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Original Article

Role of Amnioseal in Premature Rupture of Membranes

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Abstract

Objective: Interleukins (ILs) and matrix metalloproteinases actually target collagen IV in the amniochorion in premature rupture of membranes (PROM), IL-10 acts as a membrane protector. In order to counteract the immunological system involved in the pathogenesis, we have used a combination of proapoptotic bax gene P-53 inhibitor, myristoleate, with antimicrobial peptides, neutrophil defensins, and cytokine IL-10 enhancer. *Methods:* A comparative prospective randomized study was conducted from July 2006 to January 2007 in patients (46 cases and 60 controls) attending the Gynecology and Obstetrics Emergency Department, Eden Medical College, Kolkata, complaining of leakage per vagina in their antenatal period between 24 and 36 weeks of gestation. Combination of bax gene inhibitor, antimicrobial peptides, neutrophil defensins, and IL-10 enhancer in scheduled protocol was prescribed and cases followed up as per fixed protocols. *Results:* In the 24-30 weeks group, mean prolongation of gestational age was 6.16±3.21 as against 2.66±1.05 (significant), while in 31-36 weeks mean prolongation was 4.69±0.84 as against 4.6±0.632 (significant). In the first group mean birth weight in cases was 1.77±0.66 kg as against 1.2±0.43 kg (significant), while in the second it was 2.18±0.56 kg as against 1.76±0.45 kg (significant). Associated complications were less in cases. *Conclusion:* Combination of bax gene inhibitor, antimicrobial peptides, neutrophil defensins, and IL-10 enhancer as per scheduled protocol can be advocated routinely in PROM barring a few situations with excellent fetal and maternal prognosis.

Keywords: proapoptotic bax gene P53, PROM, matrix metalloproteinase inhibitor, interleukins, amnioseal.

Introduction

Preterm delivery with its associated morbidity and mortality still represents one of the major clinical problems in obstetrics in terms of both survivability and quality of life. In fact, out of every 10 deliveries conducted in the labor room 1 is a preterm; of these one third are due to preterm premature rupture of membranes (PPROM)[1].

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The amnion comprises of a single epithelial layer. Chorion is a multilayered cytotrophoblastic layer with collagen-rich connective tissue. Tensility of membranes depends on amnion while chorion is elastic[2]. Varying levels of collagen have been implicated in PROM, however consensus opinion on increased levels of matrix metalloproteinase MMP2, MMP3, MMP8, MMP9, and neutrophil elastase contributing to the condition have been obtained[2]. Increased apoptosis resulting in the breakdown of amniochorion has been reported in PPROM[2]. Infection and PPROM have been long related. Nature of immune mechanism with α plus β defensins may well be critical in permitting or preventing ascending infection. IL-1RA, TNF α promoter, MMP1 promoter, and MMP9 promoter polymorphisms have been shown to be associated with increased risks of

PROM targeting collagen IV in amniochorion[3]. IL-10 acts as a membrane protector. PROM can be diagnosed from clinical history, clinical examination, pH testing, heat test, arborization, level of IGF-BP1, α fetoprotein estimation, etc. In cases where prolongation of pregnancy is desired, apart from tocolysis, antibiotics, corticosteroids, infection monitoring, amnioinfusion, and fibrin sealing have been attempted with limited success. Hence the use of a combination of MMP inhibitors, cytokines, and defensins in the form of amnioseal was contemplated.

Aim of the Study

In order to target the immunological system involved in the pathogenesis, we have used amnioseal, a combination of proapoptotic bax gene P-53 inhibitor, myristoleate with antimicrobial peptides, α plus β defensins, neutrophil defensins, and cytokine IL-10 enhancer in order to terminate the vicious cycle and see whether it can change the course of PROM. Associated with the above are a series of supportive factors like tocopherol, glutathione, biotin, folic acid, vitamins B1, B2, B6, B12, and arginine.

