EVALUATION OF FOETAL MATURITY BY AMNIOTIC FLUID

by

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Accurate assessment of foetal maturity would be a valuable aid to the obstetrician while contemplating interruption of pregnancy in the interest of the foetus or mother. The aim of the study is to estimate foetal maturity to prevent unintentional prematurity and to decide an early delivery to effect a more satisfactory outcome for the foetus or the mother. It was therefore tried by two methods of determining foetal age, involving the study of amniotic fluid, which have given very accurate results. One is the biochemical method of estimating the creatinine content of amniotic fluid suggested by Pitkin and Zwrick (1967), Pramley and Miller (1969). The other is the cytological study of amniotic fluid by Nile blue Sulphate Staining suggested by Brossen and Gorden (1965, 66, 67), Sharpe (1968), Bishop and Corson (1968), Sharma and Trussel (1979) and others.

Material and Methods

Eighty pregnant women attending outdoor or admitted in the maternity ward of S.V.B.P. Hospital, Meerut were included in the study. Most of the patients were about 36 to 40 weeks pregnant. No special selection as to age and parity etc. was exercised. Selection of the cases was done on the following criteria:

(i) There should be no menstrual disorder.

(ii) They should know date of their last menstrual period.

(iii) Patients with past history of toxaemias, hypertension, diabetes and Rh sensitization were not included.

Liquor Creatinine Method

Ten c.c. of amniotic fluid samples were collected by abdominal Paracentesis (De *et al,* 1969) from each patient. The physical nature and characteristic of the amniotic fluid collected were studied before and after centrifugation. The samples contaminated with blood were rejected. The utmost care and precautions were taken to avoid repeated puncture in the case of failure in withdrawing the amniotic fluid.

The creatinine content was determined following Jaffi Picric Acid method and the results are expressed in mgm per cent.

Cytological Method

Amniotic fluid was collected by abdominal aminocentesis with usual aseptic precautions from 40 pregnant mothers of 34 weeks and onwards gestation. One or

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two drops of uncentrifuged amniotic fluid was put on a glass slide, and was mixed with one drop of 0.1% aqueous solution of Nile blue sulphate. This side was gently rocked until the fluid and the dye were thoroughly mixed. The slide was then gently heated for one minute and a cover slip was applied. It was subsequently examined microscopically (low power x 10) to detect mainly one type of stained cells. (i) Round or oval blue staining cells with a well defined outline and a darkly staining nucleus. These were discrete and had no tendency to clump. (ii) Polygonal, anucleated cells which were orange staining and had a tendency to clump together.

A total of 500 cells were counted noting the percentage of orange cells.

One clinical test was also performed by measuring the length of the infant from crown to heel which is divided by 5 and gives the age of infant in lunar months. This is Haas' Rule (Donald. 1972). The weight of infant was also recorded.

Observation and Results

Table I reveals that creatinine level in amiotic fluid increases significantly after 32 weeks of gestation. The creatinine value in the fluid during 32 to 34 weeks is from 1.46 to 1.72 mgm% with average of 1.586 mgm%, whereas the average values during 34 to 36 weeks, 36 to 38 weeks, 38 to 40 weeks and 41st week onward were 1.730 mg, 2.122 mg, 2.426 mg and 3.570 mg per cent respectively. This rise in creatinine content of amniotic fluid points to the progressive increases in maturity of the foetus.

Table II shows the correlation between amniotic fluid creatinine level in mgm per cent and birth weight. In 32 cases amniotic fluid creatinine was estimated to be between 1.3 to 2.0 mg per cent. But 2.1 to 2.5 mg per cent, 2.5 to 3.0 mg per cent and 3 to 4 mg per cent of creatinine level in amniotic fluid was estimated in another 30, 9 and 9 cases respectively. Since at the birth weight 3.1 to 3.5 Kg. a total 39 cases were studied, of these 26 cases were found to have creatinine concentration of more than 2 mgm per cent. This indicates that birth weight does have some bearing on amniotic fluid creatine concentration but not to be statistically significant level.

