A REVIEW OF THE ETIOLOGY EPIDEMIOLOGY PREDICTION AND INTERVENTIONS OF PRETERM PREMATURE RUPTURE OF MEMBRANES.

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ABSTRACT

A REVIEW OF THE ETIOLOGY EPIDEMIOLOGY PREDICTION AND INTERVENTIONS OF PRETERM PREMATURE RUPTURE OF MEMBRANES.

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Preterm premature rupture of membranes (PPROM) is a clinical subtype of preterm delivery (PTD) with multi factorial etiology and itself a determinant of preterm delivery. Challenges in study of PPROM include variable case definition, outcome misclassification due to inaccurate identification of the initiating event, heterogeneity of pathways leading to PPROM and inconsistent evidence in assessing risk factors. Neonatal outcomes following preterm premature rupture of membranes vary depending on gestational age and latency. Preterm premature rupture of membranes has been described as a complex auto toxic condition and its pathogenesis involves the activation and interaction of the cytokines, matrix metalloproteinases and the apoptosis pathways. Genetic variation and behavioral and environmental risk factors can add complexities to understanding these pathways. PPROM is the strongest predictor of preterm delivery but PPROM prediction has been a challenging issue. A combination of factors, short cervix, previous preterm delivery due to PPROM, and presence of fetal fibronectin seem to be the strongest predictors of PPROM at less than 37 and less than 35 weeks gestation. Intra amniotic infection as indicated by elevated cytokine levels in vaginal fluids also seems to predict PPROM with good sensitivity and modest specificity. Interventional studies to prevent PPROM have largely been unsuccessful; specifically antibiotic trials in women with bacterial vaginosis have not yielded satisfactory results.

This document is dedicated to my lifelong teachers my mother Dr Shalini Thombre, father Dr Kishor Thombre, brother Milind Thombre, my children Kaivalya Kulkarni and Bhargava Kulkarni; and my husband Dr Rajesh Kulkarni for his support and encouragement.

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Chapter 1 Background

Preterm deliveries (PTD) are currently defined as those that occur at less than 37 completed weeks of gestational age; however the lower cutoff that distinguishes preterm deliveries from spontaneous abortions lacks a consistent definition.[1] A lower cutoff of 16 weeks has been proposed and justified based on the observation that the etiological risk factors for delivery at 14-23 weeks do not seem to differ from those at 20-25 weeks.[2] Moreover women who miscarry in a previous pregnancy during second trimester also have increased risk for subsequent preterm delivery.[3] On the other hand introducing the lower cutoff at 16 weeks and an upper cutoff of 39 weeks will likely overestimate the preterm delivery rates compared to the current rates. The preterm delivery rates in the United States have increased steadily, from 9.5% in 1981 to 12.7% in 2007. [4] The overall preterm delivery rate increased by 14% among Caucasian White and decreased by 15% among African Americans from 1989 to 2000. [5] This increase in preterm delivery in Caucasian White compared to African American seems to be driven mostly by medically indicated preterm deliveries (MIPTD).[5] Temporal trends in preterm delivery at the population level have been affected by changes in the assessment of gestational age from assessments based largely on last menstrual period to those based on early ultrasound.[6] In fact there is some evidence to suggest that gestational age assessment may have differentially improved in African Americans compared with Caucasian whites especially at 28-31 weeks of gestation.[7] In 2009 the preterm delivery rate fell for the first time in decades and was reported to be 12.18% [8] which was lower than the 12.7% reported in 2007.[4] The preterm delivery rate in the United States is higher than other developed countries in Europe where it ranges from 5-11%. [9] Preterm delivery rates are increasing, mainly because of increases in multiple births, but also among singleton births. [10,11] However more research needs to be directed to understand the mechanisms by which the risk factors are related to the preterm delivery subtypes, and the disparate etiologic heterogeneity of clinical subtypes

of preterm delivery. [1,12] Insight into these factors will enable us to implement specific prevention programs and target interventions, towards the high risk subgroups.

Preterm delivery can be subdivided depending on gestational age at delivery. PTDs occurring before 28 weeks of gestation are called extreme prematurity (about 5% of PTDs), 28-31 weeks of gestation called severe prematurity (about 15% of preterm deliveries), 32-33 weeks of gestation called moderate prematurity (about 20% of preterm deliveries), and 34-36 weeks gestation called near term (about 60-70% of preterm deliveries). [1] Another way of classifying preterm deliveries is based on clinical subtypes of preterm delivery; a) Delivery for maternal and fetal indications in which labor is either induced or the neonate is delivered by pre labor caesarean section; Medically Indicated Preterm Delivery (MIPTD); b) Spontaneous preterm labor with intact membranes (SPTL), and; c) Preterm premature rupture of membranes (PPROM). Deliveries that follow spontaneous preterm labor and preterm premature rupture of membranes are grouped together as spontaneous preterm deliveries (SPTD). The stratification of PTD based on these subtypes is controversial, however if risk factors do in fact differ [14] then strategies for prevention will vary accordingly.

Preterm premature rupture of membranes complicates about 3% of pregnancies and is associated with about one third of preterm deliveries. [7,15] Among preterm singleton deliveries in the United States, currently about 69% are the result of spontaneous preterm delivery with intact membranes or PPROM, while 31% are due to medically indicated preterm deliveries.[5] Among Caucasian women preterm delivery rates following ruptured membranes declined by 23%, and among African American women preterm delivery following ruptured membranes declined by 37% from 1989 to 2000. [5] Medically indicated PTD increased by 55% in Caucasians and by 32% in African Americans during the same period. It is possible that an increase in PTD due to one subtype might decrease incidence of PTD due to another subtype.

In this thesis we consider what we know to date about the epidemiology, clinical significance, prediction, and interventions for PPROM.

Chapter 2 Diagnosis of PPROM

PPROM is usually clinically diagnosed by a history of watery vaginal discharge and confirmed on sterile speculum examination. Diagnosis of PPROM relies on the clinician's ability to document three clinical signs i) visual pooling of clear fluid in the posterior fornix of the vagina or leakage of fluid from the cervical os; ii) an alkaline ph of the cervicovaginal discharge demonstrated by the yellow nitrazine paper turning blue (nitrazine test); iii) microscopic ferning of the cervicovaginal discharge on drying. [16] Conventionally tests such as the nitrazine tests and ferning tests have been used to diagnose PPROM. The fern test refers to microscopic crystallization of amniotic fluid on drying and may give false positive results due to finger prints or contamination with semen and cervical mucus and false negative results due to technical error or contamination with blood. [17, 18] Reported sensitivity and specificity for the fern test are 51% and 70% respectively in patients without labor and 98% and 88% respectively in patients with labor. [19] These tests become progressively less accurate when more than one hour has elapsed after membrane rupture. Nitrazine test is associated with high false positive rates when cervicitis, vaginitis (bacterial vaginosis) and contamination with blood urine, semen and/or antiseptic agents. [20, 22, 26] Early and accurate diagnosis of PPROM would allow for gestational age specific obstetric interventions designed to minimize serious complications and optimize perinatal outcomes. Conversely a false positive diagnosis of PPROM may lead to unnecessary obstetric interventions including hospitalization, administration of antibiotic, corticosteroids and potentially induction of labor. [23, 24] Timely and accurate diagnosis of PPROM is critical to optimize pregnancy outcomes, so critical that amnio-dye test may be recommended if conventional tests for PPROM are equivocal and pregnancy is remote from term. This test involves amniocentesis and installation of dye (indigo caramine) into the amniotic cavity. Leakage of blue stained fluid into the vagina within 20 -30 minutes as evidenced by staining of tampon is regarded as definitive diagnosis

of PPROM. Although considered by many investigators as the gold standard test the amnio dye test is an invasive procedure. [25] A non-invasive test that determines the presence of amniotic fluid protein, the placental alpha microglobulin-1 (PAMG-1),that is abundant in the amniotic fluid (2000-25,000ng/mL), but is present in far lower concentrations in the maternal blood (5-25 ng/mL) and cervicovaginal secretions in the absence of membrane rupture (0.05-0.2ng/mL), is now being used widely in the United States. The minimum detection threshold of AmniSure immunoassay is 5ng/mL, which should be sufficiently sensitive to detect preterm PROM with an accuracy of approximately 99%. [26] In a prospective observational study of 184 consecutive patients presenting with PROM Lee et all [27] demonstrated that the PAMG-1 immunoassay confirmed rupture of membranes at initial presentation with sensitivity of 99% specificity of 88%, positive predictive value of 98% and negative predictive value of 91%. This test was reliable across a wide gestational age range (11-42) and performed better than both conventional combined clinical tests and nitrazine test alone in confirming the diagnosis of PPROM.

