

Review Article

PRETERM RUPTURE OF MEMBRANES: THE VITAMIN C FACTOR***J.A. Osaikhuwuomwan**

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Preterm prelabour rupture of membranes (PPROM) is one of the major factors that have been found to correlate with adverse pregnancy outcome¹⁻⁸. It remains a critically important clinical and public health problem. PPRM is a leading cause of preterm delivery with a third of all preterm births resulting from preterm PROM^{1-4,9-10}. It is associated with considerable increase in adverse maternal, foetal and neonatal risk⁷. The adverse perinatal outcome following preterm delivery is huge, accounting for up to 70% of perinatal mortality worldwide^{8,11-13}. A perinatal mortality rate of 52% as a result of PPRM has been reported in Nigeria⁷. Despite the challenge posed by PPRM to obstetric and paediatric care, its aetiology and thus preventive and therapeutic strategies to control the condition remains elusive¹³⁻¹⁴.

The incidence of PPRM varies for different countries and population. This is because of the multiple risk factors that have been associated with PROM and also the non-uniformity in reporting and distinguishing between preterm PROM and PROM at term^{10,13,15-16}. Some authors have reported an association with the socioeconomic status of the population studied with the incidence higher in the

most deprived segment of society¹⁵. Other studies found a statistically significant higher incidence amongst black population even after adjusting for socioeconomic and maternal medical risk factors¹⁶⁻¹⁸. The incidence of preterm PROM across the world population ranges from 0.7 . 3.5% of all births^{2,4,10,12,14,16,19}. An incidence of 2.5% was reported in southern Nigeria⁷.

The cause of preterm PROM is unknown but the pathophysiology appears to be multifactorial^{3,4}. In any given patient one or more pathophysiologic processes may be evident. Chorionic infection or inflammation appears to play an important role in aetiology of preterm PROM, with rupture of membranes being attributed to increasing physical stress that weakens it^{3,4,5}. Recent evidence suggests that membrane rupture is also related to biochemical processes such as abnormalities in collagen structure and formation as well as increased oxidative stress^{3,20}. In support of this an imbalance between synthesis and degradation of collagen within the extracellular matrix of the chorioamniotic membrane induced by an increased expression and activity of various matrix metalloproteinases has been

implicated in the aetiology of PPROM²¹⁻²³.

Micronutrients deficiencies that affect collagen formation have been shown to alter collagen structure and this has been associated with an increased risk of preterm PROM^{3,5,24}. Researches in this regard have investigated the role of Vitamin C^{5,25-27}. Vitamin C or Ascorbic Acid is a water soluble vitamin and one of the body's naturally occurring antioxidant that is not synthesized by humans²⁸⁻³⁰. Ascorbic acid oxidizes readily to dehydroascorbic acid which passes readily through the membranes where it acts to protect the body against degenerative processes resulting from oxidative stress^{29,31}. Dietary deficiencies of Vitamin C leads to scurvy which is characterized by collagen weakness and capillary haemorrhage^{29,30}. Functioning as a micronutrient/antioxidant Vitamin C directly stimulates collagen synthesis through activation of multiple genes. In addition ascorbic acid serves to strengthen and stabilize collagen by acting as an enzymatic cofactor to the enzymes, lysyl hydroxylase and prolyl hydroxylase, which are required for synthesis of hydroxyproline and hydroxylysine. Collagen requires hydroxyproline bridges across the triple helix to provide stability to it^{29,30}. Ascorbic Acid as an antioxidant acts as a reducing agent by delivering a hydrogen atom with its single electron to the single unpaired electron in the outer ring of reactive oxygen species (ROS). The ROS now with paired electron is stabilized²⁹. Ascorbic Acid also causes down regulation of the metalloproteinase-2(MMP-2) transcriptional factor, thus suppressing the expression of MMP-2 and blocking its tissue damaging effect²⁹. Thus Vitamin C is thought to participate in the equilibrium between synthesis,

stability and degradation of collagen and this may be critical in reducing the occurrence of preterm PROM^{16,29,32}.

