research was carried out on 80 female white out bread rats weighing 250-280g. Prenatal chronic hypoxia was reproduced by exposing the pregnant female rat to ambient air admixed with methylene chloride vapors (Nazyrov and Israilova, 1990). Exposition from 18 to 21 day of pregnancy caused mild hypoxia (20 rats), from 16 to 21 day of pregnancy - hypoxia of moderate severity (20 rats), from 14 to 21 day of pregnancy - severe prenatal hypoxia (20 rats). The control group was comprised of 20 animals.
The sampling of organs and tissues (blood, liver, brain, uterus, adrenal glands, placenta) was performed on 21 day of pregnancy (which is equivalent of full-term gestation) for the study of cytokines: insulin-like growth factor (IGF-1), tumor necrosis factor (TNF-α), interleukin (IL-1β, IL-4, IL-6), interferon-γ (IFN-γ).

The synthesis of cytokines by isolated lymphocytes and macrophages of placental decidua was performed in 24-hour cell culture supernatants in severe hypoxia.

The cytokines concentration was determined by solid-phase immunoenzyme method using ELISA kits “Best” (Russia) on automated analyzer PLAB Adaltis (Italy).

The severity of hypoxia of the animals was determined according to hypoxia assessment method proposed by A.T. Naziyrov, M.Z. Israilova in the certificate of authorship No 4807122/14 “Fetal hypoxia model” by the content of carboxyhemoglobin and acid-base balance profile.

The method of analysis of variance has been used for calculation of research findings. The significance of differences was determined by Student's coefficient. Only statistically significant differences were considered (p<0.05-p<0.001). The correlation analysis was performed by linear regression method using the Statgraf software.

**Research results**

See Figure 1 for the data obtained from blood cytokines profile study.

---

**Figure 1. The content of cytokines in the blood of pregnant rats with experimental hypoxia (%)**

![Graph showing cytokine levels in hypoxia conditions](image)

---

The results showed that with augmentation of experimental hypoxia, a dramatic increase in the level of pro-inflammatory cytokine TNF-α was observed. Thus, in case of severe hypoxia, the TNF-α level was 25 times higher than that of the control group. Also, the level of pro-inflammatory cytokine IL-1β grew in response to the increase of hypoxia severity. Its maximum concentration was also observed in the blood serum of rats in severe hypoxia and was 2.9 times higher than that of the control group (p<0.001).

The results showed that with augmentation of experimental hypoxia, a dramatic increase in the level of pro-inflammatory cytokine TNF-α was observed. Thus, in case of severe hypoxia, the TNF-α level was 25 times higher than that of the control group. Also, the level of pro-inflammatory cytokine IL-1β grew in response to the increase of hypoxia severity. Its maximum concentration was also observed in the blood serum of rats in severe hypoxia and was 2.9 times higher than that of the control group (p<0.001).

The level of anti-inflammatory cytokine IGF-1 with augmentation of hypoxia severity was gradually decreasing. The minimum values of IGF-1 (43.1% lower than those of the control group) were observed at severe hypoxia (p<0.001). The level of anti-inflammatory
IL-4 remained practically unchanged, showing, however, a tendency to decrease with an increase in severity of experimental hypoxia. The pattern of changes in the dynamics of IL-6 level differed from other cytokines as it demonstrated decrease of level by 17.6% in mild hypoxia. With further increase of severity of hypoxic pathology, the amount of IL-6 grew and was 30.8% higher than that of the control group (p<0.05) in severe hypoxia.

The general tendency of cytokines levels changes discovered in rats blood were the same in examined organs (Figure 2).

The studies revealed significant changes of humoral immunity factors in the brain and liver of rats with severe and moderate experimental hypoxia (p<0.001). The increase of pro-inflammatory cytokines and decrease in the level of inflammatory cytokines was observed. For example, the amount of TNF-α at severe hypoxia was 3.8 times higher than that of the control group in the brain and 92.9% higher in the liver; IL-1β was 2.5 times higher in the brain and 3 times higher in the liver.

The amount of IL-6, unlike the data obtained in the study of blood serum, uniformly grew with the increasing severity of hypoxia, and was 2 times higher than that of the control group in the brain and liver at the critical stage of hypoxic disorder. The levels of IGF-1 and IL-4 decreased 83.9% and 68.6% below the level of the control group in the brain tissue and 66.9% and 52.7% respectively in the liver tissue. It should be noted that significant decrease of IGF-1 (by 24%) and IL-4 (by 41%) in the brain was observed as early as in mild experimental hypoxia (p<0.05-0.001). The significant increase in the amount of TNF-α (by 37.9%) and decrease in the amount of IL-4 (by 25%) was observed in the liver in mild hypoxia.

The similar processes were observed in the uterus and adrenal glands of rats. The level of TNF-α at severe hypoxia was 2.3 times higher than that of the control group in the uterus, 48.7% higher in the adrenal glands; IL-1β was 84.3% higher in the uterus and 2.2 times higher in the adrenal glands. Moreover, a statistically significant increase of TNF-α in the uterus was observed as early as in mild hypoxia (p<0.001). The amount of IL-6...
uniformly grew with augmentation of hypoxia, and its value was 2.3 times higher in severe hypoxia than that of the control group in the adrenal glands and 41.3% higher in the uterus. Levels of IGF-1 and IL-4 decreased by 58.9% and 33.2% below the control level in the uterus and by 50.1% and 34% respectively in the adrenal glands (p<0.001).