Chapter 3 Adverse perinatal outcomes associated with PPROM…

3.1 PPROM a predictor of preterm delivery

About 20-40% of cases of ruptured membranes occur before 37 weeks. PPROM is itself a predictor of preterm delivery, and hence associated with short and long term maternal and neonatal outcomes of PTD.

3.2 Determinants of neonatal outcomes from PPROM

a. Latency period or gestational age at rupture associated with neonatal outcomes

Latency period is the period between membrane rupture and delivery. This period is an important clinical consideration specific to PPROM. In women with PPROM before 34 weeks, 50-60% of those conservatively managed will deliver within one week. (28) Latency period, is inversely related to the gestational age thereby increasing the risks of oligohydraamnios (amniotic fluid index ≤ 5 cm) and subsequent consequences associated with oligohydraamnios. (29) Prolonged latency also increases the risks of ascending infection in very premature infants and their mothers. The frequency and severity of maternal and fetal complications after premature rupture of fetal membranes varies with the gestational age at rupture and delivery. Depending on when in gestation membranes rupture the decision to prolong latency or deliver may be influenced by potential neonatal outcomes. There is consistent evidence that gestational age at preterm rupture of membranes and latency period are important independent determinants of perinatal death. (30) However there are conflicting studies about specific neonatal outcomes associated with latency period.

A retrospective cohort study (31) assessed if prolonged latency worsens perinatal outcomes (perinatal outcomes examined were grade III/IV intraventricular hemorrhage, periventricular leukomalacia, bronchopulmonary dysplasia). The primary neonatal outcome assessed was perinatal survival without major morbidity. The study concluded that in pregnancies complicated by PPROM latency does not appear to worsen

neonatal outcomes. The presence of perinatal morbidity was strongly correlated with the degree of prematurity, rather than the duration of the latency period. If gestational age at PPROM and not delivery is significantly associated with white matter damage [32] or adverse neonatal outcomes, delayed delivery may not change the prognosis for neurologic morbidity in the PPROM group. This may be an important finding with direct clinical application. If gestational age at rupture is the primary determinant of neonatal outcomes, then prolonging latency may not have a significant impact on those outcomes.

b. Etiologic determinants of PPROM or gestational age at rupture associated with neonatal outcomes

We seem to have an incomplete understanding of the etiologic determinants of adverse neuro developmental outcomes in infants born after PPROM. We lack specific sensitive markers to identify pregnancies at high risk for these outcomes. Cytokines have been implicated in long term adverse neonatal outcomes. The most prominent risk factors identified for white matter injury in the neonate are inflammatory conditions and cytokine exposure. [33] Whether the neonatal outcomes are determined by gestational age or by etiologic determinants of PPROM needs to be teased out.

c. Neonates born after PPROM different from neonates born after preterm labor

Preterm neonates born after PPROM may be different from neonates born after SPTL. Gestational age at delivery seems to be the primary determinant of neurologic outcome in the SPTL subgroup, while gestational age at membrane rupture is the main determinant in the PPROM group, suggesting that white matter damage may have different causative processes and those involved in PPROM may be different from those involved in other causes of prematurity. A prospective study of infants born after PPROM and infants born after SPTL with intact membranes was conducted to evaluate the impact of PPROM on the neuro developmental outcome of infants, as assessed at two years of age. [34] After adjusting for gestational age and birth weight infants born

after PPROM were more likely to have severe neuro developmental impairment (spastic tetraplegia and /or Bayley mental developmental index < 71) than the SPTL group (aOR 5.75 95%CI 1.22, 27.18).

Pulmonary hypoplasia is an important complication of PPROM occurring at less than 29 weeks of gestation. [35] Pulmonary hypoplasia seems to be associated with gestational age at the time of rupture of membranes, duration of latency and oligohydraamnios during latency. [29] Winn et al demonstrated that neonatal pulmonary hypoplasia varies with the latency period and amount of amniotic fluid in addition to gestational age at rupture of amniotic membranes. [30] Earlier the event of premature rupture of membranes occurs in the midtrimester, the greater the likelihood that pulmonary hypoplasia will occur. The more severe the oligohydraamnios or longer the latency period, the greater the impact will be on the development of pulmonary hypoplasia for earlier gestational ages. [30] Oligohydraamnios is frequently associated with premature rupture of membranes and is a risk factor for the subsequent development of clinical chorioamnionitis and neonatal sepsis. [36] Oligohydraamnios in women with PPROM is associated with an inflammatory response in the fetal, amniotic, and maternal compartments, [37] which can manifest as infection in the intrauterine environment, fetal joint contractures, and fetal pulmonary hypoplasia. [29]

3.3 Maternal morbidities associated with PPROM

Certain maternal morbidities have been reported in association with PPROM. Pregnancies complicated by PPROM that are managed expectantly are at a significant risk for placental abruption. [38] Preterm premature rupture of membranes in such cases is preceded by bleeding. [38]

The incidence of intrauterine infection increases with decreasing gestational age at membrane rupture. [39] Rupture of membranes at earlier gestational ages has been associated with infection in the chorioamnion. Chorioamnionitis has been reported in

0.5% to 71% of pregnancies with PPROM. [35, 40] The highest incidence of chorioamnionitis is associated with decreasing gestational age and prolonged latency. Conclusion: PPROM itself is a leading cause of PTD, and hence subject to neonatal outcomes of PTD. Latency and oligohydraamnios complicates PPROM even further leading to adverse maternal and neonatal outcomes. Loss of amniotic fluid due to fetal membrane rupture has important fetal and maternal consequences like maternal and fetal infection, incomplete fetal lung maturation, and fetal malformations. It is not clear whether expectant management or delivery of the neonate is a better choice, and will probably depend on the gestational age of rupture and maternal and fetal morbidities associated with PPROM. Pro inflammatory cytokines have been implicated in adverse neonatal outcomes and in PPROM however the evidence is inconsistent.

Chapter 4 Challenges in study of PPROM

4.1 Case definition and misclassification of PPROM

Spontaneous rupture of membranes is a normal component in the course of labor and delivery. Rupture of membranes that occurs before the onset of labor at term can be defined as premature rupture of membranes (PROM). Membrane rupture that occurs (>2hrs) before the onset of labor at a gestational age of less than 37 weeks has been defined as preterm premature rupture of membranes (PPROM). This definition is not very consistently used in the literature and latency period of less than two hours may also be classified as PPROM since membrane rupture was the initiating event. Inconsistent and interchangeable use of either initiating event or duration of latency in the case definition of PPROM has led to some misclassification of cases in the literature. Which definition is more “accurate” is not clear but most studies have used the initiating event as the case definition, along with certain diagnostic tests such as ferning to identify membrane rupture.

There is a potential for misclassification of preterm delivery subtypes in epidemiological studies since all three delivery circumstances can occur in the same pregnancy and hence correctly identifying the triggering event may be difficult and may depend on factors such as self-report and access to medical care.

Some outcome misclassification can occur if contractions are missed or ignored, and as a consequence membrane rupture is defined as the initiating event. Outcome misclassification can also occur when membrane rupture is not apparent and diagnostic tests have high false negative or false positive results. Outcome misclassification has implications for understanding the etiology of the subtypes of preterm deliveries.

