ROLE OF BIOCHEMICAL ASSESSMENT OF ALPHAFETOPROTEINS (AFP) IN VAGINAL SECRETIONS IN DIAGNOSIS OF PREMATURE RUPTURE OF MEMBRANES (PROM)

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SUMMARY

Premature rupture of membranes (PROM) is a common complication of pregnancy and a significant cause of maternal morbidity and neonatal morbidity and mortality. The exact causative factors are not known. The diagnosis is not always very easy, especially if the rupture is doubtful. Therefore, it was decided to evaluate presence of alphafetoprotein (AFP) levels in vaginal secretions as a marker for PROM. Optical density using Enzyme Immunoassay (EIA) was taken for assay rather than the actual value in ng/ml. We could confirm diagnosis in 80% doubtful cases of PROM. The diagnostic specificity of the test was 92%, sensitivity 94%, positive predictive value 92.1% and negative predictive value 93.8%. These results make it a good diagnostic test for the diagnosis of PROM.

INTRODUCTION

Premature rupture of membranes (PROM) is one of the most common complications of pregnancy with a reported incidence of 10%, varying from 2-18% of all pregnancies (Arias -1993;& Arulkumaran et al, 1996). It is associated with a significant increase in maternal morbidity and high rates of neonatal morbidity and mortality. It contributes to 10% of all perinatal deaths (Rochelson et al 1983).PROM is also the

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commonest cause of preterm labor and is responsible for 30% of it(Arias 1993 & Arulkumaran et al 1996).The exact causative factor of PROM is not known. The possible risk factors are :decreased strength of fetal membranes due to its infection and increased intrauterine pressure (Sharra et al-1987). The main concern in PROM is its association with prematurity which is responsible for increased fetal wastage and neonatal complications.A definitive diagnosis and appropriate management are essential to reduce the maternal morbidity and neonatal morbidity and mortality associated with PROM.

The diagnosis is easy if the clinical situation is obvious,but in doubtful cases specific tests are required to confirm the diagnosis. Various available tests to diagnose PROM are determination of vaginal pH, identification of foetal cells, estimation of diamine oxidase (DAO), prolactin and insulin like growth factors in vaginal secretions(Bortem et al 1987, Jones and Kelly - 1987,Koninck et al -1987,Phocas et al -1989, Rutanen et al 1993). Althougth, these tests are non-invasive,they are associated with increased rates of false positive and false negative results. Injection of indigo-carmine into the amniotic cavity and its detection in vaginal secretions and amnioscopy for direct visualisation, though confimatory are invasive tests.

Alphafetoprotein(AFP), a glycoprotein is secreted by foetal kidneys and is normally present in the amniotic fluid. It is neither present in vaginal secretions, nor in maternal urine. Under normal circumstances, presence of AFP in vaginal secretions is a definitive indication of PROM (Bortem et al-1987 and Rutanen et al -1993). This prospective case-controlled study was undertaken to determine the value of biochemical assessment of AFP in vaginal secretions to diagnose PROM.

MATERIAL AND METHODS

In this case controlled prospective study, 150 pregnant women in their second and third trimester were studied over a period of 10 months from May, 1995 to March, 1996. These cases were divided into three groups. Group I (control group) consisted of 50 normally pregnant women and were confirmed cases of non-premature rupture of membranes (PROM), with gestation ranging between 28-40 weeks. Group II consisted of 50 pregnant women with confirmed PROM, spontaneous / artificial rupture of membranes done for induction of labor. The period of gestation ranged from 26-40 weeks. Group III consisted of 50 pregnant women with an unconfirmed clinical diagnosis of PROM.

All patients were evaluated clinically. A detailed history of present pregnancy, menstrual history and previous obstetric history were obtained. Also, previous medical history of fever, vaginal bleeding, urinary symptoms, drug intake and exposure to radiation during the present pregnancy were elicited. History of medical disorders such as diabetes mellitus, hypertension, tuberculosis and congenital anomalies were obtained. A thorough general, physical, systemic and local examination was carried out. Routine laboratary tests done were, Hb estimation, blood counts, blood chemistry, VDRL, blood group and Rh typing and urine analysis; HVS and urine for culture and sensitivity. KFT and LFT were done in selected cases.

Measurements of a alphafetoprocin (AFP) in vaginal secretions by Enzyme Immunoassay technique was done using microwell kit procured from Melotac S.A., Barcelona, Spain. The samples were collected under direct visualization using sterile speculum and the cotton sway kept in contact with cervical secretions for at least for 10 seconds. This sample was immediately transferred to a sterile glass test tube which was capped and transferred to lab, where it was stored at -30 C temperature prior to analysis. The investigation was done in two phases. In the first phase Group I and Group II, were tested to develop the diagnostic kit and to determine a threshold vaue for AFP as a cut-off for diagnosis of PROM. Specificity, Sensitivity and positive and negative predictive values were also determined. Second stage Group III was tested and studied.

Obstetric management, conservative/active was decided upon diagnosis of PROM., confirmed by AFP determination and the period of gestation. All cases were followed till delivery for maternal and neonatal outcome.

OBSERVATIONS AND RESULTS

Cases in both control and study groups were comparable in respect to age, gravidity, parity and period of gestation (Table I). Majority cases were < 25 years of age: Group I (56%), Group II (52%), and group III (50%). The parity ranged from I-IV and majority were primigravidae in all gorups. The gestational age in majority cases was 35-40 weeks, group I (controls) (62%), group II (78%) and group III (study group) (84%) (Table I).

