EVALUATION OF AMNIOTIC FLUID TEST WITH ULTRASOUND ESTIMATION OF FOETAL MATURITY AND CO-RELATION WITH PHYSICAL AND NEURO-MUSCULAR MATURITY OF NEWBORN

by

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SUMMARY

In a country like India, where perinatal mortality is high, some reduction in perinatal mortality rate could be made by bringing about timely termination of high-risk pregnancy and at the same time avoiding prematurity and prolonged pregnancy. Again in India, where modern aids and well equipped hospitals are lacking, simpler methods should be adopted.

In this study it is very much evident that the results obtained by Amniotic fluid Creatinine level was very close to those obtained by ultrasound, followed by those of Clements Test and Nile Blue sulphate test in that order.

All or most of the above mentioned methods are used together, depending on the availability, thus giving very reliable results.

Introduction

There are many situations in which it is important to ascertain the maturity of the foetus while it is still in utero.

One of the most common problems faced by an obstetrician in day to day ante-natal practice is patients with disputed or forgotten dates of their menstrual period. Also the assesment of fetal maturity by menstrual dates, dates of quickening or by abdominal palpation can be grossly misleading, more so in pregnancies associated with complications. Assessment of foetal maturity prior to the induction of labour or an elective caesarean section is not only essential but is a must in order to obtain the full beneficial outcome of such an induction.

To minimize the morbility and mortality due to prematurity, postmaturity, intra-uterine growth retardation, Rh sensitization etc., the confirmation of foetal maturity becomes important to decide whether to terminate the pregnancy or to let it continue.

Material and Methods

The present study was conducted on 80 patienst at the Nowrosjee Wadia Maternity Hospital, Parel, Bombay.

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The patients were of two groups.

A. 40 cases were of gestation 31 to 42 weeks with known dates of the last menstrual periods. In this group cases were studied for the evaluation of all the tests and investigations and to establish the methodology.

B. 40 cases were those, where dates of last menstrual periods were not known. In this group, tests were applied to know the maturity of the foetus prior to induction of labour or before elective caeserean section.

No one test is 100% accurate, therefore several types of examinations are performed and the results are collated to help reach a conclusion.

The various tests employed were—

A. Amniotic fluid study

(1) Nile blue sulphate test.

(2) Clements test.

(3) Creatinine test.

B. Ultrasonic measurement of Bi-parietal diameter (B.P.D.).

Ultrasound measurement of Bi-parietal diameter is the most accurate and non-invasive method but it is not available in all the hospitals. Therefore, amniotic fluid study which is economic as well as simple can be performed in most of the hospitals. In the present study, the ultrasound results are compared with each of the amniotic fluid test result and thereby these tests are evaluated.

The comparative efficacy of individual tests was also known by comparing the results from the above studies with post-natal physical and neuromuscular maturity by clinical examination of the new-born.

For physical maturity, parameters looked for were skin, lanugo hair, plantar creases, breast buds, ear pinna and genitals.

For neuromuscular maturity, parameters checked for were posture, square window (wrist). arm recoil, popliteal angle, scarf and heel-to-ear sign.

Each of the above parameters were scored and hence clinical maturity estimated.

Amniotic fluid being of fetal origin, its contents vary with the period of gestation and therefore reflect the maturity of the fetus. The collection of amniotic fluid was done either by abdominal paracentesis, by vaginal route or at caesarean section.

A. I Nile Blue Sulphate Test.

One drop of amniotic fluid was mixed with one drop of 0.1% nile blue sulphate freshly prepared on a clean dry slide. Two slides were prepared. The slide was covered with a clean cover slip. It was not heated and was examined under the low power of a micrsocope.

The fields showed:

(1) Blue stained nucleated and anucleated cells.

(2) Orange stained cells.

(3) Orange droplets.

Total 100 cells were counted on each slide and the percentage of orange stained cells was counted.

Some slides showed clumping of orange stained cells at various places, an estimate of number of cells in each cluster was made according to the size of the cluster i.e. 5, 10, 20, 50. Blue stained bodies represent shed fetal epithelial cells, while the organge stained bodies originate from sebaceous glands.

II. Clements Test (Shake Test).

Volume of amniotic fluid 1, 0.75, 0.5, 0.25 and 0.2 ml were pipetted into 14 x 100 mm test tubes, chemically cleaned. Volumes of 0.25, 0.5, 0.75 and 0.8 ml of normal saline pipetted in tube Nos. 2, 3, 4 and 5 respectively. This gives 1/1, 1/1.3, 1/2, 1/4 and 1/5 dilutions, 1 ml of

95% ethanol was added to each tube. Tubes were shaken vigorously for 15 seconds and placed vertically in a test tube rack. 15 minutes later the air-liquid interface was examined in each tube.

A complete circle of stable bubbles was visible around the meniscus, 15 minutes after shaking was recorded as positive. The tube with highest dilution of liquors giving such a result was recorded. Samples were classified as:

(a) Negative—If absence of ring in first tube (1:1 dilution).