4.2 Heterogeneity of pathways

PPROM has a multi-factorial etiology and some of the causative factors are unknown. It has been proposed that since the major component of the fetal membranes that provides tensile strength is collagen, any alterations in collagen production, in terms of

quantity and quality, structure, imbalance in production and degradation of collagen, or increased collagenolytic activity, can alter the strength of fetal membranes leading to their rupture. [41] Fetal membrane rupture is likely to be a result of biochemical changes as well as physical forces. [42] Some connective tissue disorders in fetuses, like the Ehlers Danlos syndrome where the collagen content and structure are altered, are dramatic examples of preterm premature rupture of membranes. [43]

Since these pathways are assumed to operate at the maternal fetal interface it is helpful to first consider the physiological structure of the human fetal membranes.

a. Structure of fetal membranes

The human fetal membranes consist of the amnion and chorion connected by extracellular matrix (ECM). [44] This membranous layer, exclusively fetal in origin, surrounds the intrauterine cavity and provides the sac in which the fetal growth takes place. [45]

i. Amnion

The amnion is derived from the embryonic ectoderm and is made up of five distinct layers: epithelial layer, the basement membrane, the compact layer, the fibroblast layer, and the intermediate layer or the spongy layer. [45, 46] Amnion is avascular and devoid of nerves, and is in direct contact with the amniotic fluid, from which it derives its nourishment. [46] The layer in proximity with the amniotic fluid is the amniotic epithelium which secretes the collagen types III and IV and non-collagenous glycoproteins (laminins, nidogen, and fibronectin) that form the next layer, the basement membrane. [46, 48] The compact layer forms the main fibrous skeleton of the amnion, with the interstitial collagens (types I and III) predominating. [47, 48] The fibroblast layer is the thickest and consists of mesenchymal cells and macrophages within an extracellular matrix. [48] The intermediate layer (spongy layer) lies between the amnion and the chorion, contains abundant proteoglycans and glycoproteins, and contains a nonfibrillar meshwork of mostly type III collagen. [48]

ii. Chorion

The chorion is four times the thickness of the amnion but the amnion has greater tensile strength than the chorion. [49] The chorion laeve is formed from the implanted blastocyst at the pole towards the endometrial cavity that is covered by chorion fondosum and deciduas capsularis. [50] During the course of the pregnancy the blood supply to this area becomes restricted and the villi degenerate, forming the avascular chorion. [48]

iii. Chorioamnion

At three months gestation the amnion and the chorion fuse to form the chorioamnion. [51] By midterm pregnancy the uterus supports the fetal membranes, which completely line the uterine cavity. [51]

iv. Extracellular matrix

The extracellular matrix (ECM) is composed of fibrous proteins, which confer both strength and elasticity to the membranes. [48] These proteins are embedded in a polysaccharide gel and form the architectural framework of the chorioamnion. [48] Collagens form the major structural framework of the fetal membrane ECM. [48-50, 52-55] The strength of the membrane is dependent on the type of collagen that makes the ECM. [54] The major tensile strength is provided by collagen types I and III together with small amounts of types V, VI, VII. [48] The basement membrane is made up of type IV collagen, and it provides the scaffold for the assembly for other non-collagen structural proteins (laminin, entacin and proteoglycan), and plays a major role in development and maintenance of the ECM. [54] The ECM collagens undergo constant turnover and remodeling throughout the entire pregnancy to accommodate and adjust for the growing volume and tension as gestation progresses. [47] During the last eight weeks this remodeling process results in decreased collagen content of the amnion. [47]

b. Mechanisms implicated in the pathogenesis of PPROM

Most of the times in the process of parturition, labor is followed by rupture of membranes and delivery. However sometimes the process may begin with rupture of membranes, and progress to labor and delivery. If this process happens at term the implications may not be too severe however if membranes rupture before 37 weeks preterm delivery is imminent. There is some evidence that the mechanisms of term and preterm rupture may be different.

i. Relaxin

Mechanisms for rupture of membranes may be different in preterm versus term deliveries. It has been shown by investigators that some signaling events at the choriodecidual interface may differ in term and preterm premature rupture of membranes, and hence preterm premature rupture of membranes could be a pathological moiety of the term counterpart. [56] Relaxin is a collagenolytic peptide hormone that is produced by the corpus luteum and placenta during pregnancy in response to stimulation by human gonadotropin (hCG). [57] Relaxin is widely accepted as a hormone involved in uterine growth and development, myometrial contractility and cervical ripening. Relaxin causes increased production of matrix metalloproteinases (MMP's) and pro-inflammatory cytokines, and hence has been implicated in PPROM rather than preterm delivery due to preterm labor. Risk of PPROM has been related to increased levels of relaxin [58] in the placenta. In fetal membranes obtained from twelve elective C-sections before the onset of labor, human relaxin (hRLX-2) in vitro has shown to cause a 30% decline in tensile strength of the fetal membranes. [59] The expression of the two human relaxin genes was quantitated in the deciduas and placentas, of subjects. [58] This study was able to show that significantly more relaxin was expressed in the preterm deciduas from patients with PPROM when compared to patients with preterm labor or patients with c-sections for medical reasons with intact membranes and no preterm labor. [58] Relaxin was shown to have different mechanism in term and

preterm membrane rupture. Placental tissues after PPROM had a significantly higher and a uniform over expression of relaxin in the placental syncytiotrophoblast. A similar group of tissues collected at term failed to show any significant differences in relaxin expression [58] which suggests that relaxin is involved in the pathology of preterm premature rupture of fetal membranes but not in their normal rupture at term. Over expression of relaxin was shown to be independent of chorioamnionitis [60] in PPROM, which suggests a mechanism other than one associated with the infection pathway. However the different modes of action of relaxin at term and preterm have not been replicated. If the mechanisms at term and preterm membrane rupture are different then identifying triggering events will open up options for interventions and prevention of PPROM.

ii. Infection and maternal response to infection in PPROM

Microorganisms can gain access to the amniotic cavity by; ascending from the vagina and cervix; hematogenous dissemination through the placenta; accidental introduction of microorganisms at the time of procedure, and by retrograde spread through the fallopian tubes. [1] The likelihood that infection is present in the fetal compartment is inversely related to gestational age. [61, 62]The most common pathway is believed to be the ascending pathway. The timing of ascent of these pathogenic microorganisms seems to be yet unknown, but most investigators believe it happens during the second trimester, though some women may have asymptomatic endometrial colonization prior to pregnancy.[63] Most investigators now believe that the rate of microbial colonization is higher in the chorioamnion than in the amniotic cavity [64] and hence trying to estimate the rate of infection based on amniotic fluid culture might underestimate the magnitude of association. Subclinical intrauterine infection has also been implicated as a major etiologic factor in the pathogenesis of PPROM. [65-69] Intra-uterine infection has been implicated as a major etiologic factor in the pathogenesis and subsequent long term morbidity associated with PPROM. [70] When microorganisms and their

products gain access to the fetus they stimulate the production of cytokines and a systemic fetal inflammatory response syndrome (FIRS). [70] Microbial byproducts and maternal response to infection have also been associated with PPROM. Some mechanism of action of infection can be attributed to the influence of bacterial collagenases, and matrix degrading enzymes produced by bacteria. [71] These enzymes have been shown in vitro studies to significantly reduce the tensile strength and elasticity of the membranes, in a dose dependent manner leading to their rupture. [72] The controls for this study were non-collagenase producing organisms. PPROM has also been studied in women with intra amniotic infection due to organisms that are not known to produce proteases. [71] However antibacterial therapy has failed to show any significant results on reducing incidence of PPROM.

