EFFECTS OF EXPOSURE TO TOBACCO SMOKE IN PREGNANCIES COMPLICATED BY OLMOHYDRAMNIIOS AND PREMATURE RUPTURE OF THE MEMBRANES. II. ACTIVITY OF BRUSH BORDER ENZYMES IN HUMAN AMNIOTIC FLUID

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Abstract. The aim of the present study was to assess the activity of membrane enzymes: alanine aminopeptidase (AAP), γ-glutamyltransferase (GGT) and trehalase in amniotic fluid of women who smoke cigarettes during pregnancy complicated by idiopathic oligohydramnios or premature rupture of the membranes (PROM). The enzyme activity was measured between 22 and 31 (group A) and between 32 and 39 (group B) weeks of gestation. In the women of group A with idiopathic oligohydramnios, AAP activity was five times higher than in PROM women. AAP activity was declining with the progression of gestation, and in the B group women with oligohydramnios, it was over eight times lower than in group A. A threefold increase in GGT activity was found in women of group A with oligohydramnios as compared to women of group A with PROM. No statistically significant differences in trehalase activity were found in amniotic fluid of women with oligohydramnios and PROM. AAP, GGT and trehalase activity in women with idiopathic oligohydramnios correlated with the cadmium ion concentration, and AAP and GGT activity with the lead ion concentration in amniotic fluid which confirms toxic properties of these heavy metals present in cigarette smoke. It has already been confirmed that measurements of the brush border enzyme activity in amniotic fluid are very useful in prenatal diagnosis and detection of the placenta disorders.

Key words: Alanine aminopeptidase, γ-Glutamyltransferase, Trehalase, PROM, Oligohydramnios

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INTRODUCTION

The diagnosis of oligohydramnios plays a very important role from the clinical point of view as this pathology is often concurrent with the fetal developmental disorders and premature rupture of the membranes (PROM). It also occurs in women who take anti-inflammatory non-steroid drugs. Oligohydramnios most frequently results from the renal defects, disorders of the tract diverting fetal urine and inhibition of intrauterine fetal growth induced by the placenta failure [1].

Tobacco smoke components affect blood vessels of placenta which leads to its failure and changes in the tissue structure [2]. These are followed by changed enzyme activity [3,4]. The abnormalities are associated inter alia with toxic effect of cadmium present in tobacco smoke.

Brush border enzymes: alanine aminopeptidase (EC 3.4.11.2 AAP), \(\gamma\)-glutamyltransferase (EC 2.3.2.2 GGT) and \(\alpha,\alpha\)-trehalase (EC 3.2.1.28) are singled out as the most sensitive markers of Cd exposure. The determination of activity of these enzymes in amniotic fluid has been found to be very useful in prenatal clinical diagnostics.

Alanine aminopeptidase (\(\alpha\)-amino-acyl-peptid hydrolase) is an exopeptidase present in large quantities in epithelial cells of the brush borders of the small intestine, kidneys and biliary canaliculuses [5–8]. AAP activity is observed in placenta [9–11], fetal membranes [12], as well as in amniotic fluid [13–15], and increases with the progression of gestation in the serum of pregnant women [16]. Recent studies have shown that plasma AAP differs from placenta aminopeptidase N as its N-terminal 68 residues were deleted [16], however, its catalytic and immunological properties are similar. It is thought that the enzyme is released from placenta into the maternal circulation due to the deletion of the peptide segment which is responsible for binding to membranes. It has been revealed that the enzyme contains three domains: cytoplasmic, transmembrane and junctional. Histochemical localization of alanine aminopeptidase N in human placenta has confirmed its strong expression in the villious stromal cells which increases with advancing of pregnancy [12]. In fetal membranes, enzyme was identified in decidual cells in the decidua parietalis. It seems likely that placental alanine aminopeptidase N contributes significantly to the fetal growth [17] and the regulation of blood pressure in the maternal-fetal circulation as it participates in the degradation of vasoactive peptides [18]. In normal pregnancy its amniotic fluid activity declines with the progression of gestation despite the enzyme expression in placenta and amniotic fluid [12,14].

\(\gamma\)-Glutamyltransferase also known as glutathionase plays a crucial role in metabolism of glutathione and its derivatives [19–22]; participates in the transport of aminoacids through the membranes in the \(\gamma\)-glutamyl circle and production of glutathione S-conjugates [19,23]; occurs in numerous organs and fluids [23–26], as well as in all processes of absorption and secretion. Immunohistochemically, this enzyme was identified on the brush borders of renal proximal tubules, intestines and in liver and pancreas [27].

