Mar. Üniv. Ecz. Der.,
11 (1 - 2), 281 - 291 (1995)

J. Pharm. Univ. Mar.,
11 (1 - 2), 281 - 291 (1995)

ELECTROPHORETIC INVESTIGATION OF AMNIOTIC FLUID PROTEINS

Serhat PARTALCI* – Ayşen YARAT** – Kılış AYDINLI*** – Doğan CANTEKİN* – Nesrin EMEKLİ**

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 sinificant decrease with gestational week where as high molecular proteins increased although not significantly.

Key Words:Amniotic fluid,protein,electrophoresis

* Zeynep Kamil Women's and Children's Hospital, İstanbul, TURKEY.
** Marmara University, Faculty of Dentistry, Department of Biochemistry, İstanbul, TURKEY.
*** İstanbul University, Cerrahpaşa Medical Faculty, Gynaecology and Obstetrics Clinic, İstanbul, TURKEY.

INTRODUCTION

In normal development, amniotic fluid protects fetus from infections and trauma and provides a suitable constant temperature enviorenment 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).Its 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 usefull datas on fetal health. Today fetal anormalities 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 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, İstanbul,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,dansitograms of the protein bands from slab gels were obtained by dansitometer (Helena Laboratorios EDC). From the peak areas of the dansitograms, 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 haptoglobulin (86 kDa),transferin (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).

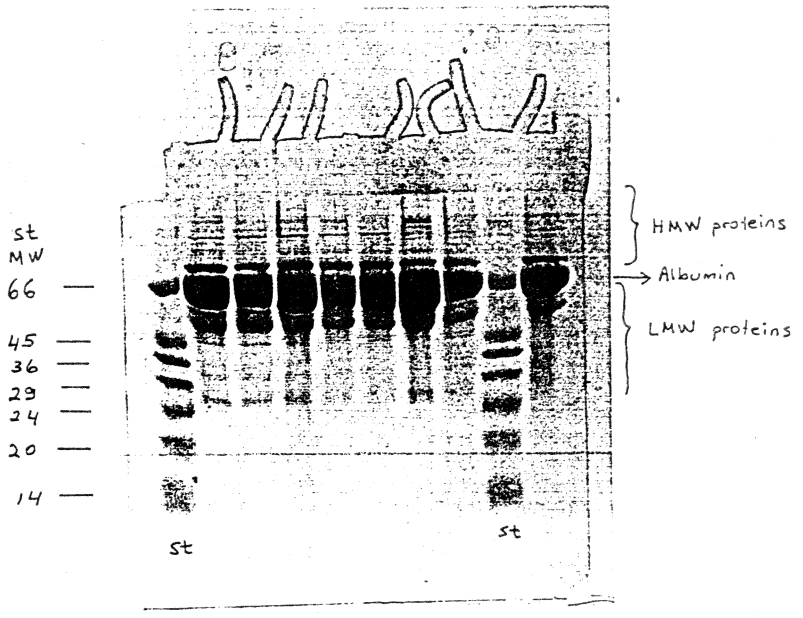

Figure 1 : SDS polyacrylamide gel electrophoretic patterns of some amniotic fluid samples(st: standard protein mixture, MW:molecular weight,kDa)

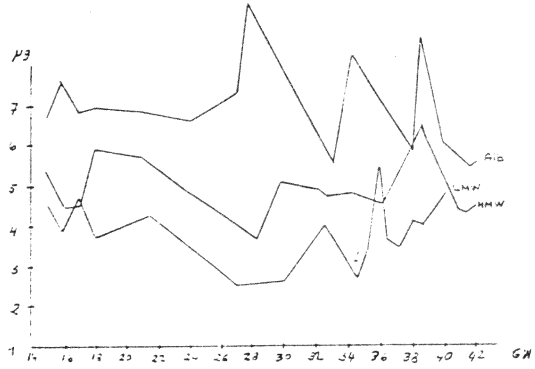

Figure 2: Distrubution of Albumin, HMW and LMW proteins according to gestational weeks.

