ELECTROPHORETIC INVESTIGATION OF AMNIOTIC FLUID PROTEINS

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ABSTRACT

Amniotic fluids obtained from 56 pregnant women in different gestational weeks were investigated. Total protein levels were determined by the Lowry method and by Laemli SDS-polyacrylamide gel electrophoresis carried out on amniotic fluid samples.

No correlation was found between gestational age and albumin nor low or high molecular weights proteins. Albumin increased linearly with gestational week. Low molecular weight proteins showed a significant decrease with gestational week where as high molecular proteins increased although not significantly.

Key Words: Amniotic fluid, protein, electrophoresis

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INTRODUCTION

In normal development, amniotic fluid protects fetus from infections and trauma and provides a suitable constant temperature environment for the development of the lungs and for fetal movement. Its quantity and composition change with gestational weeks and fetus condition (1,2,3,4). It is produced by the chorion frondosum, fetal skin, urinary and respiratory systems (5). Its production is fast and constant in the first half term of pregnancy then decreases (1). Its volume reaches a maximum at 36-37 weeks. Before keratinization of the fetal skin, fluid and other elements pass through it into the amniotic space. Amniotic fluid composition is similar to fetus extracellular fluid this period (2,6). When fetal skin permeability decreases because of keratinization amniotic fluid osmolarity and sodium concentration decrease gradually and creatinin and urea concentration increases (7). At the end of the first trimester when the metanephric kidney begins to function amniotic fluid increases significantly. Fetal urine production increases continually towards term (8,1,3).

Investigation of amniotic fluid at different time of pregnancy is therefore known to give us a good deal of useful data on fetal health. Today fetal abnormalities are among the major factors affecting families leading to sociological, psychological and financial problems which prenatal diagnosis followed where where necessary, by termination, might possibly avoid. In recent years, examination of amniotic fluid has been helpful in the diagnosis of certain pathologies and evaluation of amniotic fluid proteins has been reported as being more valuable than function tests.

In this study we examined polyacrylamide gel electrophoresis for the proving of the effective of the amniotic fluid for evaluation of certain fetal abnormalities.

MATERIAL AND METHODS

This study was performed in a private hospital in Istanbul, on 37 pregnant women by transvaginal ultrasound within 20-40 weeks pregnant. They were divided into two groups: 27-35 weeks pregnant (n=9) and 40-42 weeks pregnant (n=18). Amniotic fluid was collected by transvaginal ultrasound technique. Aliquots were collected and kept at minus 20°C until electrophoresis was performed. Electrophoretic examination was carried out by SDS-PAGE. The gel system used was a 10% acrylamide gel with the molecular weight standard proteins used.
Fluid protects fetus from lesions a suitable constant of the lungs and composition change with composition constant in the first 1, 2, 3, 4). Its produced urinary and respiratory and constant in the first s (1). Its volume reaches maturation of the fetal s through it into the osition is similar to d (2, 6). When fetal skin keratinization amniotic tion decrease gradually increases (7). At the end epidermic kidney begins to signify. Fetal urine towards term (8, 1, 3). at different time of we us at a good deal of fetal anomalies are families leading to ancial problems which where necessary, by recent years,

examination of amniotic fluid has been reported as being helpful in the diagnosis of certain pathologies and the evaluation of amniotic fluid low molecular weight proteins as being more valuable than classic renal function tests. proteins has been reported as helpful in the diagnosis of certain pathologies and amniotic fluid low molecular weight proteins evaluation as being more vauble than classic renal function tests.

In this study we examined amniotic fluid proteins by SDS polyacrylamide gel electrophoresis.

MATERIAL AND METHODS

This study was performed on 56 women attending the Zeynep Kamil Hospital, Istanbul, Turkey. They were between 15 and 42 weeks pregnant. They were divided into four groups: 1) 15-26 weeks (n=9) 2) 27-35 weeks (n=12) 3) 36-39 weeks (n=17) 4) 40-42 weeks (n=18). Amniotic fluids were obtained from 61 pregnant women by transvaginal and transabdominal ways. Each sample, which were almost 10 ml, were divided into small aliquots and kept under deep freeze. Their total protein concentrations were determined by the method of Lowry (13).

Electrophoretic examination of amniotic fluid proteins carried out by SDS polyacrylamide gel electrophoresis as described by Laemli (14). A Schleicher and Schueller profile system mini electrophoresis was performed and Sigma low molecular weight standard mixture (SDS-7 Dalton Mark VII-L) used. In each slab gel wells 20 ug denatured samples were
placed. After electrophoresis, densitograms of the protein bands from slab gels were obtained by densitometer (Helena Laboratories EDC). From the peak areas of the densitograms, the percentages and the amount of each protein in each band were calculated.

The results were evaluated by ANOVA variance analysis using the NCSS statistical computer package.

RESULTS

In the SDS polyacrylamide gel electrophoresis of amniotic fluid proteins, 5 to 9 protein bands (mean 7) could be seen (Fig 1). Their molecular weights were between 86 kDa and 13 kDa. Since some protein bands with higher or lower molecular weight than albumin were very thin and very near to each other, they were grouped as either high molecular weight (HMW) proteins and low molecular weight (LMW) proteins (Fig 1). In accordance with the literature (35), the HMW proteins were mainly haptoglobin (86 kDa), transferrin (90 kDa) and the LMW proteins a microglobulin (33 kDa), apolipoprotein (27 kDa), and retinol binding protein (22 kDa).