Thus, studies of changes of cytokines in organs of experimental animals showed unidirectionality of these processes in all organs and tissues of rats, but the greatest changes in the conditions of experimental hypoxia were observed in brain and liver indicating maximum susceptibility of these organs to hypoxic influence.

The same tendencies of changes in the dynamics of the studied parameters as in the blood serum were observed in placental tissue of rats with experimental hypoxia (Figure 3). During the study the pattern of changes of cytokines in the placenta of experimental animals, it was discovered that the levels of IL-1β and TNF-α were increasing with augmentation of hypoxia and reached maximum increase of more than 62% and 3.4 times respectively at severe disease relative to the control group data. The values of IL-4 and IGF-1 under experimental hypoxic conditions gradually decreased. Maximum rate of changes was also observed at severe hypoxia, when the levels of IL-4 and IGF-1 were decreasing by 43.7% and 60.6% relative to the control values.

The amount of IL-6 varied irregularly. For example, mild hypoxia showed significant decrease by 23.3% in the level of IL-6 relative to the control group data (p<0.05). Then a gradual increase was observed with augmentation of hypoxia, and the level of IL-6 was 38.2% higher in severe hypoxia than that of the control group (p<0.01).

The statistical analysis revealed a direct correlation dependence between the contents of TNF-α and IL-1β in the blood serum of rats (r=0.65) in severe experimental hypoxia as well as between the levels of carboxyhemoglobin and TNF-α in the blood (r=0.72) and brain tissue (r=0.68). Also correlation dependencies were revealed between the levels of IL-1β in the brain and liver of experimental animals (r=0.61) as well as an inverse correlation between TNF-α and IGF-1 in the brain (r=-0.71) and placenta (r=-0.63).

The study of TNF-α, IFN-γ, IL-4 cytokines contenting the blood as well as deciduas of placetas of experimental animals with intrauterine hypoxia when synthesized by isolated lymphocytes and macrophages allowed revealing the following intensification of Th-1 response with increase in the synthesis of pro-inflammatory cytokines TNF-α and IFN-γ on the local and systemic level. In this case, macrophages in the placenta deciduas of experimental animals showed character of local immunodeficiency in relation to the production of these cytokines rather than lymphocytes. The anti-inflammatory cytokine IL-4 did not change at the systemic level and was somewhat increased due to the activity of lymphocytes rather than macrophages locally (Table 1, 2).
### TABLE 1. BLOOD CYTOKINES LEVEL IN CASE OF SEVERE EXPERIMENTAL HYPOXIA

<table>
<thead>
<tr>
<th>Cytokine (pg/ml)</th>
<th>Hypoxia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>60.35 + 2.75*</td>
<td>2.5 + 0.05</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>125.37 + 2.44*</td>
<td>60.25 + 1.30</td>
</tr>
<tr>
<td>IL-4</td>
<td>7.04 + 1.29</td>
<td>7.91 + 1.31</td>
</tr>
</tbody>
</table>

Note: * - p<0.05 - significant differences from control group data

### TABLE 2. SECRETION OF CYTOKINES BY LYMPHOCYTES AND MACROPHAGES OF RATS DECIDUAS IN CASE OF SEVERE EXPERIMENTAL HYPOXIA

<table>
<thead>
<tr>
<th>Cytokine (pg/ml)</th>
<th>Lymphocytes</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypoxia</td>
<td>Control</td>
</tr>
<tr>
<td>TNF-α</td>
<td>150.31 + 2.74*</td>
<td>79.56 + 0.95</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>348.45 + 1.8*</td>
<td>129.61 + 1.42</td>
</tr>
<tr>
<td>IL-4</td>
<td>10.24 + 1.35*</td>
<td>6.35 + 0.99</td>
</tr>
</tbody>
</table>

Note: * - p<0.045 - significant differences from control group data

These results indicate on for multi-directional secretion of cytokines by various deciduas cells in hypoxia. It is possible that prenatal hypoxia developing in complicated pregnancy is accompanied by uncontrolled cytokine cascade locally which leads to microcirculation disorders and development of placental pathology. In this case, a local immune deficiency in reference to macrophages had been revealed.

Thus, the study of cytokines profile in the experiment allowed revealing serious violations of immune regulation at hypoxia. The growth of synthesis of pro-inflammatory cytokines has been tested in dynamics on systemic and local level. The production of the anti-inflammatory cytokine IL-4 at the systemic level did not have significant changes; moreover, local deficiency of anti-inflammatory factors was revealed. The differences in cytokine secretion by lymphocytes and macrophages were observed at the local level in the placenta.

These changes of cytokine profile, in turn, transform the direction of the local immune response to fetal antigens and contribute to normal functioning of the placenta and embryos and, therefore, are a compensatory response for adaptation to hypoxic effects, whereas the balance disorder indicates on development of decomposition phase and is the cause for complications of pregnancy, which leads to dysfunction of the system “mother-placenta-fetus” and, consequently, abnormalities of the fetus. This was tested by breaking the antigen recognizing structures on T-lymphocytes which led to increased expression of E-receptors in the response of antigen-dependent E-rosette formation with placental antigens as well as increased Th-1 response with the increase of synthesis of pro-inflammatory cytokines TNF-α and IFN-γ at the systemic and local levels. Moreover, a local immune deficiency of macrophages has been revealed.

### References

