

BIOCHEMICAL STUDIES OF AMNIOTIC FLUID IN RELATION TO GESTATIONAL AGE

by

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Introduction

An accurate antepartum estimation of fetal maturity is of paramount importance in planning early termination of pregnancies in view of maternal and fetal diseases. It also helps in anticipation of problems in the newborns in relation to maturity leading to better preparedness on the part of paediatricians to deal with them. Assessment of gestational age based on maternal history and obstetrical examinations is often misleading. The specialised investigative procedures like ultrasonic techniques and radiological methods are hazardous, expensive and impractical for routine use. In the recent past biochemical studies in the amniotic fluid in relation to gestational age have been found useful, safe, accurate and can be easily executed. However, only a few studies have been undertaken in Indian subjects mostly using single biochemical parameter (Chandiok *et al*, 1971; Gupta *et al*, 1978; Jain *et al*, 1978; Singh *et al*, 1978). The present work was undertaken to evaluate the usefulness of some of the constituents

of amniotic fluid viz. creatinine, bilirubin and lecithin-sphingomyelin ratio taken together, in all the cases in the assessment of fetal maturity.

Material and Methods

Amniotic fluid samples obtained from 57 pregnant mothers between 13-41 weeks of gestation, admitted in Queen Mary's Hospital, K.G's Medical College, Lucknow formed the case material for the study. Only women with regular menstrual cycles and definite last dates of menstrual period and uterine size corresponding to the period of amenorrhoea were included in the study. The samples were collected by vaginal needle aspiration with sterile syringe from the forewaters prior to artificial rupture of membranes, during instillation of urea for medical termination of pregnancy, during caesarean section and by transabdominal amniocentesis. Of these, 7 samples were found to be contaminated with blood etc. and were discarded.

All the mothers were healthy and free from any disease or complication related to pregnancy viz. toxæmia, hydramnios, diabetes, renal disorder or hypertension. All the women were Rh +ve. The case material was divided into 4 groups based upon the length of gestation.

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Group	Gestation period between	No. of cases
A	13-27 weeks	14
B	28-32 weeks	7
C	33-36 weeks	11
D	37 weeks and above	18

Creatinine was estimated essentially according to Clark and Thompson's method using the Jaffer's reaction. Bilirubin in amniotic fluid was estimated by measuring its optical density at 450 nm using water as a blank and was measured on Coleman Junior Spectrophotometer. Estimation of lecithin sphingomyelin ratio was done according to Folch *et al* (1951) and Gluck *et al* (1976).

Results

Table I shows creatinine concentrations in different gestational age groups. The mean creatinine concentrations of less than 1 mg./100 ml. was seen below 28

weeks of gestation. Between 28-36 weeks of gestation the mean values were below 1.8 mg./100 ml. except in 1 preterm case who had a value of 2.06 mg% at 36 weeks of gestation. Values above 2 mg/100 ml were generally observed at or above 37 weeks of gestation. The rise between different groups was highly significant.

Bilirubin levels were found to fluctuate widely between 13-26 weeks of gestation with a definite rising trend. After 26 weeks of gestation more or less progressive fall in O.D. value at 450 nm was noticed upto 36 weeks of gestation which continued more gradually upto 40 weeks.

The difference of mean O.D. value was highly significant between group B and C, and C and D ($p < .001$). None of the cases at or above 37 weeks of gestation had O.D. value above 0.03 while 13 out of 18 cases below 37 weeks had the value of more than 0.05.

The lecithin and sphingomyelin ratio, fluctuated between 13-28 weeks, without

TABLE I
Creatinine Concentrations at Different Gestational Age Groups

Group	Gestation age (weeks)	No. of cases	Creatinine (mg./10 ml.)			
			Range	Mean	S.D.	p value
A	28	14	0.59-1.30	0.94 ± 0.178		.001
B	28-32	7	0.89-1.53	1.21 ± 0.215		.001
C	33-36	11	1.48-2.06	1.75 ± 0.178		
D	37 and above	18	1.85-3.00	2.33 ± 0.523		.001

TABLE II
O.D. Value of Bilirubin at Different Gestational period

Groups and No. of cases	Weeks of Gestation	O.D. Value at 450 nm			
		Range	Mean	S.D.	p value
A 14	28	0.065-0.30	0.123 ± 0.045		
B 7	28-32	0.061-0.193	0.126 ± 0.043		0.1
C 11	33-36	0.020-0.105	0.057 ± 0.031		0.001
D 18	37 & above	0.00 -0.030	0.012 ± 0.001		0.001

showing a definite rising or falling trend, thereafter a constant rise upto 34 weeks was registered. Between 34-38 weeks there was an abrupt rise in the values, then the values levelled off to form a plateau upto 40 weeks of gestation (Table III).