Material and Methods

We conducted a comparative, prospective randomized study from July 2006 to January 2007 in patients attending the Gynecology and Obstetrics Emergency and Out Patient Department, Eden Hospital, Medical College, Kolkota, complaining of leakage per vagina between 24 and 36 weeks of gestation. Institute ethical committee approval was taken and informed consent was obtained from all the study participants. PROM was confirmed by evaporation test and pH test (pH INDICA paper). A total of 46 cases and 60 controls were studied in the study period. Excluded were patients in active labor, having multiple pregnancies, or diagnosed with fetal anomalies, with features of chorioamnionitis, profuse dribbling, or with hepatic, cardiac, or respiratory disorders. All patients were generalized with regard to their age, socioeconomic, and educational statuses to reduce bias. Relevant clinical histories were noted. Detailed general and systemic examinations were done. It was ensured that all had negative HbsAg and prevention of parent-to-child transmission of HIV (PPTCT) report and acceptable liver function test reports. Patients were randomized to either case (A1) or control (B1) group. All the patients were put on antibiotics, corticosteroids, and tocolysis after sending a high vaginal swab for culture sensitivity. In addition to these, subjects were given amnioseal (TNS Meryl Pharma). Dosage schedule was two capsules stat, two capsules after 3 hours, followed by two capsules 8 hourly for up to 72 hours. Maintenance dose was one capsule twice daily for 15 days and one capsule daily for another 15 days. Three cases and nine controls were discontinued from the study due to frank chorioamnionitis. Owing to the obvious difference in nature of prognosis, results were classified in two different categories depending on the period of gestation. Patients were followed up with daily monitoring of pulse, temperature, uterine tenderness and contractions, nature of discharge, leucocytosis, fetal heart rate, daily fetal movement count and cardiotocography, weekly ultrasounds, especially amniotic fluid index, and fortnightly liver function tests. The outcome of each pregnancy was documented in detail.

Results

Analysis was done on the two groups. The first group comprised of patients who were received at 26-30 weeks of gestation. In this group we studied 12 cases (A1) and 15 controls (B1). Pregnancy continued for another 6 weeks in seven cases in A1 and one control in B1. Mean prolongation of gestational age in A1 was 6.16±3.21 as against 2.66±1.95 weeks in B1. A "t" test performed gave a t-value of 2.6, $p<0.005$ (significant). Pregnancy reached term in three cases as against none in controls. Oligohydramnios occurred in four cases as against eight in controls. Neonatal mortality in 5 cases as against 13 in controls, septicemia in 2 cases as against 5 in controls, birth asphyxia in 4 cases as against 6 in controls, respiratory distress syndrome (RDS) in 5 cases as against 9 in controls, hypothermia and/or hypoglycemia in 6 cases as against 10 in controls, and neonatal jaundice in 7 cases as against 3 in controls (Table 1). Intraventricular hemorrhage was seen in one case and necrotizing colitis in one; none of the controls had similar outcome, possibly because very few survived to show the same. Mean birth weight in case group was 1.77±0.66 kg as against 1.2±0.43 kg. A "t" test performed showed a value of 2.7, with a significant p-value of <0.02. In subsequent follow-up, one baby in the study group was showing features of delayed milestones.

The second group comprised of patients received between 31 and 36 weeks of gestation. In this group we studied 31 cases (A2) and 36 controls (B2). Mean weeks of gestation for which the pregnancy was prolonged was 4.19±1.42 in A2 as against 3±1.54 in B2. Result was analyzed by "z" test, z-value was 3.3, $p<0.01$ (significant). Pregnancy continued till another 4 weeks in 26 cases (A2) and 15 controls (B2). Mean prolongation in them was 4.69±0.84 as against 4.6±0.632 weeks. A 't' test

