ENZYME ACTIVITIES IN THE AMNIOTIC FLUID

by

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Introduction

Amniotic fluid is the nearest environment for the foetus, so its analysis in recent years has been utilized for monitoring the foetal status *in utero*. Enzyme activities of the amniotic fluid can show the maturity of the foetus e.g. amylase (Wolf and Tawsing, 1973) or their deficiencies can predict the inherited diseases (Nadler, 1969).

However, sufficient literature is not available showing effects of different factors like gravidity of the mother, sex and weight of the foetus and maturity score of the newborn. The present study was intended to analyse the effects of such factors on amniotic fluid protein concentration and different enzyme activities. Also the levels of amniotic fluid protein, amylase and alkaline phosphatase activities were correlated with those of cord serum, maternal serum and newborn urine.

Material and Methods

Amniotic fluids of 243 normal pregnant women from S.S.G. Hospital, Baroda were studied. The amniotic fluids were collected by suction, amniocentesis or artificial rupture of the membrane. Out of 243 subjects, 50 were also studied for their corresponding cord serum, maternal serum and newborn urine. The cord blood was collected from the placental side of the severed umbilical cord without squeezing it. The maternal blood was collected within 30 minutes of delivery from the peripheral vein. The newborn urine was collected within two days of birth, and for this collection special urine collecting bags were used (purchased from Bard Cansumer, Products Division, Murry Hill, N.J. 07974 CR Bard Canada Ltd., Mississauga, Ontario). All the fluids collection was done in plain bulbs. The urine was centrifuged and supernatant was used. In case of amniotic fluid, it was first centrifuged at 3000 RPM for 10 minutes, filtered through the ordinary filter paper and then was analysed.

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TABLE I

*Amniotic Fluid Total Protein, Alkaline Phosphatase, Amylase, GOT and GPT Levels During Different Gestational Ages**

	Gestational Wteks								
	8-15	16-17	18-19	20-21	22-25	26-30	34-36	37-38	39-40
Total Protein (gm/dl)	0.53 ± 0.23 (5)	0.58 ± 0.18 (30)	0.58 ± 0.21 (35)	0.62 ± 0.18 (52)	0.63 ± 0.18 (23)	0.61 ± 0.17 (5)	0.40 ± 0.16 (15)	0.38 ± 0.13 (41)	0.38 ± 0.26 (30)
Amylase (Somogyi Units/dl)	35.2 ±11.3 (5)	34.1 ±23.8 (24)	38.5 ±23.5 (25)	42.5 ±18.5 (51)	40.7 ±21.0 (23)	32.8 ±13.6 (4)	136.5 ±97.5 (15)	130.1 ±56.8 (34)	121.6 ±74.1 (26)
Alkaline Phosphatase (KA Units/dl)	1.4 ± 2.2 (7)	2.3 ± 1.3 (30)	3.3 ± 2.8 (34)	3.1 ± 3.1 (55)	2.3 ± 2.3 (25)	3.8 ± 3.4 (5)	3.5 ± 2.1 (15)	3.6 ± 2.5 (37)	3.2 ± 2.8 (25)
GOT (IU/1)	4.0 ± 2.1 (2)	4.2 ± 1.9 (20)	5.1 ± 4.5 (16)	5.2 ± 3.5 (24)	5.3 ± 3.8 (16)	4.2 ± 1.3 (3)	7.6 ± 1.4 (3)	6.2 ± 4.7 (15)	3.4 ± 1.6 (6)
GPT (IU/1)	1.5 ± 0.7 (2)	4.9 ± 4.4 (17)	4.3 ± 3.5 (12)	3.9 ± 2.5 (22)	4.3 ± 4.3 (15)	2.0 ± 1.3 (2)	4.6 ± 5.4 (3)	2.9 ± 2.4 (11)	1.6 ± 0.5 (6)

* Mean values, ± Standard Deviation and ( ) Number of Observations.

