Pregnancy-Associated Globulin in Body Fluids of Pregnant Women

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Pregnancy-associated globulin (PAG) in saliva, urine and amniotic fluid was investigated just before and after delivery by Ouchterlony's method and immunoelectrosyneresis. In saliva, the incidence was more than 60%; 12 out of 18 samples by immunoelectrosyneresis and 11 out of 18 by Ouchterlony's method. In urine, 8 (33%) and 6 (25%) out of 24 samples were positive by immunoelectrosyneresis and Ouchterlony's method, respectively. In amniotic fluid, only 3 out of 21 were positive by Ouchterlony's method, but 19 out of 21 samples (91%) were positive by immunoelectrosyneresis. Of 12 pregnant women in whom serum, saliva, urine and amniotic fluid were tested by immunoelectrosyneresis, four showed positive reaction in the above four samples and 11 in three samples except urine.

--- pregnancy-associated globulin; saliva; urine; amniotic fluid

Sera of pregnant females are accompanied with the additional proteins which are commonly not identified in non-pregnant females and males (Smithies 1959; Afonso and Farnham 1962). These are called pregnancy-associated globulin (PAG), which is recognized immunoelectrophoretically in alpha 2 globulin region. PAG is not unique in pregnancy, and it is found also in malignant diseases (Stimson 1975a, b) and sometimes in normal females and males (MacLaren et al. 1966; Kasukawa 1973; Sagisaka and Takahashi 1976). However, PAG in body fluids such as saliva, urine and amniotic fluid has not been reported. The authors investigated PAG in these fluids just before and after delivery.

MATERIALS AND METHODS

Samples (sera 42, saliva 18, urine 24 and amniotic fluid 21) were provided from the delivery room of Akita University Hospital and Kawahara Clinic. Anti-PAG sera were prepared according to Horne et al. (1973). The rabbits were immunized with the pooled sera from 5 pregnant women near delivery. A dose of 0.5 ml antigen with the same volume of Freund's complete adjuvant was injected into foot pads or beneath the skin once a week. After 4 to 5 times immunization, the rabbit was bled from the carotid artery. The crude anti-serum was absorbed with normal male serum which did not contain PAG, and the specificity was tested by immunoelectrophoresis and Ouchterlony's method.

The immunoelectrosyneresis was performed as follows; 7 ml of 1.2% agarose mixture in agar dissolved with veronal buffer (pH 8.6, µ: 0.05) were poured on a glass plate of 10 × 6.5 cm in size. Wells of 2.5 mm in diameter were cut at cathodal and anodal sides, at 3
mm intervals in parallel. The samples (5 µl) were put into the cathodal well with microsyringe and the same volume of anti-PAG serum into the anodal well. Electrophoresis was performed in the veronal buffer (pH 8.6, µ: 0.07) at 150 V constant voltage for 45 min. Precipitation lines were observed with amido black 10B staining.

Ouchterlony’s method was as follows; 4 ml of 1.2% agarose solution dissolved with tricine-veronal buffer (pH 8.6, µ: 0.05) were poured on a glass plate of 4.5 × 2.5 cm in size. Central well of 2.5 mm in diameter and peripheral wells of 3 mm in diameter were cut at intervals of 3 mm. The anti-serum was poured in the central well and samples were poured in the peripheral wells. Reaction was observed 24 to 36 hr after amido black 10B staining.

Results

In immunoelectrophoresis, anti-PAG sera reacted strongly in alpha 2 region with sera in pregnancy but not with normal sera. In Ouchterlony’s method, the precipitation line fused with that of the anti- alpha 2 AP glycoprotein serum (Behring Institut) and no reaction was observed with normal serum.

In immunoelectrosyneresis all of 42 sera were positive, and in Ouchterlony’s method only one out of 42 was negative. As for saliva, the precipitation lines were faint and it was necessary to stain with amido black 10B, and the incidence was more than 60% (12 and 11 out of 18 samples by immunoelectrosyneresis and Ouchterlony’s method, respectively). As for urine, only 8 (33%) and 6 (25%) out of 24 samples were positive by immunoelectrosyneresis and Ouchterlony’s method, respectively. Amniotic fluid was too viscous to diffuse, and so only 3 out of 21 were positive by Ouchterlony’s method, but by immunoelectrosyneresis 19 (91%) out of 21 samples were positive. Serum, saliva, urine and amniotic fluid were available in 12 cases. By immunoelectrosyneresis, four cases of them were positive in the above four samples and 11 cases in three samples but urine.

Fig. 1. Precipitation lines to anti alpha 2 AP glycoprotein serum (Behring Institute) and anti PAG serum (absorbed with normal male serum)
P: pregnant  N: normal
Pregnancy-Associated Globulin

TABLE 1. PAG incidence on body fluid in pregnancy

<table>
<thead>
<tr>
<th>Ouchterlony’s method</th>
<th>pos.</th>
<th>neg.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>41(98%)</td>
<td>1</td>
<td>42</td>
</tr>
<tr>
<td>Saliva</td>
<td>11(61%)</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Urine</td>
<td>6(25%)</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>3(14%)</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunoelectrosyneresis</th>
<th>pos.</th>
<th>neg.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>42(100%)</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>Saliva</td>
<td>12(67%)</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Urine</td>
<td>8(33%)</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>19(91%)</td>
<td>2</td>
<td>21</td>
</tr>
</tbody>
</table>

DISCUSSION

Pregnancy-associated globulin (PAG) in sera in pregnancy or malignant diseases has been investigated by many researchers, but there is no report of PAG in saliva, urine and amniotic fluid as far as we know. The present authors examined PAG in these body fluids just before and after delivery by immunoelectrosyneresis and Ouchterlony’s method. 19 out of 21 amniotic fluid samples (91%) and more than 60% of saliva samples showed positive reaction. PAG in these fluids was not analyzed quantitatively, but the clarity of precipitation lines suggested that PAG concentration in sera was higher than that in the other fluids. The incidence of PAG in urine was the lowest among these fluids and every raw urine had to be concentrated sufficiently for the examination. The incidence of PAG in amniotic fluid was 14% by Ouchterlony’s method and 91% by immunoelectrosyneresis. Amniotic fluid was so viscous that it was difficult to centrifuge and filtrate. Therefore, the incidence by Ouchterlony’s method was so low. On the other hand, the incidence by immunoelectrosyneresis was almost similar to that of sera, and the precipitation line was clear. This suggested that PAG concentration in amniotic fluid was nearly the same as that in sera. The incidence in saliva was above 60% by both methods. PAG resisted strongly the digestive enzymes, and if the samples were concentrated, the incidence might be increased to the level of the sera.

Identification of PAG from the body fluids may give available information not only for diagnosis of pregnancy but also for criminal investigation.

Acknowledgment

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References

1) Afonso, J.P. & Farnham, N.G. (1962) Studies on a new electrophoretic zone in labor
6) Smithies, O. (1959) cited from 1).