Table 1

Groups	A1 (*n*=12 cases)	B1 (*n*=15 controls)	p-value
Pregnancy cont in weeks	6.16±3.21	2.66±1.95	<0.005*
Pregnancy continues ≤6 weeks (%)	7 (58)	1 (7)	
Till term	3 (25)	-	
Oligohydramnios	4 (33)	8 (53)	
Neonatal mort	5 (42)	13 (87)	
Septicemia	2 (17)	5 (33)	
Birth asphyxia	4 (33)	6 (40)	
RDS	5 (42)	9 (60)	
Hypothermia/ hypoglycemia	6 (50)	10 (67)	
Neonatal Jaundice	7 (58)	3 (20)	
Birth weight in kg	1.77±0.66	1.2±0.43	<0.02*

*Significant p-values.

performed did not reveal significance, t-0.36, p>0.05. Pregnancy continued to term in 21 cases (A2) as against 4 in controls (B2). The 'z' value was 5.7, p<0.005. Sonologically ultrasound found oligohydramnios in 4 cases (A2) as against 12 in controls (B2), z=2, p<0.025. Neonatal mortality was seen in 3 cases (A2) as against 12 controls (B2), z=2.32, p=0.01. Amidst morbidities, septicemia was noted in five cases as against eight in controls (B2), z=0.674, p>0.05. Birth asphyxia was seen in two cases (A2) as against seven in controls (B2), z=1.7, p<0.05. RDS was reported in two cases (A2) as against four in controls (B2), z=0.793, p>0.05. Hypothermia was seen in seven cases (A2) as against nine in controls (B2), z=0.3, p>0.05. Neonatal jaundice occurred in eight cases (A2) as against 18 in controls (B2), z=2.2, p<0.025 (Table 2). Intraventricular hemorrhage (IVH) was reported in two cases (A2) as against four in controls (B2), z=0.793, p>0.05. Mean birth weight in the case group was 2.18±0.56 kg as against 1.76±0.45 kg. A 'z' test was performed, which showed a value of 2.6, p<0.001 (significant). Incidence of cesarean section rates were slightly higher in cases and liver function tests repeated in cases where the drug was continued for 4 weeks did not reveal any significant alteration.

Discussion

Lannetta[4] in 1984 detailed a simple but reliable test for diagnosis of PROM. The endocervical material on a glass slide was heated for 1 minute, when the membranes ruptured the slide turned white and brown when intact. Brar[5] in 1997 compared the evaporation tests with various methods. Evaporation test was accurate in 96% of the cases; nile blue sulfate reproduced orange-colored cells in 94% of the cases, arborization in 91% of the cases, while pH was accurate in 80% of the cases. Evaporation test is cheap, fast, simple, and risk-free. In our study too we have combined evaporation and pH tests.

Even in the presence of infection, mechanism of preterm delivery includes release of TNF α, IL1a, 1β, 6, and 8, which result in prostaglandin release, polymorphonuclear chemotaxis, and activation of MMPs. These result in remodeling of collagen and chorioamniotic rupture. Maternal serum ILs are elevated in patients with PPROM with clinical or histologic chorioamnionitis. TNF α increases MMP9 activity in amniochorion[6]. Associations between allelic variants of the polymorphisms at position 308 in the gene for TNF α and preterm birth after PPROM have been demonstrated. Hyperresponsiveness of the gene for TNF α to genital tract infection may produce PPROM.

MMP1 is a physiologic constituent of amniotic fluid. PPROM (in both the presence and absence of infection) was associated with an increase in amniotic fluid MMP1 concentration. Neither term nor preterm parturition was associated with a significant increase in amniotic fluid concentration of MMP1. In cases of PPROM without