Apart from the action of bacterial enzymes and byproducts on the fetal membranes maternal response (to infection) in the form of maternal cytokines has also been implicated in the pathophysiological mechanisms of preterm labor complicated by PROM. Maternal serum cytokines concentrations of IL-1α, and IL-1β in women with premature labor with PPROM were significantly higher than in women in labor and PROM at term. [73] Increasing evidence suggests that unexplained cases of idiopathic preterm birth may have an underlying immune etiology.[74] Aberrant natural killer (NK) cell activation is associated with recurrent pregnancy loss. [75-77] In addition complement activation, improper regulation of the complement system [78] has also been demonstrated to lead to pregnancy loss.[79] A common factor in all of these appear to be the production of pro inflammatory cytokines such as TNF, IL-1β,and IFN-γ, which appear to be critical mediators in the induction of pregnancy loss at all stages of gestation. [80-82] Midgestation loss appears to be associated with cytokine dominated mechanism. [83] Many of these host anti and pro inflammatory processes have been associated with early and later pregnancy loss, though not necessarily with PPROM. Tumor necrosis factor alpha (TNF- α) is a pro inflammatory cytokine with multiple roles

in the immune response. TNF- α is involved in remodeling the cervix and fetal membranes by promoting production of collagen degrading matrix metalloproteinases , including MMP- 1 and MMP-9.[84] TNF- α under physiologic conditions induces trophoblast differentiation, invasion and adhesion, implantation, placental development, fetal membrane growth and remodeling.[85] Alterations in TNF- α functions can trigger adverse events like endocrine function inhibition, protease activation, and extracellular matrix degradation resulting in termination of pregnancy if it occurs early in pregnancy, or PPROM if misregulation occurs later in pregnancy.[85]

Subclinical infection manifested as vaginal bleeding during pregnancy could be a pathway to PPROM. Vaginal bleeding and consequent thrombin generation leads to a proteolytic cascade, and enhances decidual MMP-3 expression, which is capable of damaging the fetal membranes, leading to PPROM. [74]

iii. Metalloproteinases and their role in collagen degradation

In pregnancy fetal membranes undergo programmed collagenolytic remodeling [86, 87, 41] which is mediated by MMPs and each of these MMPs degrade specific substrate. Metalloproteinases use zinc-dependent catalysis to degrade extracellular matrix components. Zinc deficiency significantly increases the activity of MMPs and causes a reduced collagen type I/III ratio, and delayed cell proliferation and wound healing.[88] MMP-9 is believed to be a terminal enzyme in remodeling the extracellular matrix. In contrast to many metalloproteinases the production and release of MMP-9 can be induced under specific conditions.[89] MMP activity is regulated by control of transcription, translation, at the post translational level, and also at the tissue level by tissue inhibitors of metalloproteinases (TIMP's).[90] A balanced activity between MMP and TIMPs has been documented in the tissue remodeling process.[91] The decrease in TIMPs during labor may permit increased breakdown of extracellular matrix in the fetal membranes and deciduas at parturition, thus altering cell signaling at the feto-maternal interface and facilitating membrane rupture.[92]

Increased collagenolysis and a drop in collagen content of the membranes along with activation of the family of MMPs have been documented in PPROM. [93] A full system of MMPs, TIMPs, MMP activators, and regulatory elements are present in the fetal membranes. [92] Various MMP's have been investigated and implicated in PPROM. Most of the active forms of the MMPs (MMP 2, 8, 9) are increased in membranes that have sustained premature rupture. [94] MMP-9 expression is induced in epithelial cells, monocytes, and macrophages [95] by pro inflammatory cytokines and bacterial endotoxins. The median fetal plasma levels of MMP-9 were significantly higher (P=0.035) in fetuses with PPROM and differentiated fetuses with PPROM from those undergoing premature labor with intact membranes. [96] Excessive stretching of fetal membranes as seen in multiple fetuses is associated with increased IL-8 and MMP expression [97] which may lead to PPROM.

The rupture of membranes either preterm or at term was associated with a significant increase in the concentration of the active forms of MMP-9. [98] Significant decrease in the active forms of MMP-2 have been implicated in PPROM [98] while some investigators reported that active forms of MMP-2 in amniotic fluid are increased in PPROM. [99] The increase in MMP2 levels is associated with decrease in levels of the natural inhibitor and activator of MMP2, TIMP-2, in the amniotic fluid during premature rupture of fetal membranes. [99] This study had a smaller number of participants. Collection of sample (amniotic fluid) in the preterm premature ruptured group varied since samples were collected after delivery and women were treated with antibiotics to prolong the latency period. The TIMP's seem to have a threshold for the inhibiting function, low amounts of TIMP-2 seems to actually cause activation of MMP-2 but are not sufficient for inhibition. [100] The interaction and ratio of MMP's and the active forms present in amniotic fluid might also play a role in the rupture of membranes. The inconsistency in implicating MMP2 could also be due to the inability of these studies to measure MMP2 in active form, and even if levels of MMP2 were raised in amniotic fluid,

the active forms (of MMP2) may not be sufficient to cause membrane rupture. It may be important to distinguish between active forms of the MMPs as they may be more accurate markers than MMP concentrations alone.

iv. Genes and their role in PPROM

Induction of MMP2 may be a function of p53 gene expression increase in PROM. [71] The genatolytic activity of latent and active forms of MMP-9 is increased and the concentration of TIMP reduced in amniotic fluid of women with PROM and PPROM. [94] MMP-9 gene is induced in amniochorion during labor, PROM, and infection. [94] MMPs are also initiated by genotoxic agents, or other unknown factors. [71] Deoxyribonucleic acid fragmentation was associated with elevations in the levels of the two pro apoptotic gene products (p53 and bax) and a drop in the level of the antiapoptotic bcl-2, in PROM [71] Fetal membrane infection induces many of the apoptotic pathway genes in vitro. [66] The process of programmed cell death or apoptosis has been demonstrated to play a role in PPROM. The two major apoptotic pathways that play a role in PPROM are the Tumor necrosis factor (TNF) receptor (TNFRI) and Fas mediated pathway [99] and the p53 pathway. [71] PPROM is associated with an excess in frequency of an allele of the gene for tumor necrosis factor that increases the production of tumor necrosis factor in response to a microbial challenge, and this relationship is stronger in black women as compared to white women. [101, 102] Genetic and environmental risk factors seem to act together to increase risk for PPROM. The pathway(s) leading to the outcome if correctly mapped may have applications for interventions.

v. Reactive oxygen species

Reactive oxygen species (ROS) are unstable molecules generated in the body, which are being proposed to be responsible for damage to the chorioamniotic sac leading to their rupture. [103] In vitro studies have shown that collagen in several tissues is the primary target for ROS. [104] Normally a balance exists between production and elimination of ROS. Oxidative stress (OS) occurs when per oxidants exceeds anti-oxidants. [103] Isoprostanes (F2 IPs) are produced by ROS attack on polyunsaturated fatty acids and are sensitive and specific biomarkers of lipid per oxidation in vivo. [105] In a study by Longini et al [103] Isoprostanes (F2 IPs) were measured in amniotic fluid and they concluded that an association exists between oxidative stress and PPROM. OS caused by ROS can modify the strength and elasticity of the collagen in fetal membranes and lead to PPROM. [104, 106] Studies linking maternal smoking [107] and substance abuse, [108] infections, [104] ante-partum bleeding [104] are known to produce ROS or reduce antioxidant protection which has been hypothesized to lead to collagenolysis of the fetal membranes.

Cigarette smoke contains superoxide, hydrogen peroxide, hydroxyl ions and nitric oxide which could damage the collagen matrix or consume the anti-oxidant defenses. [109 110] Smoking has been shown to inhibit anti protease activity. [111]

Vaginal bleeding has been implicated in the etiology and pathogenesis of PPROM [14,112-116] Vaginal bleeding has been linked with PPROM as a result of release of free iron from red blood cells due to ruptured vessels and bleeding. Increased free iron has been hypothesized to catalyze the conversion of hydrogen peroxide to hydroxyl ions. Blood adjacent to the chorioamnion has been hypothesized as being a medium for subclinical microbial growth. [108]

The association of infection with PPROM has been well established. [66, 68, 69] ROS produced in the activated neutrophils could breakdown the amnion, basement membrane or the underlying collagen fibrils by releasing proteinases, which degrade

collagen and structural proteins such as fibronectin, laminin and proteoglycan. [111] Collagen is the only protein that is susceptible to fragmentation by superoxide. [109] Cocaine abuse and its relationship to PPROM are believed to be a result from ROS generated by cocaine induced ischemia and then reperfusion. [108] Maybe prospective clinical trials with antioxidant supplementation will determine if there is any beneficial effect of the antioxidant on the fetal membranes.

