LABORATORY INVESTIGATIONS IN THE RAPID DIAGNOSIS OF PREMATURE RUPTURE OF MEMBRANES

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Premature rupture of foetal membranes presents a great challenge to obstetricians. Inspite of its being common there is no universally accepted definition of it. The most commonly accepted definition is "rupture of membrances prior to onset of labour". Premature rupture of membranes leads to onset of premature labour if it occurrs before 37 weeks of pregnancy and amnionitis with subsequent neonatal and maternal infections.

In clinical practice many times pregnant woman presents with the history of watery vaginal discharge and physical examination may not verify the diagnosis of premature rupture of membranes. This is particularly so when cervical os is closed or flat membranes are felt over the presenting part on account of high rupture. Other discharges like urine, excessive vaginal discharge and inflammatory exudates may also obscure the clinical diagnosis. In such situations only laboratory diagnosis helps. Hence quick and early diagnosis is essential to improve foetal and maternal prognosis in cases of premature rupture of membranes. We are reporting the results of three laboratory tests—change in pH, arborisation test and vaginal smear staining by Nile Blue sulphate individually and collectively in early diagnosis of premature rupture of membranes.

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Material and Method

One hundred and twenty-five cases admitted to Zanana Hospital, Udaipur were studied in the year 1978-79, with or without history of premature rupture of membranes. The patients were divided into three groups.

Group A: 25 cases with clinically definite rupture of membranes, spontaneous or artificial.

Group B: 100 cases of doubtful rupture of membranes. In these cases there was history of leaking per vaginum prior to the onset ol labour pains. These cases were above 28 weeks of gestation and cervical canal was less than 2 cm. dilated.

Group C: 25 cases with intact membranes. These cases were studied in first stage of labour. This group was specially included to evaluate negative result of the test.

Detailed history was taken with special reference to age, period of gestation, duration of leaking and onset of labour pains. After that general and local examinations were done in each case to notice the escape of fluid from the cervical os and the presence or absence of membranes. Samples were collected by sterile aspiration pipette. This was followed by vaginal examination to find out the pre-

sence or absence of membranes, presentation, position of foetus and to assess the pelvis. The following tests were carried out by putting a drop of aspirate on clean dry slides.

1. *Nitrazine Paper Test*

This was done to determine pH of vagina or vaginal aspirate. A drop of vaginal aspirate on the slide was directly touched with the paper or Nitrazine paper was directly touched into the vagina. The colour changes of the paper were compared with the standard chart. Values of pH between 5 to 6 were taken to indicate intact membranes and between 6.5 to 7.5 to indicate ruptured membranes.

2. *Arborisation Test*

A drop of vaginal aspirate taken on the slide was spread and dried at room temperature. It was than examined under microscope for arborisation. Samples were not taken near the introitus and cervix to avoid false results.

3. *Nile Blue Sulphate Staining*

This dye stains neutral fat of foetal cells yellow to orange and the rest of the cytoplasm and nuclei as blue. One drop of vaginal aspirate taken on a slide was spread and smear was made. On the wet smear, one or two drops of 0.1% Nile blue sulphate was put. It was heated over a flame for few seconds and cover slip was applied. It was then seen under the low power of microscope for the presence of foetal cells.

Diagnosis of premature rupture of membranes was confirmed when pH is alkaline, positive arborisation test and presence of yellow orange foetal cells in the vaginal aspirate.

Observations

Ninety-two per cent cases in group A and C and 83 per cent cases in group B were between 36-40 weeks of gestation.

Nitrazine paper test had accuracy rate of 100, 96 and 68 per cent in Group A, B and C respectively. Arborisation test showed accuracy rate of 100, 87 and 100 per cent in Group A, B and C respectively. Nile Blue sulphate staining test had accuracy rate of 88, 90 and 100 per cent in Group A, B and C respectively (Table I, Fig. 1).

TABLE I
Accuracy Rates in Various Groups

S. No.	Group A			Group B			Group C		
	Accuracy	False +ve	False —ve	Accuracy	False +ve	False —ve	Accuracy	False +ve	False —ve
1. Nitrazin paper test	100	—	—	96	—	4	68	32	—
2. Arborization test	100	—	—	87	—	13	100	—	—
3. Nile Blue Sulphate test	88	—	12	90	—	10	100	—	—

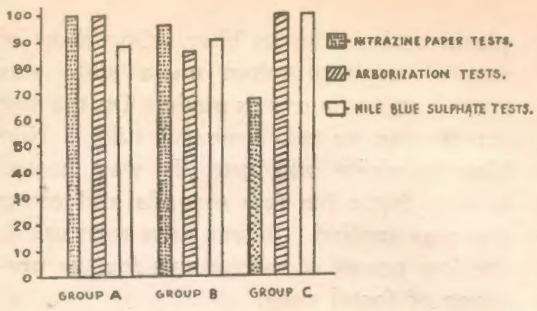

Fig. 1.

Discussion

There are numerous laboratory procedures described to help in establishing the presence of amniotic fluid in the vagina. We have chosen to study changes in pH of vagina by Nitrazine paper, arborisation test and Nile blue sulphate staining test because of their simplicity. Maximum cases were primigravida and were between 36 to 40 weeks of gestation.

Normally, pH of vagina varies between 4.5 to 5.5, whereas amniotic fluid is faintly alkaline or neutral. Hence pH of vaginal fluid changes to alkaline or neutral when it is contaminated with escaped amniotic fluid. Other discharges like infected urine, inflammatory exudates, and other non-specific discharges may also change the pH. The 32 per cent false positive results in group C may be explained by that.

Arborisation test showed 100 per cent accuracy in Group A and C, while in group B it had 13 per cent false negative results. This may be due to presence of show which obscures the crystallisation pattern. Anjaneyulu and Likhite (1967) also reported 87 per cent accuracy rate in Group B, while Gupta and Gupta (1977) reported accuracy rate of 92.5 per cent in group B.

Nile blue sulphate staining of neutral fat laden foetal cells is the basis of this test. The neutral fat, however does not appear in the foetal cells in the amniotic fluid before 34 weeks of gestation Brosens and Gordon 1965) and this test is of no use in diagnosis of premature rupture of membranes before this period. Ninety per cent accuracy rate of this test in Group B may be explained by the fact that 15% of the cases were less than 34 weeks gestation. The same fact explains accuracy rate of 88 per cent in group A as compared to 100 per cent accuracy reported by Brosens and Gorden (1965) and Saraf and Purandare (1966).

Thus it is clear that no single test is 100 per cent reliable because of false positive and negative results, but if a combination of these three tests is applied the error of one test can be corrected by others.

Summary

Three laboratory tests—Nitrazine paper test, arborisation test and Nile blue sulphate staining test have been carried out in 125 cases of premature rupture of membranes. Accuracy rate of these tests and their usefulness in the diagnosis of premature rupture of membranes have been evaluated.

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