ROLE OF BIOCHEMICAL AND CYTOLOGICAL ANALYSIS OF AMNIOTIC FLUID IN THE ASSESSMENT OF FETAL MATURITY‡

by

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Accurate antepartum estimation of fetal maturity is of paramount importance. The validity of a calculated date of conception based on gestational history may at times be doubtful because of irregular menstruation, uncertainity of date of last menstrual period or lack of menstrual data after cessation of oral contraceptives. Amniotic fluid has been analysed for creatinine, bilirubin, urea, lecithin sphingomyelin ratio and fetal fat cells by a number of workers in an attempt to assess fetal maturity (Brosens and Gordon, 1965, 1966; Gordon and Brosens, 1967; Mendelbaum *et al*, 1967; Anderson and Griffiths, 1968; Sharp, 1968; Bergneaud *et al*, 1969; Lind *et al*, 1969; White *et al*, 1969; Myers *et al*, 1975). There is disagreement among the different workers regarding the results and various workers have preferred different criteria for evaluating the post-conceptional age.

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The present work was undertaken to evaluate the usefulness of some of the constituents of amniotic fluid in assessing the maturity in our cases.

Material and Methods

Amniotic fluid samples obtained from 72 pregnant mothers between 12 to 41 weeks of gestation, admitted to the Obstetric Unit of Medical College, Rohtak formed the case material for the study. Samples were collected either at the time of caesarean section or at the time of delivery per vaginum; only the samples free from blood were taken for analysis. The period of gestation was confirmed by direct enquiry from the mother and calculated according to Naegele's rule and corroborated by the physical examination of the neonate which corresponded to the gestational age. Cases with doubtful gestational age and those whose obstetrical examination and neonatal examination were not supportive were excluded. All the mothers were healthy and free from any serious disease. All the infants also were healthy at the time of birth and free from any disease acquired in utero or any major congenital malformation. The case material was divided into 4 groups based upon length of gestation.

Group A: Gestation period 12-20 weeks: 8 cases

Group B: Gestation period 21-28 weeks: 5 cases

Group C: Gestation period 29-36 weeks: 11 cases

Group D: Gestation period 37-41 weeks: 48 cases

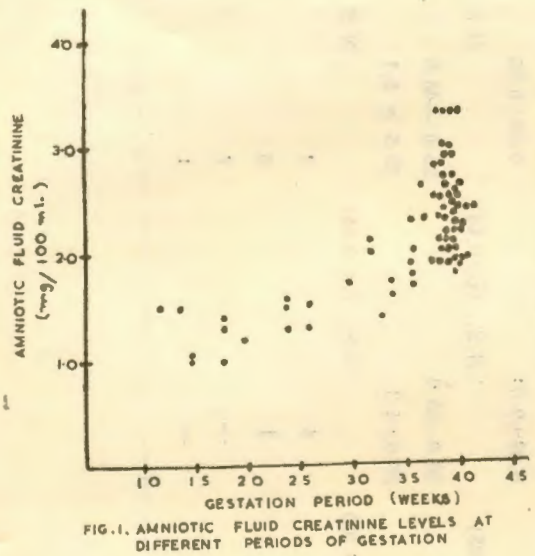

FIG. 1. AMNIOTIC FLUID CREATININE LEVELS AT DIFFERENT PERIODS OF GESTATION

Fig. 1

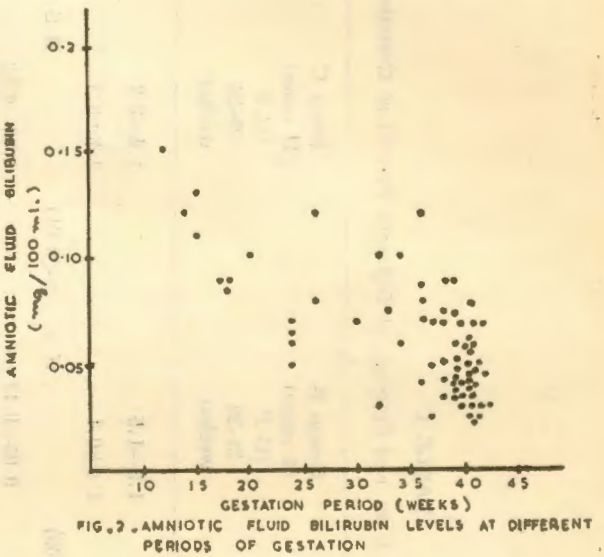

FIG. 2. AMNIOTIC FLUID BILIRUBIN LEVELS AT DIFFERENT PERIODS OF GESTATION

Fig. 2

A drop of amniotic fluid was mixed with a drop of 0.1% aqueous Nile blue sulphate solution on a clean glass slide. The slide was heated gently for 1-2 minutes and examined microscopically. Ratio of orange and blue cells was calculated by counting 100 cells.