\(\gamma\)-Glutamyltransferase present in amniotic fluid is a high-molecular protein; isolated from amniotic fluid in an early period of gestation contains more saccharide residues than the enzyme present at term [28]. Electrophoretic separation of the enzyme showed one isoenzyme in the fetal blood and two in amniotic fluid, and a higher level of activity was localized in the form of identical mobility as that of the fetal form [29]. In normal pregnancies, amniotic fluid GGT activity falls in the second trimester, the level at 20 week of gestation (WG) being 60% of that at 14 WG [3]. In prenatal diagnosis the determination of amniotic fluid GGT activity is found to be a reliable indicator of various fetal defects. In case of trisomy 21, and fetal trisomy 18 syndrome a considerable decline in GGT activity is observed as compared to normal pregnancy, which is manifested by the central nervous system defects [30–33], mucoviscidosis [34] and biliary atresia [35]. In Down's syndrome pregnancies, high GGT activity in the fetal liver and placenta, and low in the small intestine is reported [33]. In normal pregnancies GGT activity remains constant in fetal blood and maternal blood [29], declines in placenta and amniotic fluid [11], and increases in maternal urine [33]. GGT activity in fetal serum is over eight times higher than that in maternal serum [36].

Trehalase (\(\alpha,\alpha\)-trehalase glucohydroxylase), the enzyme which selectively hydrolyses the disaccharide trehalose
into two glucose molecules, is localized on the brush-border of the intestine and kidney [37,38]. In the human fetus, trehalase is present in both tissues early during the fetal development [39,40] and is released in the amniotic fluid between 14 GW and term [41,42]. During the progression of gestation a biphasic increase in the enzyme activity is observed [43]. First, it increases between 16 and 18 WG, then decreases to low values at 21 WG. During this period, the increased activity of other intestinal enzymes released in the amniotic fluid is noted. After 21 WG, the enzyme activity rises again, whereas other intestinal enzymes are virtually not excreted to amniotic fluid. It is most likely that the second rise in trehalase activity is related to renal functions. The presence of two enzymatic forms, soluble and membrane-bound, varied in isoelectric focusing ($p_I$) was indicated. The $p_I$ values were 4.37 and 4.6, respectively [41]. The assay of isoforms in amniotic fluid has been found useful in the differentiation of pregnancy pathologies (anual imperforation or polycistic kidney disease). It has been revealed that membrane-bound trehalase present in amniotic fluid after 21 GW is always of intestine origin and soluble trehalase of renal origin. Abnormal increase in amniotic fluid activity of both forms is a specific biochemical marker of fetal defects [41,44]. The significantly increased amniotic fluid soluble trehalase has been observed in fetuses with polycistic kidney disease [43,44]. It is thought that the determination of trehalase activity in fetal urine is the most specific marker to assess the damage of renal proximal tubular cells induced by both antibiotic therapy and congenital disorders [45,46]. Its determination has also been found useful in chronic human exposure to cadmium compounds [47,48]. The increase in the urinary enzyme activity is associated with the release of soluble trehalase from the external side of the brush border membrane in which the enzyme is anchored by glycosyl-phosphatidylinositol (GPI) [38,46]. Our previous study of women affected by idiopathic oligohydramnios showed a substantial exposure of this population to tobacco smoke, significantly increased blood Cd concentration, and disturbed Zn transport to fetal ovum as compared to PROM women [49]. This resulted in the altered release of molecular forms of N-acetyl-β-D-glucosaminidase into amniotic fluid [4].

The aim of the present study was to assess the activity of membrane enzymes: alanine aminopeptidase, γ-glutamyltransferase and trehalase in amniotic fluid of women who smoke cigarettes during pregnancy complicated by idio-pathic oligohydramnios or premature rupture of the membranes.

MATERIALS AND METHODS

Reagents

L-alanine-β-naphthylamide and 1-(α-D-glucopyranosyl)-D-α-D-glucopyranoside dihydrate (Sigma); Coomassie Brilliant Blue G-250 (Fluka); and 4-dimethylaminobenzaldehyde p.a. (POCH). Amniotic fluid samples were obtained from pregnant women admitted to the Obstetric Wards of the Department of Reproduction and Obstetrics, Wrocław University of Medicine.

