BIOCHEMICAL STUDIES OF AMNIOTIC FLUID: PARAMETER FOR GESTATIONAL AGE

by

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Introduction

The most valuable current need is to assess a fetus for its maturity and well being during its intrauterine life. Accurate estimation of gestational age and fetal maturity are essential before induction of labour or performance of cesarean section and for evaluating high-risk pregnancies.

There are certain biochemical parameters which vary with the length of gestation and also indicate the fetal maturity with certainty.

In the recent past a considerable data has been published as regards the composition of amniotic fluid. Most samples of amniotic fluid were analysed either from early normal pregnancy terminated between 8 and 24 weeks, or from patients at near term.

Some data regarding the concentration of some biochemical parameters of amniotic fluid of Indian women are available. Some investigators have studied only creatinine levels of amniotic fluid during second half of pregnancy, whereas amniotic fluid urea levels have been studied by some investigators between 37 to 41 weeks of gestation. Some other workers have studied both creatinine and urea concentration of amniotic fluid during second half of pregnancy. Mehta *et al* (1979) have studied creatinine, urea, orange cells, bilirubin between 12 to 41 weeks. Significance of glucose and lactic acid has been reported by Sunanda Bai *et al* (1969) between 36th and 43rd weeks of pregnancy and Raman *et al* (1978) have reported amniotic fluid glucose concentration at different gestational age.

As there was very little data available on the compostion of amniotic fluid throughout pregnancy in Indian population, the present work was undertaken to establish normal values of certain biochemical parameters throughout pregnancy. This would provide reliable information regarding the fetal maturity and prediction of length of gestation in cases of unreliable menstrual history.

A large number of amniotic fluid samples were analysed for creatinine, urea, glucose, sodium, potassium and chlorides throughout pragnancy beginning as early as 10 weeks of gestation.

Material and Methods

A total of 237 samples of amniotic fluid were obtained from women having reliable menstrual history and not suffering from any organic disease. Early pregnancy samples were obtained from cases undergoing medical termination of preg-

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nancy. In case of third trimester of pregnancy they were obtained from women undergoing amniocentesis performed by transabdominal route using 21 gauge spinal needle.

An aliquot of amniotic fluid was centrifuged at 3000 rpm for 10 min. to remove cells and sediments. Samples contaminated with blood or meconium were discarded. The clear supernatant was analysed for the following biochemical parameters.

Creatinine concentration was measured by the modified Folin Wu tungstic acid method, urea Nitrogen by diacetyl monoxime method. The levels of glucose were measured by o-toluidine method using acetic acid as well as on auto-analyzer which utilizes neocuproine reagent. Sodium and potassium were estimated by manual flame photometry using the EEL flame photometer and chlorides by the method of Schales and Schales.

Results

Creatinine and Urea N: One hundred and forty-three samples from 2nd trimester of pregnancy and 94 samples from 3rd trimester of pregnancy were analysed for these parameters. Both creatinine and urea N values showed a small but steady rise, specifically from 20 weeks onwards (Table I). There was a progressive increase in their concentration with the advancement of pregnancy. Abrupt increase was noted in the concentration of creatinine at 36 weeks of pregnancy which continued till the delivery, whereas the concentration of urea N decreased a little at 40 weeks of gestation. Both creatinine and urea N show positive correlation with gestational age which is highly significant (for creatinine $r = 0.9264$ $P < 0.001$), (for urea N $r = 0.6015$ $P < 0.001$). The positive correlation with gestational age can be clearly seen from Fig. 1 for creatinine and from Fig. 2 for urea N.

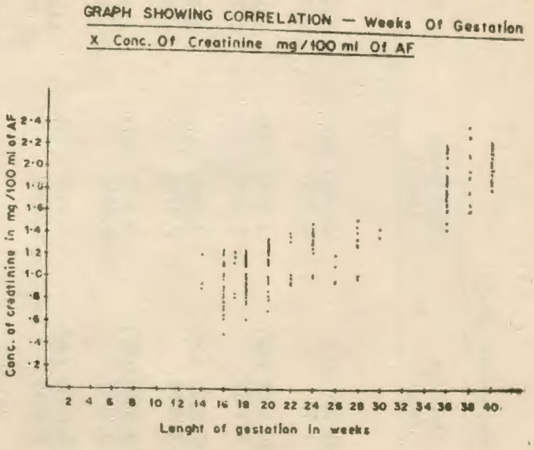

Fig. 1
Scattergram of Amniotic Fluid creatinine levels (mg/100 ml AF) as a function of gestational age.

