BIOCHEMICAL STUDIES ON AMNIOTIC FLUID AND MATERNAL SERUM WITH REFERENCE TO FOETAL MATURITY

by

M. S. P. NAIR,*

and

P. T. ANNAMALA,**

Introduction

The analysis of the constitutents in the amniotic fluid as an aid in the assessment of foetal maturity, placental function and prenatal sex determination is well documented. The contents of the amniotic fluid are supposed to be derived from maternal blood early in pregnancy, whereas foetal urine and placenta plays a major role in later stages of pregnancy (Levine *et al* 1966). As the pregnancy progresses the volume of amniotic fluid changes with resultant changes in the concentration of various constitutents. The actual knowledge of gestational age will be of great help in the early termination of pregnancy in conditions such as maternal immunisation, diabetes, toxaemia, infection, placental insufficiency etc. The constitutents creatinine, uric acid, proteins, alkaline phosphatases, sodium and potassium can be taken as good parameters to study the amniotic fluid, since they represent the various origins of the fluid, viz., foetal urine, placenta and maternal plasma.

TABLE I

Clinical Characteristics of the Subjects Selected for the Study

(The period of gestation in weeks was calculated from the last menstrual period)

Group	Period of gestation (weeks)	No. of cases	Age group	Gravida				Clinical examination
				I	II	III	IV	
I (Premature)	31-34	9	21-42	3	2	1	3	Normal
II (Premature)	35-38	12	22-44	2	2	3	5	Normal
III (Full term mature)	39-40	30	24-40	—	5	15	10	Normal
IV (Post-mature)	41-42	6	20-35	4	2	—	—	Normal

*Director and Professor, Department of Biochemistry, Medical College, Calicut, Kerala.

**Research Fellow, Department of Biochemistry, University of Kerala, Trivandrum, Kerala.

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Material and Methods

The study comprised of 57 subjects at various stages of pregnancy, ranging from 31 to 42 weeks of gestation. The subjects according to the period of gestation were classified into 4 groups. (Table I). The

period of gestation was calculated from the last menstrual period.

Amniotic fluid from premature cases was collected by transabdominal amniocentesis, and in mature and postmature cases during caesarean section. The particulate matter in the amniotic fluid was removed by centrifugation. Concomitant blood samples were taken from the superficial veins of the mother. The subjects selected for study were without any obstetric complications.

Creatinine was determined using the Jaffe' reaction (Bonsness and Tausky, 1945), uric acid by the method of Caraway (Caraway, 1963) and total proteins by the biuret method (Reinhold, 1953). The activities of alkaline phosphatases were assayed by the method of King (Kind and King, 1954) using amnioantipyrine, the heat stable variety was estimated after inactivation of the labile form at 56°C. The concentration of sodium and potassium were determined by flame photometry.

Statistical Analysis

The values given are mean ± S.D. of the number of cases in each group. The statistical significance of the data is calculated using Student's t-test (Bennet and Franklin 1967).

Results

Creatinine, Uric acid and Proteins: The results are given in Table II. There was a progressive increase in the concentration of creatinine as pregnancy progressess from a value of 1.61 ± 0.19 mg/100 ml. at 31-34 weeks of gestation to 2.22 ± 0.19 mg/100 ml. at full term, and it increased still further in postmature cases. The values at full term mature state, was significantly high ($p < .001$) from that at 35-38 weeks of gestation. It could be seen that the concentration of creatinine

TABLE II

Concentration of Creatinine, Uric Acid and Total Proteins in Amniotic Fluid and Maternal Serum at Various Stages of Pregnancies

(Values are mean ± S.D. from the number subjects in each group)

Group (period of gestation)	Amniotic Fluid			Maternal Serum		
	Creatinine mg/100 ml.	Uric acid mg/100 ml.	Total proteins mg/100 ml.	Creatinine mg/100 ml.	Uric acid mg/100 ml.	Total proteins gm/100 ml.
I (31-34)	1.61 ± 0.19	4.83 ± 0.70	310.67 ± 61.38	0.97 ± 0.21	2.96 ± 0.53	6.51 ± 0.19
II (35-38)	1.68 ± 0.17	5.37 ± 0.59	274.08 ± 34.58	0.91 ± 0.17	3.43 ± 1.25	6.35 ± 0.22
III (39-40)	2.22 ± 0.26[a,1]	7.58 ± 0.39[a,1]	216.00 ± 22.23[a,1]	1.04 ± 0.14	3.96 ± 0.67	6.42 ± 0.17
IV (41-42)	2.26 ± 0.11**	8.07 ± 0.16**	200.00 ± 11.55	0.91 ± 0.07	3.70 ± 0.55	6.52 ± 0.23

Comparison between groups

Full term nature cases (gr. III) compared with premature cases (gr. I) a, $p < .001$; b, $p < .01$; c, $p < .05$

Full term mature cases (gr. III) compared wih premature cases (gr. II) 1, $p < .001$; 2, $p < .01$; 3, $p < .05$

Post-nature cases (gr. IV) compared with full term nature cases (group III), *$p < .001$; ** $p < .01$;*** $p < .05$

in maternal serum showed no significant variation.