Prior to amniotic fluid infusion, amniocentesis was performed in 15 patients (age range 20–46 yr) between 22 and 39 GW because of premature rupture of the membranes. In addition, also prior to amniotic fluid infusion, amniocentesis was performed in 15 patients (age range 21–34 yr) between 22 and 39 GW with diagnosed idio-pathic oligohydramnios. Samples of amniotic fluid collected in polyethylene test-tubes were centrifuged at 800 • g for 10 min at 4°C, freezeed and stored at -20°C. Creatinine concentration in amniotic fluid was measured by kinetic methods using the test of Analco-GBG (No. Cat. A-292); and protein according to Bradford using bovine serum albumin (BSA) as standard [50]. Alanine aminopeptidase activity was determined colorimetrically against L-alanyl-β-naphthylamide, and released β-naphthylamide was bound to p-dimethylaminobenzaldehyde [9].

γ-Glutamyltransferase activity was determined colorimetricaly against γ-glutamyl-p.-nitroanilide using the LACHMA test (No. Cat. 1105902).

α,α-Trehalase activity was measured against l-(α-D-glucosopyranosol)-α-D-glucosopyranoside in conditions...
according to Dahlqvist [51], and the released glucose was defined colorimetrically using the Analco-GBG enzymatic test (No. Cat. A-192). Cytosol and microsomal fractions of amniotic fluid were obtained from samples (4.4 ml) centrifuged in Sorval Ultra 80 TM ultracentrifuge (Du Pont, USA) at 105 000 • g for 60 min at 4°C. Supernatant was carefully transferred to a separate container, and sediment was suspended in solution of 154 mM NaCl in amniotic fluid initial volume of 1/10.

**Statistical analysis**

The measurements of enzymes activity in patients were split into two groups, depending on the progression of gestation: group A included samples of biological fluids collected between 22 and 31 GW from 6 patients with idiopathic oligohydramnios, and 7 PROM patients; and group B comprised samples of biological fluids collected between 32 and 39 GW from 9 and 8 patients, respectively. When comparing individual parameters Student’s t-test was used to assess differences between mean values and correlation coefficients. A value of p <0.05 was considered to indicate statistical significance.

**RESULTS**

Creatinine concentration, one of the crucial markers of normal functioning of the fetal urinary tract, was measured in amniotic fluid. It was shown that in all women under study, the creatinine values in amniotic fluid from later period of gestation (group B) were higher as compared to

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Group A</th>
<th>Group B</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>x ± SD (range)</td>
<td>x ± SD (range)</td>
</tr>
<tr>
<td>PROM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 15</td>
<td>0.0904 ± 0.0136* (0.0714 – 0.1116)</td>
<td>0.1300 ± 0.0437* (0.068 – 0.212)</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td></td>
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</tr>
<tr>
<td>n = 15</td>
<td>0.0892 ± 0.0197** (0.052 – 0.108)</td>
<td>0.1497 ± 0.0482** (0.059 – 0.226)</td>
</tr>
</tbody>
</table>

* p < 0.01 – PROM group A compared with PROM group B.
** p < 0.05 – oligohydramnios group A compared with oligohydramnios group B.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>AAP activity units</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x ± SD</td>
<td>x ± SD</td>
</tr>
<tr>
<td>PROM</td>
<td>U/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 15</td>
<td>8.33 ± 3.19</td>
<td>5.56 ± 2.38</td>
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<tr>
<td></td>
<td>U/mmol creatinine</td>
<td>■ 93.83 ± 35.89* 3.08 ± 0.87</td>
<td>46.70 ± 22.17* 2.48 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>U/g protein</td>
<td>n = 7</td>
<td>n = 8</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>U/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 15</td>
<td>66.18 ± 36.63</td>
<td>8.45 ± 5.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U/mmol creatinine</td>
<td>■ 524 ± 440** 15.83 ± 7.73</td>
<td>67.76 ± 48.69** 3.29 ± 1.86</td>
</tr>
<tr>
<td></td>
<td>U/g protein</td>
<td>n = 6</td>
<td>n = 9</td>
</tr>
</tbody>
</table>

■ p < 0.02 – PROM group A compared with oligohydramnios group A.
* p < 0.001 – PROM group A compared with PROM group B.
** p < 0.01 – oligohydramnios group A compared with oligohydramnios group B.
group A (Table 1). The differences were statistically significant. But there were no significant difference in amniotic fluid creatinine concentrations between women with idiopathic oligohydramnios and those with PROM.