In summary PPROM has been described as a complex autotoxic disease and its pathogenesis involves the activation and interaction of the cytokines, MMPs and the apoptosis pathways. [94] Genetic variations and behavioral and environmental risk factors can add complexities to understanding these pathways. Relaxin seems to be involved in preterm rupture but not in term rupture of membranes independent of infection. Reactive oxygen species have been shown to induce membrane rupture in in-vitro studies. Membrane stretch has also been implicated in degrading the membrane via the IL-8 pathway and MMP pathways. The mechanism of action of infection seems to be due to the influence of bacterial collagenases and matrix degrading enzymes [71] among other factors. These enzymes have been shown in in-vitro studies to significantly reduce the tensile strength and elasticity of the membranes, in a dose dependent manner, leading to their rupture. [72] It has been shown that the amniochorionic membranes are the site of inflammatory cytokine production, [117] in response to infection. Thus infection, inflammation and host response is associated with PPROM.

4.3 Variable evidence in understanding risk factors

a. Demographic

i. Age

In a study by Berkowitz et al [14] maternal age of 35 or more has been associated with increased risk (aOR =1.5, 95%CI 1.3, 1.8) of PPROM, whereas younger women <20 years of age were at an increased risk for preterm labor. This study was able to show a trend towards increasing risk for PPROM as age increases, (adjusted OR = 1.4, 95%CI

=1.2-1.6), for women aged 30-34 years. This difference might give us some insight into the different subgroups affected by PTD subtypes. Another study [118] confirmed the positive finding of increased maternal age and PPROM, though they did not achieve statistical significance. This study by Pickett et al noted that women 35 years and older were at an increased risk for PPROM,(OR= 1.44, 95% CI 0.98,2.12) when compared to women aged 18-34 years. Data from the RADIUS study showed that maternal age greater than 30 years was associated with PPROM. [119] Increasing maternal age shows a consistent relationship or trend to be associated with preterm premature rupture of membranes.

ii. Race

A population based cohort study [120] using Missouri Department of health's maternally linked birth certificate database assessed racial effect on the occurrence and recurrence of PPROM while adjusting for socioeconomic and medical risk factors. Their results concluded that black mothers were more likely to have PPROM compared to white mothers (a OR = 2.3, 95% CI 2.0, 2.5). The magnitude of this risk was greatest at <28 weeks of gestation. Black mothers were also at a significantly increased risk of recurrence of PPROM compared to white mothers. Additionally the investigators identified a subgroup of women positive for indicators of low SES. Among these women black women had a 2.44 –fold increase in risk of PPROM compared to white women (RR =2.44, 95% CI 2.28, 2.61).The authors suggest that these findings highlight the different mechanisms associated with race and suggest a possibility of both genetic and environmental components in the pathogenesis of preterm delivery due to PPROM. Black women compared to white women, are at a significantly increased risk for PPROM as reported by Pickett et al [118] the adjusted OR =1.74 and 95% CI 1.23, 2.48, compared to 1.45 for idiopathic preterm labor, though the magnitude of association is modest. Another study [14] demonstrated an increased risk for PPROM in black women (not statistically significant) as compared to white women and Hispanic women

(aOR=1.9, 95% CI 1.5, 2.3). Some studies that have shown higher rates of preterm delivery among black women are partially explained by higher frequencies of vaginal infections in black women. [121] It is possible that differences in vaginal infection rates are responsible for the higher frequencies of PPROM in black women.

iii. Socioeconomic status

A case control study was done to estimate whether dietary or socioeconomic factors were associated with PPROM. [122] There was a significant difference between women with PPROM and their matched controls for total family income and education when using univariate analysis. The crude ORs for women with PPROM and family income <$25,000 was 6.94. However the adjusted OR was attenuated to 3.1 (95%CI 1.6 6.0). A case control study by Spinillo et al [115] concluded that low social class of the mother was an independent strong predictor of preterm PROM especially among nulliparous women. It is not clear how lower socioeconomic status predisposes women to PPROM it could be lack of access to care, different sexual hygiene habits, physical activity, or other factors associated with lower socioeconomic status like stress, depression, poor general health, or nutritional deficiencies associated with unhealthy lifestyles.

b. Obstetric

i. Role of infection, inflammation

Epidemiological studies demonstrate an association between colonization of the genital tract by group B Streptococcus, Neisseria gonorrhea, and microorganisms responsible for bacterial vaginosis, with PPROM [136]. In a case control study by Ekwo et al [136] the odds of preterm PROM were 6.0 times that of controls among women with intra-amniotic infection, 3.7 times among those with urinary tract infections, and 7.6 times among women with gonorrhea infections after controlling for effects of exposure to cigarette smoke, having previous preterm and full term PROM deliveries and ante partum bleeding. In a retrospective case-control study of high risk population of inner city women Kilpatrick et al [133] concluded that no infectious risk factors distinguished

control women from women with PPROM. Many investigators now believe that bacterial infection may be the initiator while the host response may be the culprit implicated in PPROM. [73, 124] Menon et al have reported that the amniochorionic membranes are the site of inflammatory cytokine production. [117] In a quest to identify markers of inflammation, a study by Simhan et al was able to show a significant association between the presence of vaginal neutrophil counts of >5 per high powered, and vaginal pH >= 5 with PPROM at 24 to 32 weeks, when compared with that among women with PPROM at 32 to 36 weeks and women without PPROM. [125] They also reported that women with sexually transmitted diseases had elevated vaginal pH and neutrophils more frequently than their counterparts without these infections. [125]

Bacterial Vaginosis, a condition in which the microbial flora of the vaginal tract is altered, is a risk factor for PPROM. [126, 127] Bacterial Vaginosis (BV) is asymptomatic in approximately 50% of patients. [128, 129] Kurki et al noted a 7.3 –fold increased risk of PPROM with early culture proved bacterial vaginosis compared with culture negative controls. [130] The preterm prediction study [123] was able to show a modest 2.1 fold increased relative risk (95% CI 1.1 to 7.5) for PPROM < 37 weeks with BV in nulliparous women as compared to multi parous women. A study from India reported that the incidence in the bacterial vaginosis group was 8.69% compared to 0.73% in bacterial vaginosis negative group. [131]

Chlamydia has also been shown to increase risk for PPROM by multiple authors. [126, 127] Ekwo et al noted a fivefold increased risk of PPROM with Chlamydia infections. [136] On Another case control study of high risk population of inner city women were unable to find an association of Chlamydia, or Gonorrhea with PPROM, and also the highest rate of a history of Chlamydia infection was actually in term controls. [133]

However trials have not been able to document reduced incidence of PPROM following treatment of BV. [134] Bacterial vaginosis is a condition of microbial imbalance. Various trials use various antibiotics by different routes of administration. Duration and dosage

of treatment is varied too. Later studies have noted that women who are at risk for preterm delivery may need both an environmental factor (for example BV) and a genetic predisposition to an inflammatory response that may lead to an infection associated preterm delivery [102,135] for which PPROM qualifies.

