A COMPARATIVE STUDY OF AMNIOTIC FLUID AMYLASE, NILE BLUE SULFATE TEST AND BUBBLE STABILITY TEST AS PARAMETERS OF FETAL MATURITY

by

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Assessment of maturity of fetus before its birth is the most interesting and widely discussed topic today. To minimize the morbidity and mortality due to prematurity and respiratory distress syndrome, the confirmation of fetal maturity becomes important before the pregnancy is terminated. The assessment of fetal maturity by last menstrual period, date of quickening or by abdominal palpation can be grossly misleading, more so in pregnancies associated with complications, leaving the obstetrician in a dilemma regarding the termination of pregnancy. Since the amniotic fluid has been known to be of fetal origin, its constituents vary with the period of gestation and therefore reflect the physical and functional maturity of the fetus. Almost all the constituents have been studied including creatinine, bilirubin, urea, cytology, hormones, pulmonary surfactant, fatty acids and enzymes, but none has proved to give absolute reliable results. Besides, many of the expensive and elaborate tests cannot be carried out in smaller hospitals because of the lack of facilities. Thus the need for development of simpler and reliable techniques continues. Keeping this in mind, this study was undertaken to evaluate and compare the recent biochemical test—amniotic fluid amylase with two simple parameters, the bubble stability test and the nile blue sulfate test.

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Material and Methods

The present study was conducted on 75 selected patients with known last menstrual period and regular cycles, admitted in the department of Obstetrics and Gynaecology of Lady Hardinge Medical College and S. K. Hospital, New Delhi. The patients were selected from different age groups, irrespective of their parity and were divided into three broad groups with gestational period of 36 weeks and below ½ (including abortions), 37 to 40 weeks and 41 weeks and above. Each group consisted of 17, 42 and 16 patients respectively. After a detailed history and examination, the amniotic fluid was obtained by either abdominal amniocentesis or aspiration from the sac per vaginum. The amniotic fluid obtained in each case was studied for the estimation of amylase content, percentage of orange cells by staining with nile blue sulfate and bubble stability test. Estimation of amylase content was done by Somogyi's iodine test method. For orange cells one drop of uncentrifuged well mixed amniotic fluid

was put on a clean glass slide and one drop of 0.1% commercial nile blue sulfate solution was added. The cover slip was applied and the slide was examined under the microsdope. The slide was not heated over the flame as described by Brosens and Gordon (1966). A total of 200 cells were counted and the percentage of orange cells was calculated. The orange cells have a tendency to clump together and therefore an estimation of the number of cells in each cluster was made according to the size of the cluster; 5, 10 or 20 cells. (Brosens and Gordon 1966).

The bubble stability test was performed in the same way as described by Clements *et al* (1972), taking volumes of amniotic fluid of 1.0 ml, 0.75 ml, 0.50 ml, 0.25 ml and 0.20 ml. in 5 test tubes numbered 1, 2, 3, 4 and 5 respectively. Volumes of 0.25 ml 0.50 ml, 0.75 ml and 0.80 ml of 0.9% saline were pipetted into tubes numbered 2, 3, 4 and 5 respectively. One ml of 95% ethanol was added to each tube and tubes were shaken vigrously for 15 seconds and then kept in the rack undisturbed for 15 minutes. The presence of a ring of bubbles in the first three or more tubes at the end of 15 minutes was taken as indicating 'positive' result. The absence of a ring of bubbles in the first tube indicated a 'negative' result while its presence in first or in the first and second tubes indicated an 'intermediate' result.

The results of the tests were correlated with the period of gestation, birth weight and clinical maturity of the new born for comparative evaluation.

Results

The amylase content of amniotic fluid was observed to gradually rise with the period of gestation and the mean amylase level at 36, 37 and 40 weeks of gestation was 100, 163.84, and 271.00 Somogyi Units per 100 ml respectively. A significant correlation coefficient of 0.46 was found between the period of gestation and the amniotic fluid amylase level. The mean amylase level in three broad gestational groups was observed to be 53.18, 187.68, and 186.01 Units per 100 ml respectively.

In relation to the birth weight, cases with birth weight of less than 2000 gms. had mean amylase level of 130 Somogyi Units/100 ml and the mean amylase content in cases with birth weight between 2001-2500 gms and 2501-3000 gms was 187.14 and 178.40 Somogyi Units/100 ml respectively, showing a similar correlation as that obtained with the gestational age.

Correlating the orange cell count with the period of gestation, it was observed that in group I, all the cases showed orange cell count of less than 10%, while in group II, 88.1% cases had more than 11% orange cells (54.7% showed more than 30% fat cells) and only 11.9% cases had orange cells less than 10%. In group III, most of the cases (93.75%) had orange cell count of more than 30%. This suggests a good correlation between the two (correlation coefficient—0.60). The mean orange cell percentage and mean birth weight in groups I, II and III was 1.62%, 34.59%, 52.59% and 1283 gms, 2711 gms and 3024 gms respectively.

The bubble stability test showed a highly significant correlation with the period of gestation (correlation coefficient 0.70). The data showed a negative bubble stability tests in 70.58% cases with period of gestation of 36 weeks and below and positive test in 5.88%, while 23.53% cases showed intermediate results. In group II with gestation of 37 to 40 weeks the test was negative in 7.14%, intermediate in 26.19% and positive in 66.66% cases. In group III no negative test was observed, and a positive result was observed in

93.75% cases. With birth weight of less than 2000 gms, no sample showed positive results, while with birth weight between 2001 to 2500 gms, 64.28% showed positive result, 28.57% showed intermediate result and 7.4% showed negative result. In the range of 3001 gm to 3500 gms of birth weight 78.5% positive results, 14.2% intermediate results and 7.14% negative results were obtained.

