Second-Trimester IL-15 and IL-18 Levels in the Amniotic Fluid of Fetuses with Normal Karyotypes and with Chromosome Abnormalities*

Abstract

Background. Little is known about the behavior of interleukin 15 (IL-15) and 18 (IL-18) in the amniotic fluid in the second trimester of gestations complicated by chromosomal defects in the fetus. Likewise, it has not yet been established whether a fetus with chromosome abnormalities creates its immunity mechanisms in the same way as a fetus with a normal karyotype.

Objectives. The aim of this work was to assess the concentration of IL-15 and IL-18 in the amniotic fluid in the second trimester of gestation in fetuses with normal karyotypes and with chromosome abnormalities.

Material and Methods. The material consisted of 51 samples of amniotic fluid obtained from genetic amniocenteses carried out between the 15th and the 19th weeks of gestation. On the basis of cytogenetic screening, two groups were singled out: Group I – 45 fetuses with normal karyotypes, and Group II – 6 fetuses with abnormal karyotypes. The concentrations of IL-15 and IL-18 in the amniotic fluid were assessed with ready-made assays and analyzed, and the results from both groups were compared.

Results. The differences between the IL-15 levels in the amniotic fluid from Groups I and II proved to be statistically insignificant (p = 0.054). However, the average IL-18 levels in the amniotic fluid of the fetuses with normal karyotypes were significantly higher than in the amniotic fluid of the fetuses with chromosome abnormalities (p = 0.032).

Conclusions. Some defense mechanisms in the second trimester of gestation in fetuses with chromosome abnormalities may develop in a different way than in fetuses with normal karyotypes (Adv Clin Exp Med 2012, 21, 2, 201–205).

Key words: interleukin 15, interleukin 18, amniotic fluid, chromosome aberration.

*This study was carried out with the support of a grant from Wroclaw Medical University.
The basis for non-invasive prenatal diagnoses evaluating the risk of chromosome abnormalities appearing in fetuses is ultrasound examination, such as measurement of the fetus’s nuchal translucency [1], the presence or absence of fetal nasal bones [2, 3], measurement of the length of the fetal nasal bones [4], measurement of the frontomaxillary facial angle [5], measurement of the length of the fetus’s ears [6] and assessment of Doppler blood flow in the ductus venosus [7, 8]. The ultrasound markers are usually tested between the 11th and 14th weeks of gestation, and are analyzed along with the mother’s age, the pregnancy-associated plasma protein A (PAPP-A) concentration in their blood, inhibin A, free estriol and free β-hCG, to obtain a precise evaluation of the risk of chromosome abnormalities in the fetus [9, 10]. In cases where there is a high risk of defects appearing in the fetus, the pregnant women are qualified for invasive methods: amniocentesis and cytogenetic screening of the amniotic fluid.

To date there is insufficient information about the concentration of interleukin 15 (IL-15) and interleukin 18 (IL-18) in the amniotic fluid in the second trimester of gestations complicated by chromosomal defects in the fetus. Likewise, little is known about the development of defense mechanisms in the second trimester of gestation in such fetuses. The authors of the current study were curious as to whether immune mechanisms are formed in the same way both in fetuses with chromosome abnormalities and in fetuses with normal karyotypes. This question has not yet been clarified in the available literature.

The aim of this work was to evaluate the concentration of IL-15 and IL-18 in the amniotic fluid in the second trimester of gestation in fetuses with normal karyotypes and in those with chromosome abnormalities.

Material and Methods

The clinical material consisted of 51 samples of amniotic fluid obtained from amniotic fluid tests of pregnant women being treated at Wroclaw Medical University’s Clinic for Fetal Development Disorders (Wroclaw, Poland) from 2004 to 2006.

The amniocenteses were carried out between the 15th and 19th weeks of gestation. The ages of the pregnant women ranged from 24 to 46 years (average age: 37.47 years). They had been qualified for the test on the basis of their age (over 35) and an aggravated risk of defect in the fetus indicated in biochemical tests of the mother’s blood, ultrasound screening or high-risk genetic history. The amniotic fluid was tested at the Wroclaw Medical University Department of Genetics in order to ascertain the karyotypes of the examined fetuses. On the basis of the cytogenetic screening, two groups were identified:

– Group I: 45 fetuses with normal karyotypes;
– Group II: 6 fetuses with abnormal karyotypes (including 5 fetuses with trisomy 21 and 1 with karyotype 45,X).

Samples of the amniotic fluid were centrifuged at the speed of 3000 rotations per minute for 10 minutes, and then were refrigerated at a temperature of ~82°C Celsius until the measurements were made. The concentrations of IL-15 and IL-18 in the amniotic fluid were assayed by the ELISA method using a ready-made kit (R&D Systems, Inc., Minneapolis, USA). The sensitivity of the method was > 2 pg/ml. The value of the absorbance was measured at λ = 450 nm. The Shapiro-Wilk test showed no reason to reject the normal distribution hypothesis for either group. The calculations were performed using STATISTICA 8.0 software (StatSoft, Inc.). The values of the IL-15 and IL-18 concentrations in both groups were compared using the Student t test.

Results

The analysis of IL-15 and IL-18 levels in the amniotic fluid in the second trimester of pregnancy in the examined groups is presented in Tables 1 and 2.