Table III shows the correlation between the amniotic fluid creatinine level and Haas' Rule. At 9.1 to 10 lunar months a total of 52 cases were studied and in 23 cases the creatinine level was present in

TABLE I

Creatinine Content of Amniotic Fluid in Different Duration of Pregnancy

Gestation period in week	Number of cases out of 80	Creatinine value (mgm %)	Average creatinine value (mgm%)
26	1	1.32	1.32
28 to 30	1	1.60	1.60
30 to 32	1	2.10	2.10
32 to 34	3	1.46 to 1.72	1.586
34 to 36	7	1.64 to 1.94	1.730
36 to 38	13	1.65 to 2.94	2.122
38 to 40	52	1.66 to 4.00	2.426
41st week onward	2	3.46 to 3.68	3.570

TABLE II

Correlation Between Amniotic Fluid Creatinine (mgm per cent) and Birth Weight of Infant

Birth weight of Infant in Kg.	Creatinine Values in mgm per cent			
	1.3 to 2.0	2.1 to 2.5	2.5 to 3.0	3.00 to 4.00
upto 2.5	3	4	—	—
2.51 to 3.0	14	9	3	4
3.01 to 3.50	13	16	5	5
3.51 to 4.00	2	1	1	—
Total:	32	30	9	9

$r = 0.091$, $t = 0.818$, $p > 0.05$

TABLE III

Correlation Between Amniotic Fluid Creatinine Level (mgm per cent) and Haas' Rule

Age in lunar months	Creatinine value in mgm per cent			
	1.3 to 2.0	2.1 to 2.5	2.5 to 3.0	3.0 to 4.0
6 to 8	2	—	—	—
8.1 to 9	4	3	—	—
9.1 to 10	18	23	6	5
10.1 to 11	9	4	4	2

$r = 0.552$, $t = 5.85$, $p > 0.05$.

the concentration of 2.1 to 2.5 mgm per cent. In 33 and 30 cases a values of 1.3 to 2.0 mgm per cent and 2.1 to 2.5 mgm per cent were obtained respectively. In 18 and 23 cases creatinine level was estimated as 1.3 to 2.0 mgm per cent and 2.1 to 2.5 mgm per cent at 9.1 to 10 lunar months. In all the value of correlation 'r' calculated is 0.552 with 't' 5.85, which is statistically significant. This analysis showed a direct correlation between amniotic fluid creatinine and Haas' Rule.

Table IV shows the percentage distribution of orange stained cells at different weeks of gestation. It was observed that 2 to 4 per cent of orange stained cells were present at 34 weeks of gestation. There was further rise of orange cell count 38th week upto 12 to 26 per cent. From 34th week of gestation, there was an abrupt rise of orange cell count upto 52 per cent at term. Postdated pregnancy from 41st week onward could be differentiated with still higher count having a steep rise in cell count varying between 55 to 85 per cent.

TABLE IV

Distribution of Orange Stained Cells in Amniotic Fluid at Different Weeks of Gestation

Foetal age in gestational week	No. of cases	Percentage of orange stained cells
34	2	2 to 4
36	7	6 to 10
38	16	12 to 26
40	10	27 to 52
41 onward	5	Above 55

Table V shows a comparative study of

TABLE V

Comparison Between Amniotic Fluid Creatinine Levels and Percentage of Orange Cells