Discussion

The appearance of amylase in the amniotic fluid as early as 16th week of gestation has been shown by Wolf and Taussig (1973) and its suggested sources are fetal saliva and urine. The mean amylase concentration at 36 weeks of gestation was 100 Somogyi Units/100 ml, after which a sudden rise was observed indicating the functional maturity of the fetal glands. Though good correlation was obtained between amylase content and period of gestation, not much change in amylase level was observed after 40 weeks and therefore amylase estimation is not of much value in diagnosis of post dated pregnancy.

Brosens and Gordon (1966) showed that the orange stained cells originating from the fetal sebaceous glands reflect the maturity of the fetal skin. A count of 1 to 10% was associated with 34 to 38 weeks gestation and 10 to 50% orange cells reflected maturity of 38 to 40 weeks. The results were confirmed by the same authors in 1967, 1968 and by Chandiok *et al* (1971), Sunanda Bai *et al* (1972) Roy Chowdhury *et al* (1975). In the present study, in group I, 100% samples showed orange cell count of 10% or less. With gestation of 36 weeks and below, bubble stability test was negative in 70.58% cases and positive in 5.88% cases, 23.53% showed intermediate result.

In group II, a sudden rise in amylase level was observed at 37 weeks, the mean value being 163.84 Smogyi Units/100 ml, and another peak was observed at 40 weeks. Orange cell count also increased with increasing gestation and in pregnancies of 41 weeks and above, 93.75% samples showed orange cells more than 30%. With gestation of 37 to 40 weeks 66.66% samples gave positive bubble stability test and with gestation of 41 weeks and above, all but one sample showed positive test.

When amylase level of 120 Somogyi Units per 100 ml or more is obtained, prematurity can be excluded almost with certain. Taking amylase level of 100 Somogyi Units/100 ml or more as an index of maturity, there is a risk of obtaining 11.78% false positive results and 3.45% false negative results. De Castro *et al* (1973) obtained only 1.9% false positive and 27% false negative results taking a value of 200 I.U/ litre as index of maturity. While orange cell count is quite reliable in later weeks of gestation, at gestation of 37-40 weeks, 21.9% cases had orange cell count of 10% or less. Gautheir *et al* (1972) could accurately predict a maturity of 36 weeks and above in 93% cases and stated that orange cell count is more accurate if gestation is 38 weeks or more. The results obtained in the present study are consistent with the above statements. Though Bhagwanani *et al* (1973) obtained a high percentage of false negative results with bubble stability test, in the present study, it has been shown that only 7.14% false nevative and 5.88% false positive results were obtained. However, a high percentage of intermediate results was obtained, making the interpretations difficult and limiting the value of the test.

Clinically, with negative and intermediate bubble stability test respiratory

problems were observed in 66.66% and 25% newborns respectively. With positive result, no baby had respiratory distress.

The present study shows that no single test can serve as absolute index of fetal maturity. The amniotic fluid amylase though gives a fairly good impression of the gestational age it is not of much use in diagnosis of post dated pregnancy. Moreover, it requires a biochemical laboratory. Although the orange cell counting and bubble stability test are quick, simple and side room tests, they have their own disadvantages. The orange cells have a tendency to clump together in later weeks and therefore the counting may not be accurate. In the case of bubble stability test, quite a few intermediate results are obtained which are inconclusive.

It is evident that by combining the two tests, the predictive value is higher when compared to that obtained by utilizing a single tets.

Correlation Between Three Parameters

Group	Gestation (Weeks)	No. of samples	Mean Amylase Somogyi Units per 100 ml.	Mean orange cell%	Mean bubble stability test	Mean clin. maturity (weeks)	Mean birth weight (gms)
I	36 & below	17	53.18	1.62	0.53	31.64	1288
II	37-40	42	187.68*	34.59	3.07	39.00	2711
III	41 & above	16	186.01*	52.59	3.75	41.00	3024

*One value deleted being abnormally high.

Bubble Stability
0 Negative
1, 2 Intermediate
3, 4 Positive

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References

1. Bhagwanani, S. G., Fahmy, D. and Turnbull, A. C.: Lancet. 2: 66, 1972.
2. Brosens, I. A. and Gordon, H.: Obstet. Gynec. 30: 652, 1967.
3. Chandiok, S., Gupta, A. N. and Devi, P. K.: J. Obstet. Gynec. India. 21: 547, 1971.
4. Clements, J. A., Platzker, A. C. G., Tierney, D. F., Hobel, C. J., Creasy R. K., Margolis, A. J., Thibeault, D. W., Tooley, W. H. and OH, W.: New Eng. J. Med. 286: 1077, 1972.
5. De Castro, A. F., Usateguigomez, M. and Spellacy, W. N.: Am. J. Obstet. Gynec. 116: 931, 1973.
6. Grauther, C., Despardime, P. and Mc Lean, F.: Am. J. Obstet. Gynec. 113: 344, 1972.
7. Roy Chowdhury, N. N., Mukherjee, A. K. and Paul, R. K.: J. Obstet. Gynec. India. 25: 722, 1975.
8. Sunanda Bai, K., Rohatgi, P., Lahiri, B. and Agnihotri, H.: J. Obstet. Gynec. India. 22: 152, 1972.
9. Wolf, R. O. and Taussig, L. M.: Obstet. Gynec. 41: 337, 1973.