The amniotic fluid was then centrifuged and clear fluid was used for the estimation of creatinine by alkaline—picrate reaction (Brod and Sirota, 1948). Amniotic fluid urea was estimated by Nessler's method after treatment with urease (Varley, 1976) and bilirubin was estimated by a modified diazo method (Melloy and Evelyn, 1937). In this modification instead of blood, 2 ml amniotic fluid was used as such without dilution and contents were centrifuged to remove the turbidity, if any, before noting the optical density.

Results

The results of the amniotic fluid analysis are summarised in Table I and in the figures 1, 2, and 3.

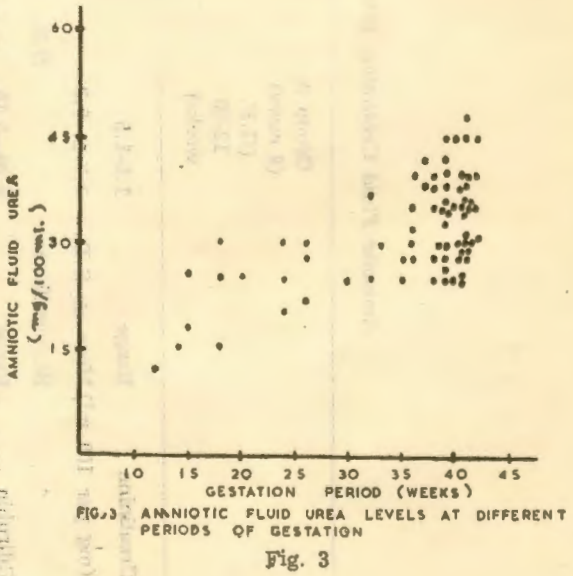

FIG. 3. AMNIOTIC FLUID UREA LEVELS AT DIFFERENT PERIODS OF GESTATION

Fig. 3

TABLE I

Amniotic Fluid Creatinine, Bilirubin, Urea and Cytology at Different Periods of Gestation

		Group A (8 cases) (G.P. 12-20 weeks)		Group B (5 cases) (G.P. 21-28 weeks)		Group C (11 cases) (G.P. 29-36 weeks)		Group D (48 cases) (37-41 weeks)
Creatinine	Range	1.0-1.5		1.3—1.5		1.4—2.3		1.8—3.3
(mg per 100 ml)	Mean ± S.D.	1.3 ± 0.2		1.4±0.1		1.8±0.3		2.4±0.4
	St. significance		N.S. (p>0.05)		H.S. (p<0.01)		H.S. (p<0.001)	
Bilirubin	Range	0.08—0.15		0.05—0.12		0.03—0.12		0.03—0.09
(mg/100 ml)	Mean ± S.D.	0.11±0.02		0.08±0.03		0.08±0.03		0.03±0.02
	St. significance		N.S. (p>0.05)		N.S. (p>0.05)		H.S. (p<0.001)	
Urea	Range	12.0-28.0		20.0—30.0		25.0 —40.0		20.0 —48.0
	Mean ± S.D.	18.5±5.5		26.6±4.3		30.5 ± 5.1		30.7 ± 6.3
	St. significance		Signif. (p<0.02)		N.S. (p>0.05)		N.S. (p>0.05)	
Orange	Nil	8		4		2		3
Cells	<10%	—		1		6		7
(No. of cases)	10-20%	—		—		2		10
	>20%	—		—		1		29

The creatinine levels (mean ± S.D.) in groups A, B, C and D were 1.3 ± 0.2, 1.0 ± 0.1, 1.8 ± 0.3 and 2.4 ± 0.4 mg per 100 ml respectively, the rise being highly significant for groups C and D. The amniotic fluid creatinine levels were found to increase with the advancement of gestation but the values revealed a wide scatter at different lengths of gestation (Fig. 1). In group D (mature) 85 per cent had amniotic fluid creatinine levels of 2 or more than 2 mg per 100 ml, whereas only 17 per cent of all cases in other groups (premature) had levels of 2 or more than 2 mg per 100 ml. There was a significant positive correlation ($r = 0.69$, $p < 0.001$) between the amniotic fluid creatinine levels and length of gestation.

The amniotic fluid bilirubin levels (mean ± S.D.) in groups A, B, C and D were 0.11 ± 0.02, 0.08 ± 0.03, 0.08 ± 0.03 and 0.05 ± 0.02 mg per 100 ml respectively. The levels differed significantly when values in groups C and D were compared ($p < 0.001$). Although the amniotic fluid bilirubin levels decreased with increasing length of gestation, there was a wide scatter at all lengths of gestation (Fig. 2). There was a significant negative correlation ($r = -0.67$, $p < 0.001$) between the bilirubin levels and gestation period. In 71 per cent of the mature cases amniotic fluid bilirubin level was less than 0.05 mg per 100 ml, whereas levels above 0.05 mg per 100 ml were encountered in 88 per cent of the preterm cases.