(b) Intermediate—when ring is present in only 1st OR 1st and 2nd tube (i.e. upto 1:1.3 dilution).

(c) Positive—If presence of complete ring in first 3 tubes OR in still higher dilutions (i.e. 1:2 dilution and onwards).

III. Creatinine level of Amniotic fluid.

All the specimens of amniotic fluid were centrifuged at the rate of 2000 sp. for 10 minutes and stored at 20°C till the creatinine estimation was carried out by the Folin—Wu (1965) adaptation of Jafes reaction. Cases with pre-eclampsia and eclampsia were encluded.

Results and Discussion

Total 80 cases.

Group A—40 cases—Amniotic fluid study was carried out for the purpose of evaluation of all three tests and results of each test compared with that of ultrasound. These patients knew their exact dates of last menstrual period. Their cycles were regular. Their fundal height corresponded to the weeks of gestation. In all cases post-natal assessment of maturity was carried out.

Nile Blue Sulphate Test

In some cases there was a high count during the earlier weeks of gestation, but most of them fell within the range shown in Table I.

Results of other authors

Brosens and Gordon

Weeks	Percentage of orange cells
Less than 34	0 to 1
34 to 38	1 to 10
39 to 40	10 to 50
More than 40	More than 50

Evaluation of present study on Brosens and Gordon (1965) scale showed that 5 cases were out of range.

Shake Test: With increasing maturity, Shake test was positive in higher dilutions, but there was no exact linear relationship. Shake test was negative before 34 weeks of gestation. The results were as shown in Table II.

TABLE I

Weeks of gestation (completed)	No. of cases	Orange cells %	Cases falling in the range	Cases out of the range
Less than 34	10	0	9	1
34 to 36	10	1 - 9	9	1
37 to 40	17	10 - 49	15	2
41 and more	3	50 - 66	2	1

TABLE II

Weeks of Gestation	Results in percentage		Positive
	Negative	Intermediate	
Less than 36	73.50	4.88	21.62
36 to 40	9.60	25.40	65.00
40 and more	0.75	2.75	96.50

Amniotic Fluid Creatinine Level

In the present series amniotic fluid creatinin level of 1.8 mg/100 c.c. was taken as a maturity index of fetus. It was supported by Karjalainen (1975); Bonner and Redman (1977).

The comparison of amniotic fluid creatinine level between different gestational age groups was statistically highly significant in both known and unknown L.M.P. groups. This result was in confirmity with the findings of Pitkin and Zwirek (1967). Creatinine level at various gestational weeks was as in Table III.

TABLE III

Weeks of gestation	Creatinine in mg/100 ml
30 to 34	1.3 mg. to 1.5 mg.
34 to 38	1.5 mg. to 1.8 mg.
38 to 42	1.8 mg. to 2.2 mg.

Comparison of results of each test by correlating with clinical outcome—80 cases as in Table IV.

TABLE IV

Test	Correlation with Clinical Out-come	
	Good %	Poor %
1. Nile Blue Sulphate Test	89	11
2. Shake test	93	7
3. Creatinine level	95	5

From the above comparison it is evident that the results obtained by estimation of creatinine level were more significant.

When used together, in 92.33% cases correlation with clinical maturity was good.

Presumably creatinine is a product of fetal urine. It is not established whether the rise in creatinine in the amniotic fluid represents (1) maturing fetal renal function, (2) a decrease in the volume of amniotic fluid as term approaches, OR (3) an increase in the fetal mass of muscle.

B. Ultrasonography:

Ultrasound has many uses and while many structures can be visualized, the technique is dispensed here only in terms of determining fetal maturity and size. The biparietal diameter of the fetal head can be measured with reasonable accuracy starting at 11.6 weeks. The following information has been noted:

(1) The biparietal diameter increases by 1.6 to 1.8 mm per week.

(2) Failure of this diameter to grow by 1.5 mm over a 2 week period indicates growth retardation.

(3) Biparietal diameters at different weeks of gestation from 1.9 cms equal to 11.6 weeks of gestation to 9.7 cms equal to 39.8 weeks of gestation.

Examination can be facilitated by allowing the patient's bladder to fill and

lowering the head of the examination couch in order to disengage the head sufficiently.

Results

Ultrasound measurement of Biperietal diameter in the present study gave the highest percentage of accuracy to know the maturity of the fetus. Accuracy rate was found to be above 98% except in cases of IUGR.

Figure 1 shows results by means of a Histogram, taking each parameter into consideration with respect to good co-relation and poor co-relation.

It is very much evident that ultrasound had the best co-relation i.e. to the extent of 98% good co-relation.

Also the results obtained from amniotic fluid creatinine level, clements test and Nile Blue Sulphate test were co-related with the results of ultrasound and their comparative evaluation and efficacy was understood.

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