The optical density (OD) was taken for final calculation and not the actual value of AFP in ng/ml as in some cases OD was very low and distinction by value of AFP in ng/ml was not possible. The mean AFP OD of group I (confirmed non-PROM)

Table I
CLINICAL CHARECTERISTICS OF THE CASES

Clinical Profile	Group I (n=50)	Group II (n=50)	Group III (n=50)
Age (years)	23.34 (19-30)	23.98 (19-32)	24.42 (19-32)
Primigravida	48%	60%	44%
Gestational Age (wks) Mean	34.6	35.8	36.5

Figures in parantheses denote the range.

Table II
SHOWING MEAN AFP VALUES IN OD IN GROUP I AND GROUP II CASES

OD cut off values of AFP	Group I False Positive	(n=50) Negative	Group II False Positive	(n=50) Negative
Mean AFP of gr.II -	0	50	15	35
Mean AFP of of Gr.I-0.5S.D. (0.1066)	1	49	29	21
Mean AFP of gr.III-1S.D. (i.e.0.0511)	4	46	47	3

Specificity is 92%, sensitivity 94%,positive predictive value (PPV) 93.1% and negative predictive value 93.8%.

was 0.00522 (0.0-0.122 OD), group II (confirmed PROM) was 0.162 (range 0.040-0.550). Taking this mean value as the threshold, resulted in no false positives but a very high degree of false negative cases (Table II).

These results translate into a test specificity of 100% which is very good, but a sensitivity of 30% with very low predictive value (58%) and this is unacceptable as diagnostic test. Hence, the threshold value was moved to mean S.D. -0.5 i.e. an O.D. value of 0.1066 which resulted in very slight loss of specificity (98%), but the sensitivity (58%) and the negative predictive value (70%) meant that these results were still not satisfactary for a diagnostic test

In the next step, the threshold cut off value was taken as mean 1 S D -1 i.e. 0.0511 O.D. The specificity was reduced to 92% but the sensitivity came upto 94% with a high positive predictive value of 92.1% and negative predictive value of 93.8%. These values are acceptable for use of the test as a diagnostic procedure as very few patients are wrongly diagnosed Therefore the threhold cut-off value was set at 0.0511 O.D. for diagnosing PROM (Table III).

In the second phase, group III consisting of 50 patients in the third trimester suspected to have PROM, were taken and diagnosed

on the basis of AFP expressed as OD of vaginal secretions. In this group, 40 patients (80%) were positive for PROM according to the above criteria and 10 (20%) were negative. Follow up management of these patients was based on these results. All cases were followed up till delivery for maternal and neonatal outcome. The diagnosis of AFP measurement was found to be correct as the 10 (20%) patients diagnosed not to have PROM, did not show any further complications of PROM.

DISCUSSION

Premature rupture of membranes always predisposed the unborn fetus to risk of infection. Diagnosis is easy when amniotic fluid is seen vaginally, but it poses a diagnostic and management dilemma in doubtful cases, especially in preterm pregnancy. The decision to use conservative/active/no treatment, requires an accurate diagnosis of PROM. Various tests are available to diagnose PROM but most of these are unreliable and associated with high rates of false positive and false negative results. Besides, some of these tests are difficult to perform and are invasive (Arias- 1993). The concentration of AFP is highest during the midtrimester of pregnancy and declines towards term. At term, the concentration of AFP in amniotic fluid is 10 times more than in maternal blood (Gaucherand et al 1994). Under normal circumstances, AFP is neither present in maternal urine nor in vaginal secretions as this protein cannot permeate through intact foetal membranes. Hence, presence in vaginal secretions is a positive indication of PROM. Fern test, a more simple, rapid and economical method is associated with very low accuracy rates (21%) and low sensitivity of 62% (Rochelson et al 1987). Besides, it is a non-specific test and is adversely affected by cervical mucus and urine. Similarly, nitrazine test, although, most commonly used, is associated with high false positive (17%) and false negative (10%) results (Freidman et al 1969). Huber et al (1983) did quantitative assessment of AFP in vaginal secretions by radioimmunoassay for the diagnosis of PROM and found it a more reliable test for confirming the presence of amniotic fluid in the vaginal secretions.

Rochelson et al (1987) also evaluated AFP in vaginal secretions in diagnosis of PROM in <36 weeks of gestation and have found excellent results with a sensitivity of 98%, regardless of duration of rupture of membranes. They also found that slight contamination of amniotic fluid with maternal blood does not affect AFP values and when membranes are intact, AFP testing was consistently negative. Moreover, the test is most accurate in preterm patients, in whom correct diagnosis is most important. Gaucherand et al (1994) in their series assessed AFP in vaginal secretions using immunoenzymatic assay to diagnose PROM and noted that this test has high sensitivity (98%) and specificity of 99% and concluded that it is a reliable, simple and rapid diagnostic test.

The Diamine Oxidase assay is one of the most commonly used tests. It is an easy, rapid and inexpensive test but is not reliable when amniotic fluid is mixed with blood. Also the Diamine oxidase allows some dialysis through intact membranes and thus is associated with high false positive results (Gahl et al 1982).

In the present study a high specificity of 92% and sensitivity of 94%, with high positive predictive value of 92% and negative predictive value of 93.8% found are comparable to the results observed in previous studies (Gaucheran et al 1994 and Rochelson et al 1987). In this study, diagnosis of PROM could be confirmed in as high as 80% of doubtful cases of PROM. The cases were managed accordingly and all had normal pregnancy outcomes.

Hence, it is concluded that biochemical assessment of AFP in vaginal secretions offers a most reliable, simple, easy and economical method to diagnose PROM in doubtful cases.

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