ii. History of PPROM or previous PTD

Many studies have consistently documented evidence for PPROM in index pregnancy in women with a previous preterm delivery and or PPROM. [14, 113, 120,123, 131, 132, 136, 137] A study by Harger et al reported after a multivariate logistic regression analysis that previous preterm delivery was an independent risk factor for PPROM with an odds ratio of 2.5 (95% CI 1.4, 2.5) compared to controls. [113] Berkowitz et al [14] also identified previous preterm delivery as a risk factor for PPROM in the index pregnancy, reporting adjusted odds ratio of 3.2 for PPROM. Mercer et al [123] reported among multiparous women in the Preterm Prediction Study a previous preterm birth with PPROM (OR 3.1, 95% CI 1.8, 5.4) and a spontaneous preterm birth (OR= 1.8 95% CI 1.1, 3.1) were both associated with subsequent preterm birth complicated by PPROM. A recent study by Shen et al [120] demonstrated that black mothers with a history of PPROM had a 3.68-fold (aOR= 3.68, 95% CI 2.07, 6.55) greater risk for recurrence compared to white women with a history of PPROM. The risk of recurrent PPROM in women with a history of prior PPROM was greater than the risk of PPROM in women without a history of prior PPROM (aOR 10.12, 95% CI 8.00-12.81).

iii. Ante-partum vaginal bleeding

Many studies have reported bleeding during pregnancy and subsequent occurrence of PPROM. [14, 112-116] About half of bleeding episodes during pregnancy have unknown causes [138, 139] and thus the reason bleeding predicts PPROM and or PTB is unclear. Vaginal bleeding has different underlying causes [140] with different consequences for preterm delivery. [74] Yang et al [116] studied vaginal bleeding during pregnancy and reported that bleeding in the first trimester only was associated with PPROM (RR= 1.9

95% CI 1.1 3.3)**.** Significant heterogeneity exists between various study results. Vaginal bleeding during pregnancy is usually a self-reported measure in most studies. All studies did not report on all the necessary components of bleeding such as bleeding frequency, quantity and trimester, most studies asked if participants had any bleeding during pregnancy, and recorded a yes/no answer.

iv. Miscellaneous

Other factors like nulliparity have been associated with PPROM by some but not others. Medical conditions like preeclampsia were noted as one of the risk factors for PPROM [115] Anemia, has been cited by some investigators as a risk factor for PPROM. [113,115, 141]

A significant obstetric history of some conditions has been associated with PPROM. A study by Evaldson et al [142] found an increased risk for PPROM in women who had increased frequencies of previous genital operations, cervical operations and lacerations. However all these findings have been inconsistently reported.

Increased risk of PPROM in multifetal pregnancies has been attributed to uterine over distension creating stress in the fetal membranes leading to their rupture. A twins Study by Mercer et al [143] reported an incidence of 7.4% vs. 3.7% of PPROM in twin gestation vs. singleton gestation (OR= 2.1 95% CI 1.71, 2.58). Other factors such as coitus in late pregnancy[114] and obstetric complications such as abnormal umbilical cord insertions, [28] polyhydra-amnios, cervical incompetence [113,136] short cervix defined as less than 25 mm [123] and procedures like cerclage, [145] previous c-section [133] have been associated with increased risk of PPROM .

c. Behavioral

i. Nutritional status

Factors that alter collagen structure and interlinking architecture have been associated with PPROM. Nutritional deficiencies that affect collagen formation have been shown to alter collagen structure. [122] The strength of collagen is maintained through its cross-

links which are formed through a series of reactions which are mediated by lysyl oxidase [146] which is a copper dependent enzyme. Women with preterm PROM have been found to have lower copper levels than women in preterm labor. [147] The study by Kiilhoma [147] demonstrated statistically significant differences in the cord copper and ceruloplasmin and also their fetal/maternal ratios were significantly lower in the group with preterm PROM than in other groups. Statistically significant differences were noted in maternal copper levels in PPROM women (2.13±0.33 vs 2.37±0.27, $p<0.05$) versus control women.

Vitamin C is a cofactor for proline hydroxylation and is essential for the formation of the triple helix structure of collagen. [47] Studies have shown an association between low vitamin C levels and preterm PROM. [148] Subsequent randomized trial [149] has confirmed the importance of supplementation with 100mg Vitamin C after 20 weeks of gestation.

Similarly high levels of homocysteine have also been associated with abnormalities in collagen cross-linking. [150] Vitamin B12 and folate are important cofactors in homocysteine metabolism, with low levels of folate leading to elevated plasma homocysteine levels. However a case control study could not detect any difference between fasting homocysteine, red blood cell folate, vitamin B12 levels and dietary intake between women who experience PPROM and term deliveries. [122] Zinc deficiency has also been associated with PPROM. Kiilhoma [147] noted lower maternal zinc levels in PPROM women compared to controls. Scholl et al [151] were able to show that zinc deficiency was associated with the risk of PTD, particularly when rupture of the membranes preceded the onset of labor (aOR = 3.46, 95% CI 1.04 11.47).

ii. Smoking, illicit drug use

Some investigators have looked at the association between cigarette smoking and the incidence of PPROM, but not all were able to demonstrate a positive association. There seems to be evidence from both sides. Among the studies that were able to show a

positive association were studies by Kilpatrick et al [133] Harger et al [113] and Ekwo et al. [136] They demonstrated a twofold to fourfold increased risk for PPROM with self-reported current cigarette smoking. A large prospective study of 30,000 deliveries by Shiono et al, demonstrated a 40% increased risk for PPROM in patients who smoked more than one pack per day. [152] The dose response relationship between smoking and PPROM has been reported by other investigators like Ekwo et al [136] Iams et al [153] who have suggested that smoking more than 10 cigarettes per day is a risk factor for PPROM. In another case control study [115] the investigators were able to demonstrate a dose-response relationship between the numbers of cigarettes smoked daily (self-reported) in women with PPROM. Women with PPROM were more than four-fold (aOR = 4.41, 95% CI 1.63-11.9) likely to be smokers than non-smokers. A case control study by Williams et al [154] reported that after confounders had been adjusted for, women with PPROM were more than two-fold (adjusted OR= 2.2, 95% CI 1.4, 3.5) likely to have smoked throughout the pregnancy, were about one and half times (1.6 (95% CI 0.8, 2.9)) more likely to have smoked during the first trimester only when compared to women who had never smoked. However they were unable to show a gradient between the number of cigarettes smoked per day and the risk of preterm PROM. They did indicate that the smoking related risk of PPROM is potentially affected by the timing of cessation with continued smoking being of highest risk when compared to those who stopped smoking during the pregnancy. Berkowitz et al [14] reported that cigarette smoking and illicit drug use were related to PPROM (aOR= 2.4; 95% CI 1.8, 3.2). Pickett et al [118] demonstrated a statistically significant though modest magnitude of association between smoking and PPROM (OR=1.52; 95%CI 1.09, 2.13).

Large studies that were unable to report an association included the study by Naeye [155] of the Collaborative prenatal project of over 10,000 pregnancies noted statistically non-significant PPROM rates of 7% among smokers and 5% among non-smokers.

d. Anthropometric

i. Body mass index (BMI)

Several studies suggest that low pre pregnancy BMI may increase the risk for PPROM. [156,157] However a study by Rudra et al was unable to confirm that low or high BMI is associated with PPROM. [158] Siega-Riz et al [159] examined spontaneous PTD and PPROM separately and found that both were associated with pre- pregnancy maternal underweight status, though not overweight status. Spinillo et al [157] reported that PPROM was more strongly associated with low second to third trimester weight gain (<0.37 kg/week) among women with BMI<19.5 kg/m^2 versus heavier women. PPROM may be more strongly related to later weight gain in pregnancy, and lower pre pregnancy body mass index. Some investigators have suggested that BMI may be an indicator of nutritional status, although BMI fails to gives any specific information about maternal micronutrient status and bio-availability of these nutrients to the fetus.

e. Genetic

A strong predictor of PPROM is a prior preterm birth due to preterm premature rupture of membranes. [115,160] Both maternal and fetal genetic factors may influence adverse pregnancy outcomes such as PPROM. Evidence suggests that maternal genetic factors contribute to PPROM, [161,162] however fetal genetic factors have not been studied well. Various genes have been studied in the etiology of PTD; these include the genes associated with response to infection, genes involved with the inflammation process, and genes involved with matrix metabolism. Some studies have identified PPROM as their outcome and those are the specific studies listed below.