Alanine aminopeptidase activity decreased with advancing gestation. In women with idiopathic oligohydramnios (group A), the AAP level was over five times higher than in PROM women of the same group (Table 2a). In the later period of gestation (group B) an over 8-fold decrease in AAP activity was found in amniotic fluid of women with idiopathic oligohydramnios as compared to group A. No significant differences in individual enzymatic forms were observed. In women with both idiopathic oligohydramnios and PROM, a cytosol AAP was about 75%, and a microsomal about 25% of the total enzyme activity (Table 2b).

Table 2b. AAP activity in microsomal and cytosol fractions of amniotic fluid in pregnant women

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Fraction</th>
<th>% of total activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A x ± SD</td>
<td>Group B x ± SD</td>
</tr>
<tr>
<td>PROM</td>
<td>microsomal</td>
<td>26.29 ± 15.57</td>
</tr>
<tr>
<td>n = 15</td>
<td>cytosol</td>
<td>76.01 ± 17.69</td>
</tr>
<tr>
<td></td>
<td>n = 7</td>
<td></td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>microsomal</td>
<td>27.18 ± 14.33</td>
</tr>
<tr>
<td>n = 15</td>
<td>cytosol</td>
<td>76.48 ± 18.03</td>
</tr>
<tr>
<td></td>
<td>n = 6</td>
<td></td>
</tr>
</tbody>
</table>

Table 3a. GGT activity in amniotic fluid of pregnant women

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>GGT activity units</th>
<th>Group A x ± SD</th>
<th>Group B x ± SD</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PROM</td>
<td>U/l</td>
<td>6.65 ± 2.62</td>
<td>6.15 ± 2.41</td>
</tr>
<tr>
<td>n = 15</td>
<td>U/mmol creatinine</td>
<td>■ 122.44 ± 107*</td>
<td>48.98 ± 22.26*</td>
</tr>
<tr>
<td></td>
<td>U/g protein</td>
<td>3.95 ± 2.72</td>
<td>2.67 ± 0.96</td>
</tr>
<tr>
<td></td>
<td>n = 7</td>
<td></td>
<td>n = 8</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>U/l</td>
<td>37.95 ± 23.98</td>
<td>9.07 ± 3.55</td>
</tr>
<tr>
<td>n = 15</td>
<td>U/mmol creatinine</td>
<td>■ 429.91 ± 222.19**</td>
<td>64.04 ± 26.62**</td>
</tr>
<tr>
<td></td>
<td>U/g protein</td>
<td>8.19 ± 2.94</td>
<td>3.92 ± 1.22</td>
</tr>
<tr>
<td></td>
<td>n = 6</td>
<td></td>
<td>n = 9</td>
</tr>
</tbody>
</table>

Table 3b. GGT activity in microsomal and cytosol fractions of amniotic fluid in pregnant women

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Fraction</th>
<th>% of total activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group Ax ± SD</td>
<td>Group Bx ± SD</td>
</tr>
<tr>
<td>PROM</td>
<td>microsomal</td>
<td>39.61 ± 24.99</td>
</tr>
<tr>
<td>n = 15</td>
<td>cytosol</td>
<td>60.39 ± 24.99</td>
</tr>
<tr>
<td></td>
<td>n = 7</td>
<td></td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>microsomal</td>
<td>35.57 ± 31.77</td>
</tr>
<tr>
<td>n = 15</td>
<td>cytosol</td>
<td>64.68 ± 31.48</td>
</tr>
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<td></td>
<td>n = 6</td>
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</tbody>
</table>
Our study revealed statistically significant differences in GGT activity related with the progression of gestation in both populations of women with idiopathic oligohydramnios and PROM (Table 3a). Women (group A) with diagnosed oligohydramnios showed a 3-fold increase in amniotic fluid GGT activity as compared to PROM women of the same group; cytosol and microsomal forms being about 65% and 35%, respectively. Slightly different values were found in the B group women with idiopathic oligohydramnios (Table 3b). However, these differences were not statistically significant.