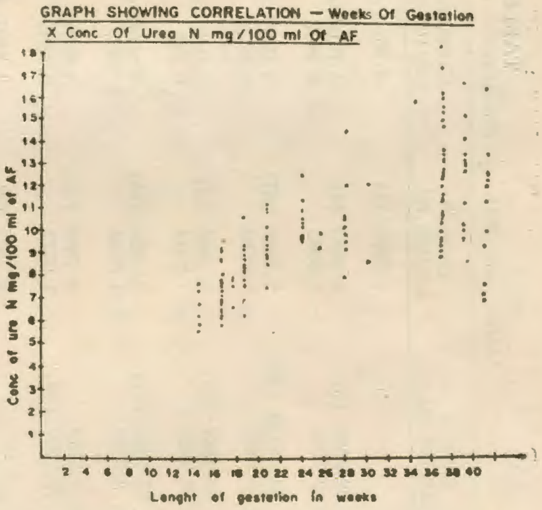

Fig. 2
Scattergram of amniotic Fluid urea N levels (mg/100 ml AF) as a function of gestational age.

Glucose, Sodium, Potassium and chlorides: One hundred samples of amniotic fluid from 2nd trimester of pregnancy and

TABLE I

Mean and S.D. of Biochemical Parameters in Amniotic Fluid

Weeks of gestation	Glucose mg/100 ml.	Urea N mg/100 ml.	Creatinine mg/100 ml.	Sodium meq/L	Potassium meq/L	Chloride meq/L
10	—	5.2400 (1)	0.5000 (1)	—	—	—
14	69.6000 (3) ± 2.7500	6.9467 (3) ± 0.9963	1.0167 (3) ± 0.1607	141.00 (3) ± 1.73	3.6000 (3) ± 0.3500	112.9000 (3) ± 5.6500
16	61.79430 (32) ± 5.9405	7.3543 (47) ± 1.6689	0.9397 (47) ± 0.1929	140.5556 (32) ± 3.1542	4.0741 (32) ± 0.3696	121.2493 (32) ± 7.4737
17	67.1667 (3) ± 2.7424	8.1960 (5) ± 1.3014	1.040 (5) ± 0.183	137.3333 (3) ± 2.3094	4.3000 (3) ± 0.1732	119.3167 (3) ± 5.0287
18	57.1459 (27) ± 3.5229	7.8820 (34) ± 1.0746	0.9936 (34) ±0.1533	139.8636 (27) ± 3.3707	4.1227 (27) ± 0.4047	119.7291 (27) ± 5.9625
20	± 6.0314 48.0237 (24)	8.7686 (32) ± 1.6573	1.0070 (32) ± 0.1895	139.0000 (24) ± 3.2318	4.0737 (24) ± 0.4293	115.3132 (24) ± 5.2543
22	—	9.7375 (9) ± 1.8742	1.1697 (9) ± 0.2175	—	—	—
24	49.9450 (11) ± 5.2625	9.9917 (12) ± 1.3619	1.2805 (12) ± 0.1896	137.0000 (11) ± 2.6726	4.1250 (11) ± 0.4862	118.2850 (11) ± 3.5807
26	45.9450 (3) ± 4.7871	9.4950 (3) ± 0.4879	1.0785 (3) ± 0.1718	134.5000 (3) ± 2.1213	4.0500 (3) ± 0.2121	114.4100 (3) ± 8.2660
28	47.8067 (11) ± 5.8466	10.4989 (11) ± 1.8814	1.3289 (11) ± 0.1827	132.6667 (11) ± 3.4641	4.0222 (11) ± 0.2489	109.1633 (11) ± 6.2213
30	28.3500 (3) ± 7.5660	10.3450 (3) ± 2.4678	1.4100 (3) ± 0.0566	131.5500 (3) ±12.0208	3.5500 (3) ± 0.4950	100.0500 (3) ± 6.5761
36	24.7707 (48) ± 6.3866	12.3277 (48) ± 2.5097	1.8689 (48) ± 0.2178	130.7045 (48) ± 4.1907	4.0295 (48) ± 0.3195	106.3164 (48) ± 6.1743
38	24.8586 (16) 24.2808 (13) ± 4.8748	12.2800 (16) ± 2.3471	1.8764 (16) ± 0.2415	132.7857 (16) ± 4.1171	4.0929 (16) ± 0.2895	105.4343 (16) ± 4.2898
40	± 5.2964	10.8923 (13) ± 2.7516	1.9615 (13) ± 0.1919	130.9231 (13) ± 3.4511	3.9308 (13) ± 0.2658	105.7077 (13) ± 6.0184