PPROM appears to aggregates in families. Plunkett et al [163] estimated the magnitude of familial aggregation as one index of possible heritable contributions. Two standard measures of familial aggregation are increase in risk to siblings of affected individuals compared to the population risk for the disorder, the sibling risk ratio, and compared to siblings of unaffected individuals, the sibling-sibling odds ratio. Risks to siblings of an

affected individual with PPROM was elevated above the population prevalence of PPROM and was indicated by λs= 8.2, 95% CI 6.5-9.9. Risks to siblings of an affected individual was similarly elevated above that of siblings of unaffected individuals, as indicated by the sib-sib OR (sib-sib OR adjusted for known risk factors) which was 9.6 and 95% CI 7.6-12.2. The investigators concluded that siblings of affected (by PPROM) individuals were at an increased risk for PPROM even after adjusting for important known environmental risk factors. The authors suggest that maternal and /or fetal genetic influences could account for some of the observed increased risk to siblings.

In a case control study by Ferrand et al [(164)] of neonates born to African American women presenting with PPROM, cases and controls were genotyped to determine the CA repeat sequence length in the MMP-9 promoter and for polymorphisms at –1562. No association was found for the -1562 polymorphism, but the 14 CA repeat was associated with a significantly increased PPROM risk compared with all other repeat sizes (OR= 3.06, 95% CI 1.77-5.27) and with two to three fold more MMP-9 expression than the 20 CA repeat size. The frequency of the 14 CA repeat in MMP-9 is lower in the African American population than in Caucasians 19% vs. 50%. [(164)]

The relationship of the TLR4 (Asp 299Gly) allele with preterm birth was examined in the Finnish population [(165)] The frequency of the TLR4 (Asp 299Gly) allele did not vary among the women, but there was a significant difference in the allele frequency between singleton term and preterm infants (P=0.024). The association of the allele was stronger with PPROM cases (p=0.021) then other causes of PTD (p=0.045).

Wang et all [(166)] conducted one of the few studies on fetal genotype in which they investigated the contribution of a functional SNP, in the promoter of the SERPINH1 gene, enriched among those of African ancestry, to PPROM. SERPINH1 encodes heat-shock protein 47, a chaperone essential for collagen synthesis. The study focused on genotype of the offspring based on the hypothesis that the genotype of the extra embryonic tissues (fetal membranes) represents the primary determinant for risk of

PPROM. The investigators extracted genomic DNA from umbilical cords, cord blood, or neonate cheek swabs. In the first case control study with 152 cases and 174 controls, they were able to demonstrate that the -656 allele is significantly more frequent in African-American neonates born from pregnancies complicated by PPROM compared with controls (OR= 3.22, 95% CI 1.50- 7.22). A follow up case control study was conducted on a different sample of 92 cases and 184 controls. This result demonstrated again that there was a significant association of PPROM with neonates carrying the -656 allele ($p< 0.0076$, OR =2.37; 95% CI 1.17, 4.79). Combining the two studies (cases =244, controls = 358) they obtained a highly significant association between the -656 allele and PPROM ($p< 0.0000045$, OR = 2.77; 95 % C 1.73, 4.95). They used population samples to estimate the -656 minor T allele frequency. They claim this study to be the first example of an ancestry-informative marker associated with PPROM in African-Americans.

Fujimoto et al demonstrated an association between fetal carriage of 2G alleles and PPROM. [167] This led them to conclude that the 2G allele has a strong promoter activity in amnion cells, and that it confers increased responsiveness of amnion cells to stimuli that induce MMP-1 and also that this polymorphism contributes to the risk of PPROM. [167] Genetic (genomic sequence variation) and epigenetic factors (DNA methylation) combine to determine MMP1 expression and influence the association with PPROM. [168]

In maternal genotype study Roberts et al [169] were able to demonstrate an association between allelic variants of the polymorphism at position -308 in the gene for tumor necrosis factor alpha and preterm birth after PPROM. They observed that the common allele of the TNFA-308 polymorphism (TNFA*2) is over-represented in a population of women who were themselves delivered preterm after PROM. The authors suggest that this demonstrates an endogenous susceptibility to idiopathic preterm birth. They concluded that the presence of a single TNFA*2 allele is a risk factor for PPROM in

African American women. DNA variants in a maternal gene involved in the extracellular matrix metabolism doubled the risk of PPROM. A SNP in TIMP2 in mothers was significantly associated with PPROM (OR= 2.12 95% CI 1.47 3.07).

These studies indicate that maternal and fetal genetic factors contribute to PPROM and genetic variation plays a significant role in predisposition to PPROM. This involves DNA variants in genes that participate in the inflammatory response and extracellular matrix degradation. Genome wide studies on PTD and subsequently PPROM have yet to be reported, and the existing literature is based almost exclusively on candidate genes using the classical case-control study design. Most studies on PTD have small sample sizes and consequently lack the power, and run into the possibility of type 2 error where we fail to find a difference even when one exists, hence challenging the validity of negative associations in these studies. When positive associations have been reported the magnitude of associations has been small, and statistical significance only marginal. Replication studies, a necessity when studies are based on small sample sizes have not been widely done. Multiple genes may be involved in the etiology of PPROM. Furthermore the impact of these genes may be influenced by environmental factors. Additionally maternal and fetal genes may play a role in the etiology of PPROM each acting independently or the two acting in synergy.

Chapter 5 Prediction of PPROM

Overtreatment for women presenting with vague symptoms before term is common, due to the morbidity and mortality associated with PTD. Women may be exposed to unnecessary interventions such as hospitalizations, tacolytic drugs and prolonged bed rest. So identifying a test to predict PPROM will correctly identify women at risk and reduce anxiety and costs associated with these unwarranted interventions. PPROM is a useful marker in itself for PTD. The preterm prediction study [123] reported that in multiparous women short cervix, previous PTD due to PPROM, and presence of fetal fibronectin (fFn) were the strongest predictors for both PTD following PPROM at <37 and <35 weeks gestation. Women with positive fetal fibronectin screening results and short cervix had greater risks for PTD due to PROM at <37 weeks' gestation (RR= 4.9) and greater risk of PTD caused by membrane rupture at <35 weeks' gestation (RR=13.5). Multiparous women with all three risk factors had a 31.3 fold increased risk of PTD due to premature rupture of membranes at <35weeks' gestation. In nulliparous and multiparous women short cervical length was consistently associated with PPROM at <37 and <35 weeks gestation. In a nested case control study [112] women with PPROM had increased median plasma TAT (thrombin-anti thrombin 3 complex) levels in second trimester and third trimester. In the second trimester the odds ratio for PTD due to preterm PROM with a TAT level of > 3.9ng/mL was 6.0 (95% CI, 1.67- 21.1) and this value predicted PPROM with sensitivity, specificity, positive predictive value and negative predictive value of 88%, 68%, 82%, and 97% respectively.

Although the optimal use of pathway specific markers of PTD have yet to be developed a clinically useful biochemical approach to the detection of final common pathways has been developed. This approach involves the measurement of fetal fibronectin in the cervicovaginal secretions. Goldenberg et al [171] evaluated series of asymptomatic women at low risk for PTD who were screened every two weeks from 22 to 30 weeks' gestation. The investigators observed sensitivity, specificity, positive predictive value

and negative predictive value for delivery before 28 weeks' gestation of as 63%, 98%, 15% and 100% respectively. Measurement of fetal fibronectin in the cervicovaginal secretions seems to have a high negative predictive value but a comparatively lower positive predictive value. The risk of PTD increases with increasing fetal FN values from 20 to 300ng/mL. [172] Another study reported that fetal FN was equally predictive of PTD due to PROM or PTL and on an average fetal FN was detected in cervicovaginal secretions three or more weeks before PTD. [173] Among the markers, fetal FN in the amniotic fluid seems to be the best marker for diagnosis of PPROM with a sensitivity of 94%, and specificity of 97%. [174] However amniotic fluid screening for fetal fibronectin seems to be an invasive procedure to include in routine prenatal protocol.