All these samples were stored in the deep freeze and were analysed within 1 week. Amniotic fluid was analysed for total protein (Lowry *et al*, 1951), amylase (Carrawa, 1959), alkaline phosphatase (Kind and King, 1954), GOT and GPT (Mohun and Cook, 1957). The cord serum, maternal serum and newborn urine were analysed for total protein, amylase and alkaline phosphatase by the same methods. The maturity score was calculated by the method of Chikermane *et al* (1969).

Results

The subjects were divided into 9 gestational age groups as shown in Table I to study the effect of gestational age. Then in each group the amniotic fluid total protein, amylase, alkaline phosphatase, GOT and GPT levels were correlated with different variants like the gravida of the mother and weight of the foetus. In 34-40 weeks, the levels were also correlated with sex and maturity score of the newborns. In 34-40 weeks age groups the amniotic fluid levels of total protein, amylase and alkaline phosphatase were correlated with those of cord serum, maternal serum and newborn urine.

Total protein concentration in amniotic fluid increased upto 22-25 weeks and then dropped significantly ($P < 0.001$). There were no effects of different factors like gravida, sex, weight and maturity score. No significant correlation was found between the total protein concentrations of amniotic fluid and those of cord serum, maternal serum or newborn urine. In amniotic fluid protein was almost half of the urine and was about 1/20th of the cord or maternal serum (Table II).

Amylase activity of the amniotic fluid did not change appreciably upto 26-30 weeks of gestation then there was a sudden and significant rise ($P < 0.001$). There were no consistent effects of weight, gravida and maturity score. In female foetus bearing mother, amniotic fluid amylase was almost 20% higher. The levels of amniotic fluid amylase showed some positive correlations with those of cord and maternal sera but these

TABLE II

Total Protein Concentrations in the Amniotic Fluid, Cord Serum, Maternal Serum and Newborn Urne During 34-40 Weeks

Gestational weeks	Total Protein (gm/dl)			
	Amniotic fluid	Cord serum	Maternal serum	Newborn urine
34-36	0.41 ± 0.12 (12)	7.59 ± 1.36 (12)	8.06 ± 1.50 (13)	0.71 ± 0.93 (5)
37-38	0.37 ± 0.11 (19)	7.00 ± 1.04 (19)	7.73 ± 1.07 (16)	0.94 ± 0.81 (11)
39-40	0.32 ± 0.14 (17)	6.95 ± 1.64 (14)	7.68 ± 0.81 (14)	0.56 ± 0.42 (10)

* Mean values, ± Standard Deviation and ( ) Number of Observations.

were statistically non-significant. In amniotic fluid amylase level was highest amongst the four studied fluids (Table III).

There were no consistent changes found in alkaline phosphatase activity with advance in gestation and the amniotic fluid alkaline phosphatase activity was negatively correlated with gravida, which was non-significant. With increase of maturity score low values of alkaline phosphatase were observed in 39-40 weeks age group ($P < 0.05$). There were no effects of sex and gravida (Table IV).

TABLE III

*Amylase Activity in the Amniotic Fluid, Cord Serum, Maternal Serum and Newborn Urine During 34-40 Weeks of Gestation**

Gestational weeks	Amylases (Somogyi Units/dl)			
	Amniotic fluid	Cord serum	Maternal serum	Newborn urine
34-36	130.9 ± 93.0 (12)	67.3 ± 27.1 (8)	99.6 ± 38.4 (8)	66.0 ± 52.6 (3)
37-38	117.4 ± 50.4 (17)	68.6 ± 33.0 (13)	78.3 ± 48.4 (16)	31.7 ± 20.2 (8)
39-40	133.6 ±101.0 (14)	66.8 ± 30.8 (10)	72.5 ± 35.9 (11)	80.8 ± 51.8 (9)

* Mean values, ± Standard Deviation and ( ) Number of observations.