The figures in the brackets indicate the number of observations.

94 samples from 3rd trimester of pregnancy were analysed for these parameters. Glucose was estimated by O- toluidine method and autoanalyser technique which showed no difference in the values. The levels of glucose showed a progressive decline with advancing gestation—Table I. Considerable decline in the values was noted from 20 weeks onwards to 40 weeks of gestation indicating a highly significant negative correlation with gestational age-Fig 3 demonstrates the negative correlation of glucose with advancing gestational age. ($r = -0.92446$ $P < 0.001$).

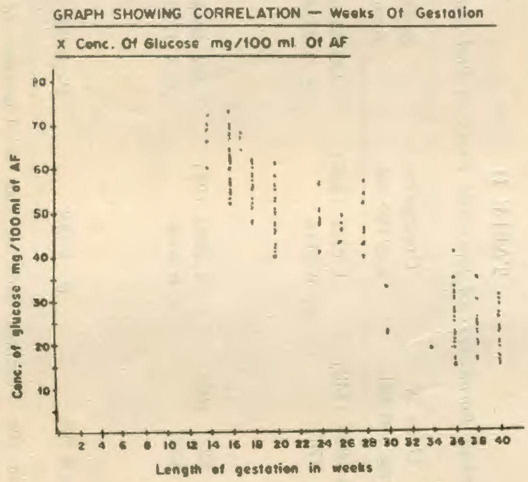

Fig. 3
Scattergram of Amniotic Fluid glucose levels (mg/100 ml AF) as a function of gestational age.

Sodium and chlorides showed a downward trend. As it is seen from Table I there was small but steady decrease in the concentration of sodium and chlorides.

There was no appreciable change in the levels of potassium throughout pregnancy (Mean 4.08 meq/I. and 4.01 meq/L for 2nd and 3rd trimester of pregnancy respectively).

Discussion

It is now a well established fact that amniotic fluid is not a static pool. Vosburgh and Associates (1948) through their isotopic studies have concluded that the amniotic fluid was completely replaced every 2.9 hours. It is assumed that an equal amount of water is transferred in the opposite direction i.e. from amniotic fluid to mother, leading to a continuous exchange of water between these two compartments. There is a steady state exchange of water and electrolytes between intrauterine compartments in pregnancy, involving fetal swallowing, urinary excretion and other significant exchanges of solute and water by means of as yet ill-defined pathways. The morphology of fetal skin in early and mid-pregnancy is compatible with a role in the exchange of water and electrolytes between amniotic fluid and fetus. (Abramovich 1968) Lind *et al* (1972) confirmed that sodium and therefore water can pass readily through fetal skin during the first half of pregnancy, although the rate of diffusion slows down as the skin thickness increases and probably ceases altogether when keratinization occurs. Due to this there is no continuity between amniotic fluid and fetal body fluids and it then becomes an exteriorized fluid.