Like creatinine, the amniotic fluid urea levels also increased with pregnancy and showed a wide scatter (Fig. 3). There was a significant positive correlation ($r = 0.63$, $p < 0.001$) between amniotic fluid urea concentration and length of gestation. There was no significant difference in the values when groups C and D were compared ($p > 0.05$). Values above 30 mg per 100 ml were found in 69 per cent of the term cases but as many as 31 per cent of the preterm cases had also values equal or higher than 30 mg per 100 ml.

There was a significant negative correlation ($r = 0.58$, $p < 0.001$) between amniotic fluid creatinine and bilirubin levels and a significant positive correlation ($r = 0.37$, $p < 0.01$) between creatinine and urea levels of the amniotic fluid.

No orange cells could be detected in the amniotic fluid of women with gestation period below 30 weeks, except in 2 cases where 1-2 per cent occasional cells were seen. Only 1 case from group C had more than 20 per cent orange staining cells in its amniotic fluid, whereas 60 per cent of the cases in group D had more than 20 per cent orange staining cells in their amniotic fluid.

Table II shows the comparative usefulness of different parameters in the evaluation of fetal maturity. Amniotic fluid creatinine levels seem to be the most reliable followed by fetal cell count and bilirubin levels.

Discussion

The liquor amnii in the initial stages is assumed to be an ultrafiltrate of plasma and with the advancing pregnancy it becomes richer in the constituents found in the urine. The decrease in amniotic fluid bilirubin concentration and increase in amniotic fluid creatinine and urea concentrations as observed in this and other studies (Lind *et al*, 1969; White *et al*, 1969; Bergneaud *et al*, 1969; and Myers *et al*, 1975) can be explained on this basis. But there is no unanimity of opinion among different workers as to the critical values of these ingredients to serve as an index of maturity. A concentration of 2

TABLE II

Comparison of Various Amniotic Fluid Constituents in the Assessment of Gestational Age

Observation	Mature value	Percentage accuracy in assessing gestational age				
		Group A	Group B	Group C	Total pre-term (group A+B+C)	Term (group D)
Creatinine	2.0 mg%	100	100	64	83	85
Bilirubin	0.05 mg%	100	80	82	87	71
Urea	30.0 mg%	100	60	46	67	69
Orange staining cells	10%	100	100	73	87	81
	20%	100	100	93	93	60

mg per 100 ml creatinine in the amniotic fluid has been taken as a critical value by some workers (Pitkin and Zwirek, 1957; White *et al* 1969; Myers *et al* 1975) and using this level we could assess maturity in 85 per cent of the term cases and results are similar to those reported by these workers. The variations encountered in the amniotic fluid creatinine is probably because of the multifunctional aspect of this compound. Its concentration depends upon muscle mass, kidney excretion and maternal serum levels.

The urea concentration was more variable than creatinine and only 69 per cent of the term cases could be diagnosed by keeping 30 mg per 100 ml as the critical level for maturity. The variability can be explained on almost the same grounds as for creatinine; in addition urea is more sensitive to dietary habits.

Amniotic fluid bilirubin levels below 0.05 mg per 100 ml could accurately assess maturity in 71 per cent of the cases. Similar results were observed by White et al (1969). However, Myers et al (1975) and Mandelbaum et al (1967) found this parameter to be comparatively more useful in diagnosing maturity. The variability in amniotic fluid bilirubin levels may be a reflection of the variable serum bilirubin levels.

The presence in the amniotic fluid of orange staining cells, thought to be shed from the fetal sebaceous glands has been extensively used for evaluating gestational age (Kittrich and Gebertsh, 1963; Brosens and Gordon, 1965; 1966; Anderson and Griffith, 1968; Sharp, 1968; White et al, 1969; Myers et al, 1975). In the present study the orange staining cells could be rarely detected in the amniotic fluid before 30 weeks of gestation, a finding similar to that reported by the above workers. Of the term cases 80 per cent had more than 10 per cent and 60 per cent of them had more than 20 per cent of these cells in their amniotic fluid. But some of the term cases also either did not have or had less than 10 per cent orange staining cells. Sometimes, it is not possible to accurately count the number of these cells when they are present in clusters, a difficulty also reported by White *et al* (1969). The false negative results obtained with the fetal fat cell content has been reported by Henemann *et al* (1970) but no suitable explanation has been given. There may be a probability that a population of fetal fat cells have lost their fat

content and appear as empty cells that do not stain orange with nile blue sulphate.

Summary

Amniotic fluid samples obtained from 72 pregnant mothers at different stages of gestation were analysed for creatinine, urea, bilirubin and fetal fat cells. While creatinine and urea levels increased with the advancement of pregnancy, the bilirubin levels decreased. However, there was a wide scatter for all these values.

The amniotic fluid creatinine levels above 2 mg/100 ml alongwith amniotic fluid cytology could be used for the detection of maturity in about 90 per cent of the cases and the bilirubin levels could be used as a supporting test for this purpose.

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