Some studies have found elevated matrix metalloproteinase-9 concentration in amniotic fluid [175] and plasma [176] in women with preterm PROM. This enzyme though is not specific for preterm PROM, it is found in the amniotic fluid in the presence of infection which may or may not lead to preterm PROM.

MMP-8 has also been examined in the amniotic fluid at midtrimester as a potential biomarker for preterm PROM. 26% of asymptomatic women who had preterm PROM had a midtrimester amniotic fluid MMP-8 concentrations above the 90th percentile compared with only 10% of term controls. [177]

C-reactive protein measurements during gestation remains controversial, and cannot specifically predict PPROM, as some studies have concluded that the CRP levels may increase with advancing normal gestation and also during labor and the post-partum period. [178] The use of maternal C-reactive protein (CRP) measurements in the diagnosis of chorioamnionitis and puerperal and neonatal infectious morbidity was studied in 147 patients with PPROM. [179] The overall test performance of CRP was poor suggesting that elevated ante-partum CRP may be misleading for diagnosis of chorioamnionitis. In the diagnosis of acute chorioamnionitis, ante partum CRP of >= 12 mg/l showed high sensitivity (94%) and high negative predictive value (97%), but low

specificity (50%) and low positive predictive value (35%). However serial CRP measurements increase the test performance. [179]

IL- 6 in cervical secretions has also been investigated independently to determine its value in diagnosing microbial invasion of the amniotic cavity in patients with PPROM. An IL-6 level in cervical secretions >200pg/ml had a sensitivity of 78.5% a specificity of 73.1% and a relative risk of 4.6 for intra amniotic infection. The study concluded that intra-amniotic infection is associated with increased levels of IL-6 and concentrations in cervical secretions are related to amniotic levels. [180]

Relaxin is a polypeptide hormone that is produced in the corpus luteum and the placenta during pregnancy. [57] Relaxin stimulates collagenases in the cervix [181] and human fetal membranes. [182, 183] Raised serum relaxin concentrations in the 30th gestational week are associated with preterm delivery, more so in women with PPROM [184] as compared to women with spontaneous PTD without rupture if membranes. The risk of spontaneous PTD has been related to decreased cervical length and increased maternal serum relaxin levels. A study was conducted with an aim to test the correlation of relaxin levels with cervical length and risk of spontaneous PTD in women with twin pregnancies. [185] However no association could be demonstrated between relaxin and cervical length which led the investigators to conclude that relaxin does not explain the inverse correlation between cervical length and spontaneous PTD in women with spontaneous twin pregnancies. [185] Only reliable predictors will give us some insight into prevention strategies.

Chapter 6 Preventive and interventional studies involving PPROM

Current interventions to prevent preterm PROM can be divided into 3 categories; interventional trials to prevent modifiable risk factors for example, smoking and urogenital infections; broad based preventive measures like antioxidant therapy and supplementation with Vitamin C and E; and treatment of non-modifiable risk factors like previous history of preterm birth and short cervix. Most of the interventional trials were not designed exclusively for PPROM. The trials were designed to assess reduction in PTD as their primary outcome and some have investigated PPROM as secondary outcome.

Morales et all [186] conducted a double blind placebo controlled trial to determine whether treatment of bacterial vaginosis with metronidazole in patients with PTD in the penultimate pregnancy from PTL or PPROM reduces the risk of subsequent PTD in the index pregnancy. Their analysis demonstrated that compared with the placebo group patients in the metronidazole group had significantly fewer hospital admissions for PPROM. They concluded that treatment with metronidazole was effective in reducing the PPROM rate in the index pregnancy in women with a history of PTD.

Kuhnert et al have shown that maternal zinc intake is related to fetal zinc status and not to maternal zinc status in a normal pregnancy. [170] Some trials to assess the effect of Zinc supplementation on reduction in PPROM incidence have been done. Jonsson et al [187] conducted a double blind randomized controlled trial in a normal healthy middle class population in Denmark to assess the effect of Zinc supplementation (vs. placebo) in pregnancy on various outcomes such of large for gestational age, small for gestational age, PPROM, preterm labor, preeclampsia and bleeding in the second or third trimester. They concluded that Zinc supplementation during pregnancy in this population did not offer any benefits to the mother or the fetus. It would be interesting if these findings can be replicated in micronutrient starved populations.

Casanueva et all [188] conducted a randomized double blind placebo controlled trial to evaluate the effectiveness of 100 mg Vitamin C/day in preventing PROM. The reported incidence of PROM was 24.5% in the placebo group and 7.69% in the supplemented group (RR = 0.26; 95% CI 0.078, 0.837).The investigators concluded that daily supplementation with 100mg Vitamin C after 20 weeks of gestation effectively reduced the incidence of PROM. However the investigators did not assess the incidence of PPROM. It is not clear if similar prevention strategies (with Vitamin C) will be effective in PPROM since mechanisms or pathways involved in membrane rupture differ in PROM and PPROM.

Chapter 7 Conclusion

In conclusion PPROM is a complex condition with a wide ranging etiology, and itself is a strong predictor of preterm delivery, with potential adverse neonatal outcomes. The proportion of preterm neonates surviving following PPROM is increasing. The long term complications and effects of PPROM on neuro developmental outcomes have potential for further investigation in longitudinal studies with long follow up windows.

A consistent case definition will reduce misclassification which will assist in determining etiological pathways and give accurate estimates of the subtypes of preterm delivery. Relaxin has been associated with PPROM, but not with rupture at term, indicating that preterm rupture is a pathological moiety of the term counterpart. Since relaxin stimulates collagenase production, raised serum relaxin concentrations in the last trimester along with short cervical length are strong predictors of PPROM. Relaxin can operate independent of infection causing degradation of the membranes; however the trigger for this collagen degradation is not clear. Pro inflammatory cytokines, and /or infection have been associated with PPROM suggesting inflammation to be a strong etiologic factor associated with PPROM. Inflammation caused by infection and sterile inflammation caused by reactive oxygen species triggers membrane rupture. In this scenario inflammation can be described as a proximal predictor of PPROM, but determinants of inflammation can be described as more upstream predictors of PPROM. It might be naïve to suggest anti-inflammatory trials for PPROM since the upstream factors associated with PPROM are multiple and diverse. However therapeutic agents to down regulate the pro inflammatory cytokines and reduce the active forms of MMPs should be explored as possible options for randomized trials to reduce the incidence of PPROM.

Infection has been implied as a causative factor in many studies of preterm delivery and PPROM although most randomized clinical trials of antibiotics fail to show any beneficial effect on reduction of PTD rate. Since infection has been associated with

early PPROM, antibiotic administration may have to be initiated early in gestation. Inception cohorts of high risk women in their reproductive ages might be useful in screening and monitoring in the first or early second trimester antibiotic administration. However teratogenicity and toxicity of the anti-microbial agents to the fetus along with long term administration may be constraints in administering these agents.

PPROM is the strongest predictor of preterm delivery but PPROM prediction has been a challenging issue. A combination of factors such as short cervix, previous preterm delivery due to PPROM, and presence of fetal fibronectin seem to be the strongest predictors of PPROM at <37 and <35 weeks gestation. Intra amniotic infection as indicated by elevated cytokine levels in vaginal fluids also seems to predict PPROM with good sensitivity and modest specificity. It will be interesting to investigate the microbial flora associated with elevated IL-6 in vaginal fluids. However some of these predictors may be hard to measure since amniotic fluid measurements cannot be routinely advocated as protocol in prenatal care.

Since PPROM is directly associated with oligohydraamnios certain fetal conditions are more likely with PPROM than other subtypes of PTD. Pulmonary hypoplasia, fetal infection after membrane rupture, and fetal joint contractures due to oligohydraamnios are specific consequences of PPROM. Prevention of PPROM will reduce incidence of long term morbidities associated with these conditions.

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