Analysis of the Concentrations of Interleukin 15 in Amniotic Fluid in the Second and the Third Trimesters of Pregnancy*
Cytokines play an essential role in the regulation of immunological responses. They interact within a complex network of connections within which the production of one particular cytokine influences the secretion of another cytokine [1].

Interleukin 15 (IL-15) is a glycoprotein secreted primarily by macrophages and monocytes. The gene that codes IL-15 has its locus in the chromosome 4q31 [2]. As far as molecular structure and biological characteristics are concerned, IL-15 is similar to IL-2 [2–6]. IL-15 plays an important role in the immunological response of the cellular type. IL-15 acts as a stimulator for proliferation and activity of the T lymphocyte and the NK (Natural Killer) cells. In tandem with IL-2, IL-15 stimulates the secretion of other cytokines: interferon (IFN)-gamma and TNF (tumor necrosis factor) by NK cells [2, 4, 5, 7–9]. IL-15 also plays a key role outside the immunological system – it is a stimulating factor in the angiogenesis process as well as a strong apoptosis inhibitor [10, 11]. It has been shown that the concentration of IL-15 and mRNA IL-15 in the placental tissue in pregnancies complicated by high blood pressure is significantly lower in comparison with pregnancies that are physiologically normal, which confirms the role of IL-15 in the process of human placenta invasion as a factor facilitating the process of angiogenesis [10]. IL-15 is present in the amniotic fluid beginning with the third trimester of gestation [12].

IL-15 and mRNA IL-15 has also been proven to be found in fetal membrane – the amniotic, amniochorial and deciduous membranes, as well as in the placenta [3, 9, 12–14]. Expression of IL-15 and its mRNA in the endometrium during the secretory phase as well as in the deciduous membrane points to the role of this cytokine in a regular monthly cycle as well as in gestation development during the phase of blastocyst implantation as well as the early phase of trophoblast invasion by way of stimulation of the proliferation of deciduous NK cells [7, 8, 15, 16]. IL-15 synthesis in the endometrium and deciduala are hormonally controlled – primarily by progesterone [7, 8, 16].

The objective of this study was to evaluate the fetus’s defense mechanisms on the basis of an analysis of the concentration of interleukin IL-15 in the amniotic fluids in the second and third trimesters of normal gestation.

Material and Methods

The clinical material consisted of 74 samples of amniotic fluid taken by way of amniocentesis from pregnant women being treated at the Clinic for Fetal Development Disorders of the Medical University Wrocław during 2004–2006. The study had the approval of the Bioethical Committee of the Medical University of Wrocław.

The gathered material was divided into two groups:

Group I – 45 pregnant women in their second trimester who had qualified for genetic amniocentesis due to: their age (over 35 years old), an increased risk of fetal defects as shown in double or triple testing, an abnormal ultrasonogram examination of the nuchal translucency and/or a high-risk genetic medical history.

The age of the pregnant women was between 24 and 46 years of age (with an average of 38.5 years old). Genetic amniocentesis was carried out in the second trimester between the 15th and 19th weeks. The amniotic fluids were sent to the Genetic Laboratory of the Wroclaw Medical University in order to determine the karyotype of the fetuses under examination. There were no abnormalities found in the results of the fetal cytogenetic tests.

Group II – 29 pregnant women in their third trimesters (between the 36th and 40th weeks) who had qualified for diagnostic amniocentesis in order to evaluate the biological maturity of the fetus. The age of the pregnant women was between 22 and 38 years old (with an average of 28.5).

The amniotic fluid samples were centrifuged at a speed of 3,000 spins per minute for 10 minutes, and then were refrigerated at a temperature of –82°C until such time as measurement were made. The concentration of IL-15 was marked with the ELISA method using a kit made by the R&D company. Method sensitivity was > 2 pg/ml.

Absorbance value was read at a wavelength of 450 nm.

Based on the Shapiro-Wilk test there was no reason to reject the hypothesis of normal schedule. The calculations were performed by using system STATISTICA 8.0, StatSoft, Inc. 2007. IL-15 concentration in the study groups were compared.
with each other using the Student t-test. The value \( p < 0.05 \) was assumed as statistically significant.

**Results**

Comparison of IL-15 concentration levels in amniotic fluids of groups I and II, i.e., in the second and third trimesters of gestation, are presented in Table 1.

The average level of IL-15 concentration in the amniotic fluid in second trimester gestation was 6.54 pg/ml, whereas in the third trimester it was 18.62 pg/ml. These differences were found to be statistically significant (\( p < 0.05 \)) – Fig. 1.