A gradual variation could be observed in the concentration of uric acid in amniotic fluid with increasing gestational age. The concentration of uric acid in amniotic fluid from full term cases was significantly more compared to that observed at 31-34 weeks ($p < .001$) and 35-38 weeks ($p < .001$) of gestation. The concentration of uric acid maternal serum remained without any significant variation.

The concentration of total proteins in amniotic fluid was very low (200-310 mg/100 ml) compared to that in maternal serum (6-7 g/100 ml). The concentration of proteins in amniotic fluid showed a significant decline from a value of 310.67 ± 61.38 mg/100 ml at 31-34 weeks tp 216 ± 22.23 mg/100 ml at full term ($p < .001$). The concentration of total proteins in maternal serum showed no significant changes due to the increase in the period of gestation.

Total and Heat Stable Alkaline Phophatases: The results are shown in Table III. The concentration of total alkaline phosphatase in amniotic fluid, the whole of which were heat stable variety, showed very wide deviation from the mean values. The concentration of total and heat stable alkaline phosphatases in maternal serum showed a significant correlation with the period of gestation. There was significant difference in the values of both the total and heat stable alkaline phosphatases at full term when compared to those at 31-34 weeks ($p < .001$, $p < .001$) and at 35-38 weeks ($p < .001$, $p < .001$) of gestation.

Sodium and Potassium: The resuts are given in Table IV. The concentration of sodium in amniotic fluid showed a significant increase at full term when compared to premature cases—group I and II—($p < .001$, $p < 001$), whereas its concentration in maternal serum showed a slightly significant variation ($p < .05$).

The concentration of potassium in amniotic fluid and maternal serum showed no notable change with the period of gestation.

Discussion

Pitkin *et al*, (1968) and Chandiok *et al* (1971) observed that a concentration of creatinine of 2 mg/100 ml. amniotic fluid closely related with a pregnancy of 36 weeks gestation and beyond. Our results are in agreement with the above reports.

TABLE III

Concentration of Total and Heat Stable Alkaline Phosphatases in Amniotic Fluid and Maternal Serum at Various Stages of Pregnancy

(Values, mean ± S.D. are expressed in K.A. units/100 ml.)

Group (Period of gestation)	Concentration of alkaline phosphatase			
	Amniotic fluid	Maternal serum		
		Total	Heat stable	Percentage of heat stable
I (31-34)	4.52 ± 4.91	13.5 ± 3.05	6.22 ± 1.67	45.06 ± 3.15
II (35-38)	9.36 ± 18.36	13.65 ± 2.67	7.17 ± 1.28	52.87 ± 4.41
III (39-40)	28.50 ± 37.11	19.53 ± 2.45$^{\alpha,1}$	13.01 ± 2.30$^{\alpha,1}$	66.23 ± 6.11$^{\alpha,1}$
IV (41-42)	105.58 ± 72.48*	20.58 ± 2.45	15.08 ± 3.21	73.00 ± 8.21***

For comparison between groups see Lengend under Table II.

TABLE IV

Concentration of Sodium and Potassium in Amniotic Fluid and Maternal Serum at Various Stages of Pregnancy

(Values, mean ± S.D. are expressed in m.Eq/l)

Group (Period of Gestation)	Amniotic Fluid		Maternal Serum	
	Sodium	Potassium	Sodium	Potassium
I (31-34)	129.6 ± 2.45	3.89 ± 0.13	132.0 ± 2.49	4.04 ± 0.26
II (35-38)	129.8 ± 4.36	3.93 ± 0.58	132.2 ± 4.45	4.07 ± 0.14
III (39-40)	123.8 ± 3.11[a,1]	3.96 ± 0.14	129.5 ± 2.51[c,3]	4.01 ± 0.20
IV (41-42)	119.67 ± 0.74**	4.1 ± 0.32	127.7 ± 1.37	3.95 ± 0.20

For comparison between groups see legends under Table II.