No statistically significant differences in α,α-trehalase activity were observed between women with idiopathic oligohydramnios and PROM (Table 4a). An downward
trend in the enzyme activity with advancing pregnancy and a slightly higher activity in women with idiopathic oligohydramnios were found. Cytosol and microsomal forms were about 85% and 15% of the total amniotic fluid disaccharidase activity, respectively (Table 4b).

The determination of AAP, GGT and trehalase activity were correlated with the values of amniotic fluid Cd and Pb concentrations. A linear positive correlation between activity of the enzymes and Cd and Pb concentrations was found in women with idiopathic oligohydramnios (Table 5a). No correlation was observed only between trehalase activity and amniotic fluid Pb concentration. In PROM women, linear positive correlation was noted between trehalase activity and Cd concentration, as well as between GGT activity and Pb concentration (Table 5b).

DISCUSSION

Pregnancy complicated by oligohydramnios is a vital diagnostic and therapeutic problem of the present obstetrics [52]. Oligohydramnios means a serious deficiency of amniotic fluid or its complete lack [1]. Retrogressive changes in the placenta and disorders of amniotic fluid excretion by the amnion covering placental chorion plate are the direct causes of oligohydramnios [53]. In 30% of cases, the urinary system defects are responsible for the incidence of oligohydramnios. It is suggested that the creatinine concentration and activity of urinary enzymes present in amniotic fluid are reliable markers of the renal damage [15,54].

The present study revealed that amniotic fluid creatinine concentration significantly increased with advancing of pregnancy in women with both PROM and idiopathic oligohydramnios. Higher concentrations were found in amniotic fluid in later weeks of gestation (group B). The values determined (0.091 ± 0.0142 mmol/L) were close to those obtained by Ring et al. [54] for normal pregnancy between 28 and 31 GW. Such values point to the normal functioning of the fetal urinary track as it is thought that the increase in amniotic fluid creatinine concentration at term is associated with the increasing filtration of fetal renal tubules and their maturity [14]. The examination of AAP activity is very useful in assessing the development of fetal kidneys. A dramatic decrease in the activity of this enzyme after 20 GW has been reported; a 28-fold decrease in activity between 28 and 32 GW as compared to that between 15 and 19 GW. Such a decrease of AAP activity in amniotic fluid is attributed to a stronger enzyme binding to the brush borders of the membrane in the fetal renal tubules [14]. The same authors investigated AAP activity at progressive stages of gestation, and showed a twofold lower enzyme activity in amniotic fluid between 32 and 36 GW as compared to that found between 28 and 32 GW. Our present study revealed a similar regularity (Table 2a). AAP activity in group B of women with PROM was twice as low as in group A. The decrease in the enzyme activity with advancing of gestation was observed in women with idiopathic oligohydramnios. It should be noted that the decrease was more dramatic and resulted from much higher AAP activity in the group A women with idiopathic oligohydramnios; it was over five times higher than that found in the group A women with PROM. It is not likely that the increase in activity was induced by defects of the fetal urinary track as such defects were not observed in autopsy [49]. Most probably the placenta and amniotic fluid disorders were responsible for the elevated activity. As already indicated, the group of women in question was mostly exposed to tobacco smoke [49]. In normal pregnancy, despite increased AAP activity in syncytiotrophoblast cells and decidual cells in the decidue parietalis [12], the decrease in the amniotic fluid enzyme activity is observed. However, it has been reported that in amniotic fluid of women with EPH-gestosis, AAP activity rises by 3–7 times [13]. The increase observed in AAP activity in amniotic fluid of women with oligohydramnios results most probably from morphological changes in the placenta structure noted earlier in pregnancies complicated by oligohydramnios [55]. Heavy metals (Pb and Cd) could play a significant role in the enzyme release from the tissue structures.

The present study indicated a positive linear correlation between AAP activity and Cd concentration ($r = 0.89; p < 0.001$) and Pb concentration ($r = 0.90; p < 0.01$). Interestingly, a similar percentage of microsomal and
cytosol forms were found in amniotic fluid of women with idiopathic oligohydramnios and PROM (Table 2b) despite differences in the enzyme activity. It is assumed that in normal urine microsomal form makes 45% of AAP activity [56]. Nothing about the participation of these forms in amniotic fluid has been yet reported. Our study showed that microsomal form constituted about 25% of the total enzyme activity which means less than in urine [10]. It seems that this fact also confirms the placental origin of the enzyme present in amniotic fluid investigated.