Foetal age in gestational week	Mean amniotic fluid creatinine in mgm per cent	Percentage of orange stained cells
34	1.58	2 to 4
36	1.73	6 to 10
38	2.122	12 to 26
40	2.44	27 to 52
41 onwards	3.57	Above 55

mean amniotic fluid creatinine and percentage of orange stained cells at various gestational periods. At 34 weeks of gestation, the mean creatinine level and orange cells in amniotic fluid were present 1.58 mgm per cent and 2 to 4 per cent respectively. 1.73 mgm per cent of creatinine level and 6 to 10 per cent of orange cells in amniotic fluid were noted at 36 weeks of gestation period. An orange cell count of 12 to 26 per cent and creatinine level of 2.12 mgm per cent in amniotic fluid indicated the foetal maturity of 38 weeks. At 40 weeks of gestation period, creatinine level and orange cells were observed 2.44 mgm pr cent and 27 to 52 per cent respectively. It is evident, that both the amount of creatinine (mgm per cent) and percentage of orange cells increases with respect to the gestation period and the creatinine level of 2.122 mgm per cent and orange cell count at least 12 per cent indicated maturity of 38 weeks of gestation period in the present study.

Discussion

In the present study, mean creatinine level in amniotic fluid was 2.122 mgm per cent, which indicates a mature foetus and gestation period of 37 weeks. These findings coincide with other workers (Pitkin and Zwirek, 1967; Chandoik *et al*, 1971; Issac and Lillie, 1972; Sinha, 1975 and Shah and Patil, 1975). Roopnarine Singh, 1970; and Chowdhury *et al*, 1945 reported the creatinine level lower than our study at the same gestation period. A level of over 3 mgm per cent is supposed to indicate postmaturity (Shah and Patil, 1975) which coincides to our study.

Present study reveals a poor correlation between amniotic fluid creatinine level and birth weight. Pitkin and Zwirek, 1967; Chandoik, *et al*, 1970; Issac and Lillie, 1972, have well correlated amniotic fluid creatinine level of 2 mgm per cent and a birth weight of 2500 gm or more. Their findings are contrary to ours. Doran, Bjerre, and Porter, 1970 reported that co-efficient of correlation between liquor creatinine and birth weight was 0.66, while in the present study, it is 0.091. Our study well coincides with Mandlebaum and Evans, 1969; Makowski, 1969 and Harrison, 1973; where no correlation was established between creatinine level and birth weight.

In the present study, amniotic fluid creatinine was correlated with foetal age in lunar months as assessed by Haas' Rule. Value of correlation 'r' calculated in 0.552 with 't' 5.85 which is statistically significant. Hence a direct correlation between amniotic fluid creatinine level and Haas' Rule has been established.

As observed in this study, 2 to 4 per cent of orange cells were present at 34 weeks of gestation period. The percentage of orange cells increased with the gestation period. The steep rise rise of orange cells count from 2 to 4 per cent at 34th week to 10 per cent at 36th week and still further rise ranging between 12 to 52 per cent at 38 to 40 weeks of gestation is highly significant. From 41st week onward the

orange cell count was constantly varying between 55 to 85 per cent. This result compares favourably with Sharma and Trusell (1970) and Chowdhry *et al* (1975). The appearance of orange cells is due to active secretion of sebaccous glands (Potter, 1961). Subsequent rise in the orange cell count may be explained by the progressive functional maturity of the sebaceous glands with advancement of pregnancy.

Amniotic fluid creatinine values at various periods of gestation were compared with percentage of orange cells. At 2.122 mgm per cent of amniotic fluid creatinine level orange count ranged from 12 to 26 per cent. Other workers have carried out the studies including both parameters, but they did not correlate the two; so a comparision could not be made out in the present study.

Summary and Conclusions

Pregnant women of different ages, parity were selected for study of creatinine level and orange cell count in amniotic fluid, creatine level correlation with birth weight and Haas' Rule with aim to estimate the maturity of the foetus.

All the values studied gradually and constantly rise according to the duration of pregnancy. It is concluded from the present study the creatinine level and orange cells count are very useful for evaluation of foetal maturity. It is also concluded that Haas' Rule is highly significant for the determination of foetal maturity, which has some correlation to the creatinine level and orange stained cells of amniotic fluid. This knowledge helps in assessing the degree of maturity in doubtful cases. All these tests combinely further enhance the accuracy.

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