1967). In the present study it was found that creatinine value below 1.8 mg/100 ml always signified prematurity, while 2.0 mg/100 ml and above, almost always signified maturity. Only 1 case of 'C' group had a creatinine level of 2.06 mg/100 ml. The creatinine levels between 1.8-1.99 mg/

TABLE III
L/S ratio at Different Periods of Gestation

Group and No. of cases	Gestation age in weeks	L/S Ratio			
		Range	Mean	S.D.	
A 14	28	0.72-1.14	0.85	± 0.16	0.1
B 7	28-32	0.63-1.10	0.88	± 0.15	0.001
C 11	33-36	1.20-2.75	1.65	± 0.45	0.001
D 18	37-41	1.82-3.20	2.21	± 0.39	

Upto 32 weeks of gestation mean L/S ratio of less than 1.0 was seen. A significant rise in the ratio was observed after 32 weeks, which was more significant after 36 weeks of gestation when the mean value was 2.21. None of the cases with 37 weeks of pregnancy had L/S ratio below 1.8 while, 15 out of 18 between 28-36 weeks had the ratio below this level, only 3 preterm cases had L/S ratio of more than 1.8. At and above L/S ratio of 2, out of 12 out of 18 term and 1 out of 18 preterm cases were seen.

Discussion

The amniotic fluid is assumed to be the ultrafiltrate of plasma in initial stages of pregnancy, but it becomes richer in the constituents found in the urine with advancing gestation age. A linear rise of mean creatinine concentration level was noted with increasing gestational age, and abrupt rise was noted at 37 weeks of gestation. Other workers have also reported similar findings (Chandiok *et al*, 1971; Gupta *et al*, 1978; Pitkin and Zwirek,

1967). In the present study it was found that creatinine value below 1.8 mg/100 ml always signified prematurity, while 2.0 mg/100 ml and above, almost always signified maturity. Only 1 case of 'C' group had a creatinine level of 2.06 mg/100 ml. The creatinine levels between 1.8-1.99 mg/

100 ml appeared to be the zone of transition from prematurity to maturity. The amniotic fluid bilirubin level as observed by O.D. value at 450 nm correlated well with gestational age. The O.D. values of 0.01 or less at 450 nm. always signified a mature gestation of 37 weeks or more and no false results were encountered. However, at higher levels the correlation with fetal maturity was poor as four term babies had O.D. values between 0.040-0.011. Similar were the results of Quinilivan *et al* (1973) and Bhatnagar and Ramesh (1978). They also found a direct and definite correlation of amniotic fluid bilirubin levels with fetal maturity. On the other hand Bentrem *et al* (1970) found the test of dubious value in the assessment of fetal maturity.

In the present study L/S ratio below 1.6 always signified preterm gestation while ratio above 2.0 signified term gestation in most of the cases (94%). However, 33% of our mature cases had values between 1.8-1.99.

As shown in Table IV taking the L.S. ratio of 1.8 as the dividing line between

TABLE IV
L/S Ratio in Relation to Gestation

Gestational age in weeks	Total No. of cases	L/S Ratio		Accurate results percentage	False results percentage
		Below 1.8	Above 1.8		
28-36	18	16	2	89	11
37 & above	18	0	18	100	0
Total	36	16	20	34.4	5.6

preterm and maturity all the mature babies had a ratio above 1.8, only 11% of preterm babies showed false +ve results.

The study has revealed that these biochemical parameters in amniotic fluid have a good correlation with gestational age. Individually the tests are more reliable predictors of prematurity than maturity. The use of multiple parameters is desirable for accurate assessment of gestational age.

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