γ-Glutamyltransferase, a key enzyme in γ-glutamyl cycle responsible for the transport of aminoacids through membranes, plays a vital role in the fetal development. Its presence is localized in placental microvillous membranes [35,57]. The investigation carried out on a group of pregnant rats revealed that the inhibition of GGT activity reduced placental transport of alanine and its subsequent incorporation into placental proteins and fetus [58]. With advancing of normal pregnancy placental and amniotic fluid GGT activity usually decreases [35], whereas in pregnant women who smoke, the increased GGT activity in amniotic fluid was observed [59]. The present study revealed that GGT activity decreased with advancing of pregnancy (Table 3a). Like in AAP, the activity of GGT was higher in the group A women with idiopathic oligohydramnios than in PROM women of the same group. The percent of microsomal and cytosol GGT activity was defined in amniotic fluid; the values were 34% and 75%, respectively (Table 3b). In urine of healthy people the proportion of microsomal form accounts for 75% [56].

A positive linear correlation between amniotic fluid GGT activity and Cd (r = 0.66; p < 0.02) and Pb (r = 0.66; p < 0.001) concentrations were observed in women with idiopathic oligohydramnios, and positive correlation between GGT activity and Pb concentration (r = 0.58; p < 0.05) in women with PROM.

Both AAP and GGT play a crucial role in fetal nutrition, and their release from placental structures into amniotic fluid may impair considerably this process which is observed in the case of idiopathic oligohydramnios. As very well known, tobacco smoke is one of important sources of the environmental Cd exposure. Cd toxic effect on placenta, fetal membranes and fetus has been evidenced in numerous studies. Investigations into the human term placenta perfused with cadmium chloride solution showed a 60-fold increase in Cd concentration in the cytosol fraction, as well as pathologic changes in the tissue ultrastructure. However, no significant changes were found in the oxygen use and glucose consumption [60].

In the study described, activity of trehalase, an enzyme hydrolizing selectively trehalose into two glucose molecules and also playing an important role in the sugar transport was assayed [7,8]. The physiological role of the enzyme has not as yet been fully elucidated [61]. Amniotic fluid trehalase activity is also determined in different pathologies of the fetal intestine and kidneys [34,61]. It has been evidenced that among many enzymes (N-acetyl-β-D-glucosaminidase, γ-glutamyltranspeptidase, lactate dehydrogenase etc.) trehalase is the most reliable marker of cellular proliferation and/or renal proximal tubular damage in newborn infants [61]. Our studies did not reveal significant differences in the trehalase activity in amniotic fluid in women with PROM and idiopathic oligohydramnios. Enzyme activity decreased with advancing of pregnancy (Table 4a). Slightly higher activity was observed in women with idiopathic oligohydramnios. In the A group of women with PROM, cytosol form made 87.94 ± 5.99%, and in women with oligohydramnios 85.08 ± 7.09% of the total enzyme activity. These values were very close to the results obtained by Elsliger et al. who found 96 ± 3% of cytosol form in amniotic fluid of normal pregnancy after 21 GW [40]. In pathologies of the urinary tract the proportion of cytosol form increased up to 99% [40].

Positive linear correlation was indicated between trehalase activity and amniotic fluid Cd concentration (r = 0.84; p < 0.01) in women with idiopathic oligohydramnios and also in women with PROM (r = 0.48; p < 0.05). There was no correlation between the enzyme activity and Pb concentration. Positive correlation between the enzyme activity and cadmium ions may confirm the toxic effect of cadmium and the release of trehalase regarded as the most specific marker of nephrotoxicity [47].
CONCLUSION

Significant differences in amniotic fluid AAP and GGT activity between women with PROM and idiopathic oligohydramnios highlighted significant disorders of the placenta functioning in the case of oligohydramnios, especially in early stages of gestation, and the adverse Cd effect manifested inter alia by release of enzymes into amniotic fluid – enzymes which play a crucial role in the fetal nutrition.

The absence of differences in trehalase activity in amniotic fluid of women with idiopathic oligohydramnios and PROM confirmed the fact that oligohydramnios was not accompanied by defects of the fetal urinary tract [49] and the usefulness of trehalase activity assays in the detection of such defects. Positive correlation between trehalase activity between women with PROM and idiopathic oligohydramnios and fetal nutrition.

REFERENCES


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