Although there was no significant difference in albumin or HMW protein levels between any the gestational groups, there was an observable increase towards term in albumin. LMW levels decreased significantly between consecutive groups towards term. There was an increase in high molecular proteins; however, this was not significant (Table 1, Fig 2).
rams of the protein by densitometer (Helena is of the densitograms, protein in each band.

Anova variance analysis package.

Electrophoresis of amniotic (mean 7) could be seen between 86 kDa and 137 higher or lower molecular and very near to each high molecular weight (HMW) W) proteins (Fig 1). In the HMW proteins were in (90 kDa) and the LMW apolipoprotein (27 kDa), difference in albumin or stational groups, there rm in albumin. LMW levels evitive groups towards lecular proteins however g 2).

Figure 1: SDS polyacrylamide gel electrophoretic patterns of some amniotic fluid samples (st: standard protein mixture, MW: molecular weight, kDa).

Figure 2: Distribution of Albumin, HMW and LMW proteins according to gestational weeks.
Table 1: Comparison of amniotic fluid total protein levels and protein bands (albumin, LMW and HMW proteins) in respect to gestational week (w) and significance of differences (SD: Standard deviation)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Protein (mg/dl) Mean</th>
<th>SD</th>
<th>HMW Proteins (µg) Mean</th>
<th>SD</th>
<th>Albumin (µg) Mean</th>
<th>SD</th>
<th>LWM Proteins (µg) Mean</th>
<th>SD</th>
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</thead>
<tbody>
<tr>
<td>(15-25 w)</td>
<td>366.53</td>
<td>136.43</td>
<td>4.10</td>
<td>0.91</td>
<td>8.51</td>
<td>0.70</td>
<td>7.20</td>
<td>0.77</td>
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<td>(n=9)</td>
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<td>Group 2</td>
<td>456.8</td>
<td>224.09</td>
<td>3.31</td>
<td>0.94</td>
<td>8.21</td>
<td>1.73</td>
<td>8.27</td>
<td>0.64</td>
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<tr>
<td>(27-35 w)</td>
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<td>(n=12)</td>
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<tr>
<td>Group 3</td>
<td>309.43</td>
<td>95.29</td>
<td>4.04</td>
<td>1.19</td>
<td>8.89</td>
<td>1.78</td>
<td>6.76</td>
<td>1.67</td>
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<td>(36-38 w)</td>
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<td>(n=17)</td>
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<tr>
<td>Group 4</td>
<td>382.00</td>
<td>188.48</td>
<td>4.1</td>
<td>0.88</td>
<td>9.88</td>
<td>1.28</td>
<td>5.84</td>
<td>0.51</td>
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<tr>
<td>(39-42 w)</td>
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<td>(n=18)</td>
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<td>P</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
<td>&lt;0.01</td>
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DISCUSSION

In our study we generally confirm that electrophoresis and define the molecular weight proteins.

Burghard found total protein levels of amniotic fluids to decrease in the second trimester. He also noted that albumin levels stopped an increase towards term and decreased similar to Burghard's findings. LMW proteins is generally a marker of tubular function. In the study, hypoxemia conditions were found from the literature that the excreting LMW proteins increase with gestational age. However, filtered from glomerulus with gestational age, the presence of LMW proteins is an early measure of kidney maturation, normal conditions, LMW protein levels have been reported as being...
DISCUSSION

In our study we generally obtained seven proteins bands by electrophoresis and defined them as albumin, low and high molecular weight proteins.

Burghard found total protein, albumin, low molecular weight proteins, α microglobulin and B2 microglobulin levels in amniotic fluids to decrease significantly towards the end of the second trimester. He also reported that the decrease in albumin levels stopped and became constant but that low molecular weight proteins continued to decrease (16). In our study albumin levels were lowest in Group 2 and they increased towards term in Group 3 and 4. LMW proteins decreased similar to Burghard’s study. The decrease in LMW proteins is generally attributed to the development of tubular function. In the present study the changes in hypoxemia conditions were not investigated. But it is clear from the literature that the tubules respond to hypoxemia by excreting LMW proteins (17,18). It would seem to follow that the production of LMW proteins decrease with increasing gestational age. However, the amount of these proteins filtered from glomerulus has been shown to actually increase with gestational age (16,19). It should be noted that the presence of LMW proteins is a necessary but not sufficient measure of kidney maturation. After 30 gestational weeks in normal conditions, LMW proteins α1 and B and microglobulins have been reported as being not more than 0.25 g/L, 30 mg/L.
and 8 mg/L respectively (16).

Since the levels of these proteins in amniotic fluid and fetal urine are similar, the source of LMW proteins are believed to be fetal (16). In our study these proteins disappeared after 35 weeks but after 32 weeks in Burghard's study (16).

HMW proteins and albumin are not related to fetal kidney maturation. They may however be used in the diagnosis of congenital nephrotic syndrome. In our study HMW proteins did not change significantly during the pregnancy, which is consistent with Burghard's study (16).

The presently conducted urea, creatinine tests are not sufficiently indicative of fetal kidney function (20). Urine flow, Na, Cl and osmolarity have been used to evaluate fetal kidney development. In recent years due to the work of Holzgreve, LMW proteins have been added to these parameters (21). In obstructive uropathies, LMW proteins increase, because of insufficient reabsorption due to damage of the proximal tubulus (21).

The examination of amniotic fluid proteins has gained a lot of importance in prenatal diagnosis, however further studies are still necessary.

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