Vitamin C cannot be synthesized by the body hence it is only gotten from dietary sources or supplementation³². Dietary or supplemental dose of 60-100mg of Vitamin C has been recommended to maintain normal plasma levels^{28,31}. Assessing micronutrients status in human sample is challenging due to their labile nature, however results are validated by the method of assay³¹. Vitamin C can be measured in plasma, white blood cells or urine. In humans the determination of Vitamin C status is commonly done using plasma³¹. Fasting plasma samples are collected and preserved quickly by freezing in order to improve result reliability^{31,33}. The method of assay could be by chromatography, spectrophotometry or automated enzyme procedure.. The commonest assay method used is the reverse phase high performance liquid chromatography^{31,33}. Reference values for Vitamin C seems to be influenced by the assay method and study design, albeit the widely accepted reference value for normal of fasting samples is 26.1-84.6 μ mol/l (>0.6mg/dl, >20 μ g/10⁸cells, >114nmol/10⁸ cells), while for deficiency state is a value of <11 μ mol/l (<0.2mg/dl, <10 μ g/10⁸ cells, <57nmol/10⁸ cells)^{31,33-37}. Two separate studies in northern and southern Nigeria assessing the Vitamin C plasma levels amongst pregnant and non pregnant women reported values within the international reference range³⁸⁻³⁹. These local studies further corroborated previous reports, by demonstrating a fall in Vitamin C levels in pregnancy and a steady decline observed with increasing gestational age^{28,36-42}. It has been suggested that the lower levels observed in pregnancy when

compared to non pregnant controls may be due to the physiologic hemodilution in pregnancy, increased demand from oxidative stress or a reduced dietary intake^{15,41,43-44}. Pregnancy has also been shown to promote oxidative stress^{28,38,45-46}. A study in University of Benin Teaching Hospital on the effect of pregnancy on antioxidant capacity demonstrated that the total antioxidant capacity was low amongst pregnant women⁴⁵. These studies proposed that use of exogenous antioxidant supplement during pregnancy to mitigate against this oxidative stress may be necessary to prevent pregnancy complications^{38,39,41,44-46}. Despite these beneficial effects of antioxidants in pregnancy more detailed and specific research is still advocated^{45,47}. Therefore deficiency of Vitamin C has been hypothesized to play a role in preterm PROM^{16,21,32}.

The role of Vitamin C in PROM/preterm delivery has been previously investigated. Pioneer work in this regard dates back to 1964 when Wideman and colleagues²⁵ reported an association between low ascorbic acid levels and preterm rupture of foetal membranes. Since then, other studies have examined the role of Vitamin C in maintaining membrane integrity, dietary intake and also emphasis on measurement of ascorbic acid concentration in plasma, leukocyte and cord blood as it affects preterm PROM and preterm birth^{27,28,31,48-49}.

Kanayama and colleagues were able to substantiate Wideman's work by demonstrating low collagen content in the membranes of patients with PROM²⁶. Their finding was corroborated by *in-vitro* studies carried out by Plessinger *et al*²⁷ who noted that excessive damage to *in-vitro* amniotic epithelium and collagen

results from exposure to hypochlorous acid that was dose related. They went further to confirm that pre-treatment with Vitamin C and E (as antioxidants) prevented damage in all cases.

Woods⁵⁰ in his review of reactive oxygen species and preterm PROM hypothesized that reactive oxygen generated by the body's response to diverse insults, such as infection, smoking and bleeding could activate collagenolytic enzymes and impair foetal membrane integrity. He proposed that supplementation with Vitamin C may substantially reduce the risk of PPROM and thus suggested the prospects of therapeutic trials^{23,29}. Another *in-vitro* study done showed proportional degradation of collagen the lower the Vitamin C concentration⁵¹. Similar studies in Nigeria observed that Vitamin C may protect against lipid peroxidation and hypochlorous induced damage in pregnant women⁴⁶. These studies are however *in-vitro* studies and may not reflect exactly the *in-vivo* effects of Vitamin C. In a nested case control study among a cohort of pregnant women, those with PROM had decreased Vitamin C concentration and were also shown to have quantitative deficiency and abnormal crosslinking of collagen²². The assumptions from this study were however limited by the small sample size. Barrett and colleagues evaluating the potential role of ascorbic acid in prevention of PROM found only a positive association between low amniotic fluid Vitamin C and not serum Vitamin C as an important determinant of PROM⁴⁸.