TABLE IV

*Alkaline Phosphatase Activity in the Amniotic Fluid, Cord Serum, Maternal Serum and Newborn Urine During 34-40 Weeks of Gestation**

Gestational weeks	Alkaline Phosphatase (KA Units/dl)			
	Amniotic fluid	Cord serum	Maternal serum	Newborn urine
34-36	3.9 ± 2.1 (10)	9.1 ± 4.4 (11)	9.0 ± 3.9 (12)	2.6 ± 2.4 (4)
37-38	6.7 ± 8.4 (20)	7.3 ± 3.9 (20)	10.6 ± 5.0 (17)	1.3 ± 1.9 (10)
39-40	3.2 ± 2.2 (14)	6.8 ± 5.2 (12)	7.7 ± 4.7 (16)	1.5 ± 1.4 (9)

* Mean values, ± Standard Deviation and ( ) Number of observations.

The GOT and GPT activities did not show any definite trend, so after studying 105 samples, their estimations were dropped. Also no effect of different factors were observed.

Discussion

Many studies reported the peak level of amniotic fluid total protein concentration at about 25 weeks and then a drop (Queenan, 1971; Sutcliffe and Brock, 1972 and Armstrong *et al*, 1976). It is difficult to explain the initial increase, however, the decrease after 25 weeks can be due to utilization of amniotic fluid protein by foetus. Thomas (1968) stated that production of proteolytic enzyme starts by 24 weeks of gestation. Queenan (1971) observed low concentration of amniotic fluid protein in mothers of having heavier babies, however, such effect was not observed in the present study.

Wolf and Tawsing (1973) observed a sharp rise in the amniotic fluid amylase activity at 34 weeks of gestation. They also concluded that due to wide variations in the values, it could not be used as maturity index. In the present study, also a sudden and significant rise was observed in 34-36 weeks age group. Sachio *et al* (1978) studied isoamylases from the amniotic fluid, and found that the increase in the salivary gland type of amylase was faster than that of pancrease, suggesting that at 34 weeks salivary gland matures to secrete amylase. Fernandez *et al* (1973) suggested that to avoid the variation in the amniotic fluid amylase activity due to volume, the ratio between amylase and total protein concentration in amniotic fluid could be a better maturity index. From the present study, it was observed that the mean coefficient of variations during 34-40 weeks of gestation were 56.0% and 60.7% in the amylase and amylase to protein ratio respectively. Thus, it is better to use absolute amylase level instead of the ratio.

Sutcliffe *et al* (1962) and Hahneman and Soreson (1974) reported two peaks of alkaline phosphatase activity in amniotic fluid during gestation. In the present study peaks were observed at 18-19 weeks and 26-28 weeks. Mischel (1960) reported higher alkaline phosphatase activity in amniotic fluid when compared with those of cord and maternal sera, and there were no correlation between amniotic fluid, cord serum, and maternal serum. Gupta *et al* (1970) also found no such correlations. The present study agrees with these observations.

There are no reports on GOT and GPT activities in amniotic fluid at different gestational periods. Lapman and Briedman (1962) and Thaler *et al* (1979) studied these enzymes near term, and reported wide variations. The later study also noted that meconium contamination had no effect on the enzyme activities.

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Summary

The present study deals with the analysis of amniotic fluids from 243 normal pregnant women. Out of 243 subjects, 50 were studied for their cord serum, maternal serum and newborn urine. The total protein concentration of amniotic fluid increased upto 25 weeks of gestation and then dropped. Amylase activity did not show appreciable change

upto 26-30 weeks and then suddenly rose, while no definite trend was found in alkaline phosphatase, GOT and GPT activities. No significant effects of different factors like gravida of the mother, sex and weight of the foetus and maturity score of the newborn were observed. Also no significant correlation of the amniotic fluid total protein, alkaline phosphatase, and amylase activities were found with those of cord serum, maternal serum and newborn urine.

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