As can be seen in Table 1, the difference between the average concentration of IL-15 in Groups I and II borders on statistical significance (p = 0.054). As Table 2 shows, the average concentration of IL-18 was significantly higher in fetuses with normal karyotypes than in fetuses with chromosome abnormalities (p = 0.032).
Discussion

Interleukin 15 is a glycoprotein secreted mainly by macrophages and monocytes. In terms of its molecular structure and biological properties, IL-15 is similar to IL-2 [11–13]. IL-15 participates in the cell-mediated immune response. It also plays a significant role beyond the immune system: It stimulates the process of angiogenesis and is also a strong inhibitor of apoptosis [14, 15]. It is found in the amniotic fluid starting from the second trimester of pregnancy. The presence of IL-15 and the mRNA of IL-15 has been demonstrated in fetal membranes – the amnion, chorion and decidua, as well as in the placenta [16, 17].

The main sources of interleukin 18 are macrophages and the epithelial cells, keratinocytes and osteoblasts [18–20]. IL-18 induces both the cell-mediated immune response and the humoral immune response [21, 22]. IL-18’s receptor (IL-18R) is found in the cells of the syncytiotrophoblast, interstitial villi and fetal blood cells. The presence of IL-18R in the decidua has been observed as early as the 6th to 10th week of gestation [23]. IL-18 is present in the amniotic fluid and in the fetal membranes, and increases in its expression are connected with intrauterine infections, premature ruptures of fetal membranes and/or risk of premature delivery [24–26].

In the available literature, IL-15 and IL-18 levels in the amniotic fluid have been analyzed mainly in relation to their participation in the development of intrauterine infections, risk of premature delivery and premature ruptures of fetal membranes [17, 24–26]. The authors have not found any research presenting an analysis of the relationship between the levels of IL-15 and IL-18 in the amniotic fluid and the results of cytogenetic screening in the fetus.

In fetuses with trisomy 21, various authors have investigated the concentrations of inhibin A, inhibin B, inhibin pro-alpha C, activin and follistatin, as well as IL-2 and Transforming Growth Factor beta (TGF beta) [27–30]. In gestations complicated by trisomy 21, significantly lower levels of inhibin A, inhibin C and activin in the amniotic fluid have been observed (in the 14th through 19th weeks of gestation) [27, 28]; active IL-2 has not been found, only a soluble form of its receptor IL-2R [29, 30]. However, the concentration of a bioactive form of TGF beta in the amniotic fluid has been found to be significantly higher in fetuses with an abnormal karyotype [30].

The statistical analysis of the results that the current authors obtained showed that there is a relationship between abnormal results in cytogenetic screening of the fetus and the concentration of IL-18 in the amniotic fluid in the second trimester:

**Table 1. The concentration of IL-15 in the amniotic fluid in the second trimester of pregnancy in fetuses with normal (Group I) and abnormal (Group II) karyotypes**

<table>
<thead>
<tr>
<th>Concentration of IL-15 (pg/ml)</th>
<th>Minimum value</th>
<th>Maximum Value</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I n = 45 (Grupa I)</td>
<td>2.31</td>
<td>15.87</td>
<td>5.73</td>
<td>3.24</td>
</tr>
<tr>
<td>Group II n = 6 (Grupa II)</td>
<td>1.64</td>
<td>17.70</td>
<td>8.07</td>
<td>6.0</td>
</tr>
</tbody>
</table>

**Table 2. The concentration of IL-18 in the amniotic fluid in the second trimester of pregnancy in fetuses with normal (Group I) and abnormal (Group II) karyotypes**

<table>
<thead>
<tr>
<th>Concentration of IL-18 (pg/ml)</th>
<th>Minimum value</th>
<th>Maximum Value</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I n = 45 (Grupa I)</td>
<td>129.66</td>
<td>1118.83</td>
<td>416.33</td>
<td>228.21</td>
</tr>
<tr>
<td>Group II n = 6 (Grupa II)</td>
<td>53.41</td>
<td>583.41</td>
<td>201.75</td>
<td>190.05</td>
</tr>
</tbody>
</table>
The average concentration of IL-18 was significantly higher in the fetuses with normal karyotypes in comparison with the fetuses with abnormal karyotypes. On the other hand, the difference between the average IL-15 levels in the two groups did not reach the boundary of statistical significance.

Because the levels of IL-15 and IL-18 in the amniotic fluid in the fetuses with chromosome abnormalities were measured only once, this study does not provide enough information about the development of some defense mechanisms. As noted above, the available literature offers no research about concentrations of IL-15 and IL-18 in the amniotic fluid in fetuses with abnormal karyotypes. In the authors' opinion, the results obtained in this study strongly indicate the potential importance of further research on IL-15 and IL-18 concentrations in the amniotic fluid in the fetuses with chromosome abnormalities.

The authors concluded that some defense mechanisms in the second trimester of gestation in fetuses with chromosome abnormalities may develop in a different way than in fetuses with normal karyotypes.

References

[7] Klimkiewicz-Blok et al. 204

Address for correspondence:
Dominika Klimkiewicz-Blok
Second Department of Gynecology, Obstetrics and Neonatology
Wroclaw Medical University
Borowska 213
50-528 Wroclaw
Poland
Tel.: +48 71 733 14 00, +48 600 010 055
E-mail: nikablok@o2.pl

Conflict of interest: None declared

Received: 31.03.2011
Revised: 13.11.2011
Accepted: 29.03.2012