On this background, Casanueva *et al*^{15,52-54} under-took several studies to establish Vitamin C status in pregnancy and its association with premature rupture of membranes. They had observed altered pattern of

collagen synthesis and diminished Vitamin C concentration in pregnancy which was associated with subsequent occurrence of PPROM⁵⁴. They went further to use PROM as a functional assessment to establish cut off points for Vitamin C throughout pregnancy⁵³. Vitamin C levels were determined by the reverse phase high performance liquid chromatography and it was found that plasma Vitamin C concentration below a critical level of $18\mu\text{g}$ (102mmol)/ 10^8 cells is associated with a substantially increased risk of PROM^{24,34,53,55}.

The effectiveness of Vitamin C at a dose of 100mg/day in preventing PPROM was evaluated in a randomized double-blind placebo controlled trial where it was found that 100mg of Vitamin C per day was sufficient to maintain leukocyte ascorbic acid concentration at a level above $18\mu\text{g}/10^8$ cells and this can protect against PPROM⁵². Furthermore it was noted that though daily supplementation with 100mg Vitamin C after 20 weeks gestation effectively reduced the incidence of PROM there was no significant difference in Vitamin C concentration between the two groups and mean plasma Vitamin C concentration decreased throughout pregnancy in both groups⁵².

Borna and colleagues in addition found in their study a statistically significant increase in latency period for those that had Vitamin C and E supplementation after preterm PROM⁵⁶. Similarly in another prospective cohort study of 2,064 pregnant women it was found that women with preconception total Vitamin C intake of less than 10th percentile had twice the risk of preterm delivery because of PROM and thus it has been suggested that vitamin

supplementation may be a valuable interventional strategy⁵⁷.

On the other hand, in a randomised double-blind placebo-control trial; where antioxidant supplementation and PROM was planned secondary analysis. It was observed contrary to expectation that Vitamin C caused an increase in the incidence of PROM⁵⁸. They concluded that empirical use of antioxidant supplementation should be discouraged and future investigations on antioxidants supplement to prevent PROM should be done with caution. In this study, the population investigated were mainly chronic hypertensives or those with history of previous pre-eclampsia and this may have flawed the results obtained. Furthermore, the high dose of antioxidant supplements used and the pharmacology of the selected drug were limitations in the study which makes it difficult to challenge the validity of previous work by Casanueva and co-workers⁵² supporting the role of Vitamin C supplementation to prevent PROM. Another randomized double-blind placebo-controlled trial of ascorbic acid for the prevention of preterm labour did not show any significant change in Vitamin C levels or incidence of preterm labour with Vitamin C supplementation and they concluded that the proposed effect of Vitamin C supplementation may only be apparent in the patient with a bonafide Vitamin C deficiency³². They suggested the prospects of performing the study in a more rural population.

In Nigeria and sub-Saharan Africa research efforts have focussed on assessment of Vitamin C status and antioxidant capacity in pregnancy. These studies reported increased oxidative stress in pregnancy, inadequate dietary intake and low plasma Vitamin C levels amongst pregnant Nigerian women^{38-41,43-46}.

The work done in UBTH by Idogun *et al*, found that total antioxidant capacity was lower in pregnancy and supported the need for antioxidant supplementation in pregnancy⁴⁵; they recommended the need for more detailed research on specific antioxidants.

There exists an obvious paucity of research on the relationship between preterm PROM and Vitamin C in sub-Saharan Africa (where ironically the incidence is commoner), majority of published work in our locale have been on bacteriology of PROM and antibiotic use^{6,7,11-13,16-18,59-60}. The dearth of research effort on prevention of preterm PROM in our environment is worrisome, owing to the fact that while in the developed society the survival rates of premature babies have greatly improved as a result of improved neonatal care¹⁴, the reverse is the case in developing countries where neonatal care especially for preterm babies is quite challenging, still expensive and beyond the reach of most people. Other factors like ignorance, poverty and even power failure impact negatively on the care of preterm neonates in our locale^{11,14}, thus perpetuating the high perinatal mortality rate of over 50% often reported^{7,12,17}.

The devastating consequences of preterm PROM and prematurity makes it necessary to develop health strategies aimed at improving outcome by predicting, preventing and treating preterm PROM and preterm labour. Hence it becomes imperative to carry out studies in our locale that seek to evaluate the relationship between preterm PROM and Vitamin C concentration in blood. If this is established, advocacy could be made for strategies such as Vitamin C supplementation that represents a safe, non-invasive and cost effective

approach to the prevention of preterm PROM and its adverse consequences.

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