Tubular proteins and enzyme content in the amniotic fluid

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Abstract

Amniotic fluid is the product of many substances and fetal urine is considered to be one of the principal components. Only a few reports have been published describing the concentration of microglobulins and urinary enzymes in the amniotic fluid. We determined the levels of alpha,-m, beta,-m, AAP and NAG, in 154 samples of amniotic fluid (103 early determinations and 51 late determinations) as a function of gestational age. We observed a statistically significant decrease in concentration of alpha,-m (P < 0.001), beta,-m (P < 0.01) and AAP (P < 0.001) when early and late amniotic fluid samples were compared. A statistically significant increase of NAG (P < 0.01) and creatinine (P < 0.01) was also found. A significant correlation was observed between alpha,-m and betal-m, and between AAP and NAG, respectively. The potential role of urinary enzyme and microglobulin determination in amniotic fluid as an index of fetal kidney development, is discussed.

Keywords: alpha,-Microglobulin; beta,-Microglobulin; Alanine aminopeptidase; N-acetyl-β-D-glucosaminidase; Amniotic fluid

1. Introduction

Amniotic fluid is the product of many substances and fetal urine has been shown to be one of the main components. alpha,-Microglobulin (alpha,-m) and beta,-microglobulin (beta,-m) are two proteins of low molecular weight, 33 000 and 11 800 Da, respectively, which have the property of being readily filtered by the glomerulus and in normal conditions are always nearly completely re-absorbed by the proximal tubular cells [1,2]. However, in the presence of impaired tubular function, the amount re-absorbed decreases while the urinary excretion increases [3–8].

Alanine aminopeptidase (AAP) and N-acetyl-β-D-glucosaminidase (NAG) are two enzymes located in the brush border of the tubular cells and in the lysosomes, respectively. Because of their high molecular weight (240 000 and 136 000 Da) these enzymes, normally, are unable to pass throughout the glomerulus and it is commonly reported that their urinary levels are derived from the kidney [9–12]. Several reports concerning the levels of beta,-m in amniotic fluid have been published [13–15], but limited data are at present available on the concentration of alpha,-m [15,16]. The purpose of our study was to evaluate the levels of alpha,-m, beta,-m, AAP, NAG, and creatinine in the amniotic fluid at different gestational ages.

2. Materials and methods

Amniotic fluid samples were obtained from 155 healthy pregnant women at various stages of gestation by abdominal amniocentesis. In 101 cases (group A) amniocentesis was performed at an early stage (mean gestational age, 16 ± 2 weeks; range, 15–19 weeks) for chromosome analysis. In the remaining 54 cases (group B) amniocentesis was performed later (mean gestational
age, 31.6 ± 2.5 weeks; range, 28–36 weeks) to evaluate fetal pulmonary maturation and in order to define the appropriate timing of birth.

In all cases congenital defects, pregnancy-related maternal disease and maternal or fetal infection and impaired development and maturation of the fetus were excluded. Furthermore, functional indexes of the fetal-placental unit were normal in all women without history of drugs consumption.

The gestational age was determined by history and clinical examination and by ultrasonographic assessment of the biparietal diameter.

Immediately after being drawn, the amniotic fluid was divided into several samples, one of which was stored at −20°C until the chemical tests were performed. The concentrations of alpha-1-m and beta-2-m in the amniotic fluid were measured immuno-turbidimetrically, by Latex Agglutination Photometric Immunoassay (LAPIA), using anti-human specific antibodies (anti-alpha-1-m and anti-beta-2-m, respectively) coated with polystyrene latex particles of 0.79 μm in diameter (Eiken Chemical Co Ltd, Tokyo, Japan). The assay was performed on an automatic turbidimeter (LA System 2000, Poli diagnostic division, Milan, Italy). AAP activity was measured at 410 nm and 37°C [17] by monitoring the increase of absorbance due to the release of 4-nitroaniline catalysed by AAP during 15 min (FAR Divisione Diagnostici, Verona, Italy). To determine the activity of NAG in amniotic fluid, the procedure of Findlay et al., modified by Gressner and Roebruck [18], was performed. The increase of absorbance due to release of p-nitrophenol was measured at 405 nm and 37°C (FAR Divisione Diagnostici, Verona, Italy). Both AAP and NAG assays were automatically performed on the COBAS FARA II analyzer (Roche Divisione Diagnostici, Milan, Italy). Creatinine was determined enzymatically.

Statistical analysis was performed with the StatView SE+Graphics™ package (version 1.03) and with the Cricket Graph package (version 1.3), using a Macintosh SE/30 personal computer (Apple Computer Inc., Cupertino, CA, USA).

We calculated mean values, standard deviation and standard error of the variables. The relationship between two independent variables was analysed by linear regression and Pearson’s correlation coefficient, r, was calculated. Student’s unpaired t-test (one-tailed) was performed to evaluate differences between the results obtained in samples from early and late amniocentesis.

3. Results

Results (mean ± S.E.) are summarized in Table 1. The behaviour of alpha-1-m and beta-2-m (mean values expressed as mg/l and mg/mmol creatinine) are presented in Fig. 1.