confirmed infection, although there was no significant change in maternal serum cytokines, two thirds of them revealed elevated amniotic fluid cytokines. Babies born preterm when such elevated cytokines are present are more prone to suffer from bronchopulmonary dysplasia and periventricular leukonalacia[7]. It has been observed that babies born in presence of increased concentration of white blood cells and cytokines such as IL-6 and IL-8 in amniotic fluid are more likely to develop cerebral palsy at long-term follow-up[8]. Apart from proinflammatory cytokines, IL-6, TNF-α, IL-1, IL-2, IL-8, IL-12, and some antiinflammatory cytokines like IL-10, IL-4 also exist to downregulate Th1 helper T-cell functions[9]. The antiinflammatory action of IL-10 challenge the proinflammatory cytokine leading to decreased likelihood of preterm birth in preterm prelabor membrane rupture and also prevents lipopolysaccharide stimulated PGE2 production in membranes as well as cytokine and prostaglandin production by choriodecidual tissue[10]. Use of these antiinflammatory cytokines for manipulating the physiologically protective mechanisms or defenses may possibly be the most effective method of preventing the onset of SPTL[11]. MMP function is modulated by cytokines. Cytokine activation of cells led to increased expression of MMP, and cytokines and their receptors can serve as substrates for MMP action. TNF α in conjunction with IFN-α induces apoptosis of trophoblasts, which led to weakening of the fetal membranes, especially the chorion, prior to rupture. Our use of the drug containing immune factor modulator amides was initiated to terminate the series of cytokine activation and reduce the number of activated MMP2 and 9, thereby reducing tissue inhibitor of matrix metalloprotein (TIMP) level in fetal membranes, increase expression of proapoptotic bax gene and p53 inhibitor, and enhance cytokine IL-10. Ribosomally synthesized antimicrobial peptides including bactericidal permeability increasing protein, a defensins, and β defensins help in prevention of infection. The drug enhances antiapoptotic BCL-2 protein and therefore possibly encourages resealing of membranes.

The most important factor studied was latency, i.e., the period to which the patient remained undelivered. Two peer-reviewed metaanalyses found that antibiotics increased the chance of prolonging pregnancy by 7 days[12]. In our study, in the case group of 26-30 weeks, pregnancy continued ≤6 weeks in 58% of the cases as against only 7% in control; 25% of them reached term. In the case group 31-36 weeks, both pregnancy continuation in weeks and remaining undelivered to term reached significantly higher proportions. Only antibiotics and steroids were not able to provide any significant improvement. In two separate studies the overall incidence of chorioamnionitis ranged from 4.2%[13] to 10.5%[14]. Occurrence of chorioamnionitis following PROM was found to be higher in hospitals catering to the low socioeconomic than affluent. In our study 6.52% of the cases developed chorioamnionitis, while 15% of the cases did so in the control group. In a series in Yale New Haven Hospital, 29.8% of the perinatal deaths before 36 weeks were caused by RDS due to hyaline membrane disease[15]. In our series in the group between 26 and 30 weeks, 42% of the cases had RDS as against 60% in controls. In the group between 31 and 36 weeks it was 6% in cases as against 11% in controls. Labor, intrapartum hypoxia, and blood pressure fluctuations are important factors associated with IVH and subarachnoid hemorrhage seen in the preterm infant[16]. In the group between 31 and 36 weeks, IVH was less in cases as against controls. Incidences of birth asphyxia, neonatal septicemia, hypothermia, hypoglycemia, and neonatal jaundice were all less in cases as against controls.

Conclusion

PROM is an obstetrical problem requiring high level of professional skill management for better outcome. Corticosteroids, tocolysis, and antibiotics have been used for a long time. More recent treatments have included lung surfactants, amnioinfusion, and fibrin sealing. Comprehending the basic pathology at the molecular level, use of antiinflammatory cytokines to modify the physiologically protective mechanisms or defenses may be the most effective method of preventing the onset of spontaneous preterm labor in PROM. Hence the idea cropped up to terminate the series of cytokine activation and reduce the number of activated MMP. Till now it seems we have a good option in amnioseal, which is a combination of cytokine IL-10 enhancer, myristoleate in the esterified form (esterified carboxylate) with antimicrobial peptides, and other supportive factors tocopherol, glutathione, biotin, folic acid, vitamins B1, B2, B6, B12, C and arginine. It encourages resealing of torn membranes, torn edges merge faster and ensure safe containment of amniotic fluid and fetus. What is to be carefully monitored is whether these babies subsequently develop cognitive functional differences.

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