In the second half of pregnancy by 20 weeks fetal kidneys start functioning and they play a role in the control of the composition and volume of amniotic fluid. As the gestation advances fetal urine which is hypotonic as compared with fetal or maternal plasma makes an important contribution to the amniotid fluid. Due to this, the amniotic fluid which is isotonic with maternal plasma becomes hypotonic with fetal or maternal plasma reflecting the gradual fall in the concentration of sodium and chlorides with advancing gestation. The levels of sodium fall from 139.56 mEq/L to 106.4237 mEq/L at term. This fall in mean amniotic fluid sodium is quite significant (P 0.001). With the advancement of pregnancy concentration of chloride decreases from 118.8006 mEq/L to 106.4237 mEq/L which shows high levels of significance ($P < 0.001$). Table II. This decrease in the concentration of sodium and chlorides with the advancing gestation was also observed by Gillibrand (1969), Sinha and Carlton (1970), Nusbaum and Zettner (1973).

The concentration of potassium neither increases nor decreases throughout pregnancy. Hence there is no significant change in the values of second and third trimesters of pregnancy for potassium. (P 0.05), as shown in Table II.

The results of the previous studies have shown that there is a gradual increase in creatinine and urea concentration up to 36 weeks of pregnancy followed by a marked rise in their concentration thereafter. The normal mean for amniotic fluid creatinine from 36 weeks to term is 1.88 mg/100 ml of amniotic fluid and the mean for urea N levels from 36 to 38 weeks is 12.3038 mg/100 ml of amniotic fluid with a little decrease at 40 weeks i.e. 10.8923 mg/100 ml amniotic fluid.

This increase in the concentration of urea N and creatinine can be explained on the basis that with advancing gestation fetal urine makes an increasingly important contribution to the amniotic fluid. As the fetal excretory products do not enter the maternal circulation they are voided in the surrounding amniotic fluid. Hence it is expected that as the fetus grows with the increasing maturity of its renal function, there is a gradual rise in the concentration of creatinine and urea. This indicates that the fetal micturition in

TABLE II

Comparative Study of Biochemical Parameters of Amniotic Fluid in 2nd and 3rd Trimester of Pregnancy

	Glucose mg/100 ml.	Urea N mg/100 ml.	Creatinine mg/100 ml.	Sodium meq/L	Potassium meq/L	Chloride meq/L
2nd trimester of pregnancy 10 to 24 weeks of gestation	56.8912 (100) ± 7.9357	8.0996 (143) ± 1.7767	1.0337 (143) ± 0.2116	139.5610 (100) ± 3.2588	4.0829 (100) ± 0.4030	118.8006 (100) ± 6.4971
3rd trimester of pregnancy 26 to 40 weeks of gestation	28.0051 (94) ±10.3268	11.8221 (94) ± 2.5263	1.7969 (94) ± 0.3008	131.4048 (94) ± 4.1772	4.0131 (94) ± 0.3053	106.4237 (94) ± 6.0428
	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P>0.05$	$P<0.001$

The figures in the brackets indicate the total number of observations.

utero acts as the possible source of the increasing concentration of these substances. It has been also noted that as pregnancy progresses the concentration of protein in amniotic fluid gradually decreases (unpublished data). Creatinine and urea being metabolic waste products of protein metabolism, the opposite trends exhibited by their concentrations can suggest the possible use of amniotic fluid proteins as a source of nutrition by the growing fetus. This naturally reflects the progressive increase in the concentration of creatinine and urea with advancing pregnancy.

Spellacy *et al* (1955), Wood and Sherline (1975) have studied the levels of glucose in amniotic fluid and have stated its decreasing concentration with progressing gestation. Glucose which appears in the amniotic fluid is probably derived from several sources, including active secretion by the fetal kidneys and possibly the bowel. Some may diffuse across the membranes or umbilical cord. Since maternal and fetal glucose levels are relatively unchanged from early to late pregnancy, this diffusion would be relatively constant. But still a downward trend of glucose concentration is noted. This can be explained on the basis that due to improper establishment of tubular function during first half of pregnancy glucose is excreted in large amounts. But as the tubular reabsorptive power increases with advancing gestation the excretion of glucose gradually decreases which is noted by 20 weeks onwards.