There was a statistically significant decrease in concentration of alpha-1-m (P < 0.001), beta-2-m (P < 0.01) and AAP (P < 0.001) as pregnancy progressed. A statistically significant increase of NAG (P < 0.01) and creatinine (P < 0.01) was found during pregnancy. Additionally, we observed a significant correlation between alpha-1-m and beta-2-m both in the early and late amniotic fluid samples either expressed as mg/l (Fig. 2) or as mg/mmol creatinine (Fig. 3). Finally, in all samples there was no significant correlation between AAP and NAG.

No statistically significant correlations between tubular proteins and enzymes, tubular proteins and creatinine, enzymes and creatinine could be demonstrated.

4. Discussion

Our study is the first to our knowledge to assess both enzymes and microglobulins in amniotic fluid.

Our data confirm the results of Burghard et al. [15] on alpha-1-m, showing a progressive decrease of the microglobulin alpha-1-m as gestational age advances. However, we found lower values in our study; this was probably due to the fact that Burghard et al. employed a different method (alpha-1-m was assayed by radial immunodiffusion and beta-2-m was determined by an
enzyme immunoassay). On the other hand, there is considerable literature focusing on the concentration of beta-m in the amniotic fluid. All those studies have shown a progressive fall of this microglobulin during pregnancy [13,15].

The cause for the decrease of alpha-m and beta-m in the amniotic fluid is not well understood and the biological role of these proteins is still uncertain [1-4]. It is evident that beta-m plays a basic role in the structure of membrane surfaces and HLA molecules [2,3]. Moreover, alpha-m interferes with chemotaxis and the interaction between leukocytes and antigens [1,4,5].

Several hypotheses have been advocated to explain the fall in alpha-m in the amniotic fluid [15,16].

The first hypothesis takes into account a reduced uptake of microglobulin from maternal blood as a result of chronic changes of placental circulation and/or amniotic–chorionic tissue. However, many studies have provided evidence which challenges this hypothesis. In fact, the serum concentration of alpha-m during pregnancy is similar to that of controls [7,15,16]. There is no
correlation between the concentration of alpha₂-m and beta₂-m in cord and maternal blood [15]. Moreover, in contrast to most other proteins, the concentration of alpha₁-m and beta₂-m is higher in the amniotic fluid than in maternal serum and it is unlikely that a counter-current mechanism transfer can occur [13,16,19].

A second hypothesis is based on a specific mechanism of dilution. It is well known, in fact, that the volume of the amniotic fluid increases 30-fold between the 10th and 30th week of gestation [19]. However, this explanation seems too simplistic [13].

A third hypothesis postulates a decrease in the fetal production of alpha₂-m and beta₂-m during the last trimester. The observation that production of beta₂-m in the fetus correlates with the development of fetal lymphoid tissue starting around the 8th week of gestation [2,3], and that production of alpha₁-m is probably related to the progressive development of the liver during intrauterine life [1,4,5] are against this theory. Furthermore, the concentration of alpha₁-m determined in the umbilical cord is higher when compared with other pediatric ages [16]. Nolte et al. [16] documented that umbilical cord concentrations of alpha₁-m are lower in infants born at term than in premature babies as a consequence of glomerular renal development. Levels of alpha₁-m are higher in the urine of newborns than in older children or adults [4]. We have recently published data on the urinary excretion of alpha₁-m in healthy newborns, born at term vaginally or by caesarean section [5].

The last hypothesis concerns the development of renal tubular function. It is currently assumed that alpha₁-m and beta₂-m in amniotic fluid are largely produced by the fetus itself mainly as a consequence of fetal urinary excretion [1]. According to the literature alpha₁-m and beta₂-m accumulate in the amniotic fluid until the reabsorption ability of the proximal tubular cells in the fetal kidney increases. Burghard et al. [15] have demonstrated a strong correlation between the concentrations of alpha₁-m in amniotic fluid and in the first voided urine of preterm infants of corresponding gestational age. The same correlation holds true in the presence of pathological conditions affecting the fetal kidney. The correlation that we have observed between alpha₁-m and beta₂-m both in early and late amniocenteses suggest that these microglobulins might be representative of the same tubular function of fetal kidney.

As far as the enzymes are concerned, our data on NAG match with those previously published [21,22]. The activity of NAG in the urine voided by term infants during the first 12 h of life, which reflects the composition of fetal urine, is lower than NAG activity in the amniotic fluid at term [11]. The increase of NAG in amniotic fluid during pregnancy seems, therefore, to be related to extra-fetal factors. Conversely, the urinary concentration of AAP during the first few hours of life corresponds to the concentration in the amniotic fluid at the same gestational age [11]. The different behaviour of these two enzymes is assumed to be related to the different position inside the proximal tubular cell (AAP is more superficial than NAG) [9,10,12,29].

In conclusion, we believe that our study can provide valuable reference data for further investigations in order to define the link among maternal and fetal metabolism of microglobulins and enzymes. Further studies are needed to assess the diagnostic value of alpha₁-m in evaluating fetal kidney function, in normal and pathological conditions.

References