Table 1: Comparison of amniotic fluid total protein levels and protein bands (albumin, LMW and HMW proteins) in respect to gestationel week (w) and significance of differences. (SD: Standard deviation)

	Protein (mg/dl)		HMW Proteins (µg)		Albunin (µg)		LWM Proteins (µg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group 1 (15-26 w) (n=9)	366.53	136.43	4.10	0.91	8.51	0.70	7.20	0.77
Group 2 (27-35 w) (n=12)	456.8	224.09	3.31	0.94	8.21	1.73	8.27	0.64
Group 3 (36-38 w) (n=17)	309.43	95.29	4.04	1.19	8.89	1.78	6.76	1.67
Group 4 (39-42 w) (n=18)	382.00	188.48	4.1	0.88	9.88	1.28	5.84	0.51
P	>0.1		>0.1		>0.1		<0.01	

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, a microglobulin and B2 microglobulin levels in amniotic fluids to decrease significantly towards the end of the second timester. 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 sufficent measure of kidney maturation. After 30 gestational weeks in normal conditions, LMW proteins o1 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.

REFERENCES

1 - Wallenburg HC: The amniotic fluid: water and electrolyte homeostasis. J Perinat Med, 3:191, 1977.

2 - Lind T , Kendal A, Hytten FE : The role of the fetus in the formation of amniotic fluid. J Obstet Gynaecol Br Commw, 49:287, 1972.

3 - Wladimiroff JW and Campbell S: Fetal urine production rates in normal and complicated pregnancy. Lancet,1:151, 1974

4 - Pritchard JA: Fetal Swalling and amniotic fluid wolume obstet. J Gynaecol Br Comm, 28:606, 1966.

5 - Bissonnette J: Placental and fetal physiology. In: Gabbe SG, Niebyl JR and Simpson JL (eds) : Obstetritcs normal and problem pregnancies, New York, Churchill Livingston p.109, 1986.

6 - Fox H: The placenta. In: Philipp BJ and Newton M (eds) Scientific Foundations of Obstetrics and Gynecology, London Year Book Medical publishers.p.236, 1986.

7 - C Savona-Ventura: Amniocentesis for fetal maturity (Review) Obstetrical and Gynecol. Survey Williams and Wilkins, vol.42, no.12, 1987.

8 - Harrison MR, Mitchel SG, Roy AF. The unborn patient, Prenatal Diagnosis and treatment.2nd ed. W.B.Saunders Company Philadelphia, 1991.

9 - Şentürk L, Hekim N. Prenatal tanıda noninvazif yöntemler, Prenatal Tanı ve Tedavi,bölüm.5, Doç.Dr. K.Aydınlı, Perspektiv Yayın, İstanbul,1992.

10- Apak MY. Genetik Hastalıklara Yaklaşım ve Genetik Danışma (B1.1) Prenatal Tanı ve Tedavi, Doç.Dr.K.Aydınlı, Perspektiv Yayın, İstanbul,1992.

11- Bevis DCA: The antenatal prediction of hemolytic disease of the newborn. Lancet,1:395, 1952.

12- Johnson A. and Hodmilon L: Genetic amniocentesis at weeks or less. Clin Obstet Gynecol, 31:345, 1988.

13- Lowry OH, Rosenberg WI, Farr AL, Randal RJ : Protein measurement with the foli phenol reagent. J Biol Chem. 193: 265-275,1951.

14- Laemli UK: Cleavage of structural proteins during the assembly of the head of bacterophage T4. Nature, 227: 680-685,1970.

15- Holzgreve W, Bulla M, Evans M, Lison A, Kaufman C:Protein analysis to determine fetal kidney function. Am J Obstet Gynecol, 164:336, 1991.

16- Burghard R et al: Microproteins in amniotic fluid as an index of changes in fetal renal function during development. Pediatr Nephrol, 1:574-580, 1987.

17- Tsukahara H et al: Assessment of tubular function in neonates using urinary B2-microglobin. Pediatr Nephrol, 4:512-514, 1990.

18- Cole JW, Portman RJ, Lim Y, Perlman JM, Robson AM. urinary B2-MG in full-term newborns.Evidence for proximal tubular dysfunction in infants with Meconium-stained amniotic fluid. Pediatrics, Vol.76,No.6, 1985.

19- Assadi FK, John EG, Justice P, Fornell L:B2-microglobulin clearence in neonates:index of tubular maturation. Kidney International, 28:153-157, 1985.

20- Tack DE, Perlman MJ, Robson AM: Renal injury in sick new infants. A prospective evalution using urinary B2 microglobulin concentrations. Pediatrics, vol.81, no.3, 1988.

21- Holzgreve W. Fetal Tedavi, Prenatal Tanı ve Tedavi, Doç.Dr.K.Aydınlı (Ed.) Perspektiv yayın, İstanbul,1992.