The progressive rise in the concentration of creatinine in amniotic fluid can well be correlated with the period of gestation and the rise may be attributed to the increased contribution of urine to the fluid. The concentration of creatinine in maternal serum remains unaltered which indicates that it plays no significant role in the formation of amniotic fluid.

The concentration of uric acid is found to be higher in amniotic fluid than that in the maternal and foetal blood in later part of pregnancy (Serr *et al*, 1963). In the present study it is found that there is a definite increase in the concentration of uric acid in amniotic fluid in the third trimester of pregnancy. The observation that there is no significat fall or rise of uric acid in maternal serum is well in agreement with those observed in normal pregnancies. A level of 7.58 ± 0.39 mg./100 ml. of uric acid in amniotic fluid may be taken as an evidence of full term mature pregnancy.

Queenan *et al* (1970) reported that, at term, the average concentration of total proteins in amniotic fluid was 250 mg/100 ml. The present study shows a gradual lowering in the concentration of total proteins in amniotic fluid as pregnancy progresses, which again helps in the prediction of foetal maturity. The very low concentration of total proteins in amniotic fluid compared to that in maternal serum shows that amniotic fluid may be an ultrafiltrate of maternal plasma.

The increase in the activities of alkaline phosphatases in amniotic fluid and maternal serum may be attributed to the contribution by the placenta (McMaster *et al*, 1964). Levine *et al* (1966) and Tiwana *et al* (1971) suggested the role of alkaline phosphatase in maternal serum as an aid in the assessment of placental function and foetal maturity. It is observed from the present investigation that the concentration of alkaline phosphatase in amniotic fluid cannot be correlated with the period of gestation because of the wide deviation from the mean values. But, the concentration of total and heat stable alkaline phosphatases in maternal serum show a very good correlation with gestational age. If precautions are taken to avoid conditions such as bone and liver diseases, alkaline phosphatase in maternal serum can serve as a reliable index in the assessment of placental function and foetal maturity.

Amniotic fluid can be considered as an ultrafiltrate of plasma early in pregnancy and the decrease observed in its osmolar concentration may be attributed to the

decrease in the concentration of sodium (Seeds and Barnes, 1972). The slight decrease observed in the concentration of sodium in amniotic fluid as pregnancy progresses, is not so significant as to consider it as an index in the assessment of gestational age. Sodium in maternal serum shows slight decrease with advancing pregnancy which may be attributed to the increased body water content. The concentrations of potassium neither in maternal serum nor in amniotic fluid show any variation with the period of gestation which again proves that amniotic fluid is a pool of foetal urine and the ultrafiltrate of maternal plasma.

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Summary

Creatinine, uric acid, total proteins, total and heat stable alkaline phosphatases, sodium and potassium were analysed in amniotic fluid and the concomitant maternal serum of 57 subjects at various stages of pregnancy. The concentration of creatinine and uric acid in amniotic fluid showed a progressive rise with increasing gestational age and they can serve as good indices in the assessment of foetal maturity. The concentration of total proteins in amniotic fluid showed a gradual significant decrease as pregnancy progressed and it can serve as an additional index in the assessment of the period of gestation. The activity of total and heat stable alkaline phosphatases in maternal serum showed a definite increase as the pregnancy approached term. A knowledge of the activity of the heat stable alkaline phosphatase in maternal serum can be of great help in the assessment of foetal age. The above observations also confirm the earlier views that foetal urine may be the major source of amniotic fluid in the last trimester of pregnency.

References

1. Chandiock, S., Gupta, A. N. and Devi, P. K.: J. Obstet. Gynec. India. 21: 551, 1971 (a, b).
2. Levine, B., Cecil, W. E. and Warren, W. E.: Am. J. Obstet. Gynec. 96: 1155, 1966.
3. Mc Master, Y., Tennant, R., Clubb, J. S., Neale, F. C. and Posen, S.: J. Obstet. Gynec. Brit. C'wlth. 71: 735, 1964.
4. Pitkin, R. M., Reynold, W. A. and Burchell, R. C.: Am. J. Obstet. Gynec. 100: 834, 1968.
5. Queenan, J. T. Gadow, E. C., Bachner, P. and Kubarych, S. F.: Am. J. Obstet. Gynec. 108: 406, 1970.
6. Tiwana, S., Devi, P. K. and Amma, M. K. P.: J. Obstet Gynec. India. 21: 144, 1971.
7. Seeds, A. E. and Barnes, A. C.: "The Water Metabolism of the Foetus", 1972 (C. O. Thomas, Springfield, Illinois).
8. Serr, M. D., Czaczkes, J. W. and Zuckerman, H.: Obstet. Gynec. 21: 551, 1963.