It is observed that during the second trimester of pregnancy fetal liver contains glycogen in low concentration, but near term there is a rapid and marked increase in glycogen content. This can also be the possible cause of gradual decrease in the concentration of glucose with the length of gestation. Hence glucose shows negative correlation with the length of gestation which is statistically highly significant ($r = -0.92446, P < 0.001$).

Considering the upward trends shown by creatinine and urea concentrations and downward trend of glucose concentrations, it can be said that these three parameters indicate the progressive maturity of fetal kidneys.

Table III presents the mean values of the biochemical parameters of amniotic fluid studied by various investigators. As may be seen, our results for sodium and potassium are in agreement with those of Gillibrand (1969), whereas values for chlorides agree with those stated by Sinha and Carlton (1970) for second trimester of pregnancy.

Our study reports low levels of creatinine and urea and high levels of glucose than those reported by Western investigators indicated in Table III. As the maternal diet plays an important role in the nutrition of the fetus, this difference in the values can be attributed to the dietary status of the Indian mothers. There is evidence (Pritchard and Macdonald, 1980) that glucose and the naturally occuring forms of amnio acids readily cross the placenta to the fetus. The diet which contains large proportion of carbohydrates than proteins can contribute to the high levels of glucose and low levels of creatinine and urea (reported as urea N) which are metabolic waste products of proteins, in the amniotic fluid.

From the presented data we conclude that analysis of amniotic fluid is a valuable tool which furnishes information regarding the functional development of the fetus. As the data shows the changes in the composition of amniotic fluid which are closely related to the length of gestation, reflecting the functional maturity of

TABLE III

Mean Values of Biochemical Parameters of Amniotic Fluid Reported by Various Authors

Name of the author	Duration of preg-nancy (weeks)	Glucose mg/ 100 ml	Urea mg/ 100 ml	Crea-tinine mg/ 100 ml	Sodium meq/L	Potas-sium meq/L	Chlori-des meq/L
Battaglia et al (1959)	9-19	—	—	—	137.40 (4)	4.0 (4)	—
	37-term	—	—	—	125.30 (7)	4.0 (7)	—
Gillibrand (1969)	10-22	—	20.18 (27)	—	138.00 (27)	3.8 (27)	107.8 (27)
	29-44	—	31.00 (32)	—	130.53 (32)	3.8 (32)	102.5 (32)
Sinha and Carlton (1970)	8-24	49.0 (28)	190 (27)	1.4 (26)	135.00 (28)	4.3 (28)	118.0 (28)
Pitkins S. J. Zwirek (1967)	37 or more	—	—	2.0 or more	—	—	—
Doran et al (1970)	13-29½	—	—	0.97 (31)	136.74 (27)	4.0 (27)	112.7 (27)
	35-term	—	—	1.88 (47)	132.89 (39)	4.5 (39)	111.5 (39)
Roopnarine Singh (1973)	37	—	—	1.72 (94)	—	—	—
	41	—	—	2.74 (6)	—	—	—
C. Wood et al (1963)	36-43	13.0 (80)	—	—	—	—	—
Bevis (1953)	36-40	26.4	—	—	—	—	—
Wood and Sherline (1975)	32-36	<30 (26)	—	—	—	—	—
Present series	10-24	56.89 (100)	17.33 (143)	1.03 (143)	139.56 (100)	4.1 (100)	118.8 (100)
	26-40	28.00 (94)	25.29 (94)	1.80 (94)	131.40 (94)	4.0 (94)	106.4 (94)

Figures in the brackets indicate No. of observations.

the fetus, it can provide a valuable technique to the obstetrician in estimating the gastational age with certainty atleast in 90% of cases.

Summary

A tota of 237 samples of amniotic fluid were analysed to establish the normal composition of amniotic fluid throughout pregnancy. Concentration of sodium and chloride decreases as the pregnancy progresses, whereas there is no specific trend shown by the concentration of potassium. The increasing concentration of creatinine and urea N and decreasing concentration of glucose with the length of gestation indicate the maturity of fetal renal function. The close relation of all these parameters with the length of gestation when studied simultaneusly would provide a reliable information about the maturity of the fetus and would help the obstetrician to estimate the gestational age in cases of unreliable menstrual history.

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