**Discussion**

The presence of cytokine and changes in their concentration in amniotic fluids (IL-1alfa, IL-1beta, IL-4, IL-6, IL-8, TNF – tumor necrosis factor) were analyzed primarily in light of their role in the development of intrauterine infection, risk of preterm birth, or preterm rupture of fetal membranes [17–23]. In accordance with the data presented in the literature, the concentration of proinflammatory cytokines (IL-1alfa, IL-1beta, IL-6, IL-8, IL-16, TNF) as well as IL-4 and IL-10 in the amniotic fluid are significantly higher in pregnancies affected by chorioamnionitis and are associated with an increased risk of preterm contraction activity [17–24].

Studies by Witczak et al. [23] showed that there is no correlation between levels of interleukin concentration (IL-1, IL-6, IL-8) in the amniotic fluid and that in the serum of pregnant women with complications of premature rupture of membranes. The authors suggest that there is a barrier that makes it impossible for cytokine to transfer through the placenta, meaning that there are two environments, that of the mother and that of the fetus, that function independently of each other and in which interleukin is secreted through the cells of the immunological system.

The development of the fetal immunological system begins at a very early stage of gestation – at about the 7th week. During this period, multi-potential cells develop into precursory cells for each of the specific lines of the immunological system. During its intrauterine development period, the undeveloped fetus is capable of immunological responses and of activating its own mechanisms of humoral and cellular defense, but the immunological response of the fetus and the newborn is not fully developed and therefore not fully effective [25].

Publication regarding interleukin 15 (IL-15) concentration in the amniotic fluid are few and are not unanimous [3, 12, 13]. The values of IL-15 concentration in the amniotic fluid in gestation development that the current study showed differ

<table>
<thead>
<tr>
<th>IL-15 Concentration (Stężenie IL-15) (pg/ml)</th>
<th>Minimum value (Wartość minimalna)</th>
<th>Maximum value (Wartość maksymalna)</th>
<th>Median (Mediana)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Grupa I) (n = 45)</td>
<td>2.31</td>
<td>15.87</td>
<td>5.73</td>
<td>3.24</td>
</tr>
<tr>
<td>Group II (Grupa II) (n = 29)</td>
<td>2.59</td>
<td>42.45</td>
<td>18.07</td>
<td>10.33</td>
</tr>
</tbody>
</table>

\( p < 0.05 \).

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from those found by the authors of other studies, though the current authors feel that it must be emphasized that the literature has very little to say on this subject.

The current study results confirm those reported by Fortunato et al. [12], namely, that IL-15 is present in the amniotic fluid from the beginning of the second trimester of gestation. According to Fortunato et al. the concentration of IL-15 in the amniotic fluid is highest during the second trimester of gestation (115 pg/ml), and in at term pregnancies (third trimester), one can observe a significant drop in the level of concentration to 61 pg/ml. The current study showed that the IL-15 concentration in the amniotic fluid was decidedly lower compared with the results given by Fortunato et al. and were 6.54 and 18.62 pg/ml in the second and third trimesters, respectively. Furthermore, the current study found that there was a significant increase in IL-15 concentration in the amniotic fluid as gestation progressed, a fact that was not observed by Fortunato et al.

In addition Fortunato et al. indicated that in the case of regular uterine contractions at term as well as in the case of pregnancies with a higher risk of preterm birth the concentration of IL-15 in the amniotic fluid could reach a value as high as 179–224 pg/ml.

A study by Heikkinen et al. [13] observed IL-15 concentration levels in the amniotic fluid in the second trimester of gestation at an average value of 47 pg/ml which was completely at odds with those indicated by Fortunato et al. [12] (2.4 times lower) as well as with the current study (almost 8 times higher). Heikkinen et al. did not observe any significant differences in the level of IL-15 in amniotic fluid over the course of gestation (median – 42 pg/ml), which also differs from observations by both Fortunato and the current study.

Searle et al. [26] reported that while one may observe the presence of IL-15 (average concentration – 41 pg/ml) in the amniotic fluid in the second trimester, it does not show full biological activity, and enhancement of cytotoxic lymphoblasts to proliferation takes place solely by way of a beta-chain being a fragment of IL-15.

On the basis of the literature cited here and their own study, the authors can state that IL-15 is present in the amniotic fluid at the beginning of the second trimester of gestation, though the value of concentration in the second and third trimesters provided by different authors does differ significantly. Neither do the relevant studies concur as regards changes in the level of IL-15 in the amniotic fluid over the course of the gestation period.

The authors concluded that the presence of IL-15 in the amniotic fluid in the second trimester of gestation indicates an early activation of the fetus’s defensive mechanisms. An average concentration of IL-15 in the amniotic fluid is significantly higher in the third trimester of pregnancy than the second trimester, which can indicate the development of the immune response in the fetus.

References


[23] Witzczak M, Torbe A, Czajka R: Maternal serum and amniotic fluid IL-1alpha, IL-1beta, IL-6 and IL-8 in preterm and term labor complicated by PROM. Gin Pol 2003, 74, 1343–1347.


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