

The Efficacy and Safety of a Combination of Amino Acids, Vitamins and Probiotics in PROM and PPRM

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ABSTRACT

The prevalence and severity of prelabor rupture of the membranes (PROM)/preterm PROM (PPROM) are a worldwide public health concern. PROM is the result of a cascade of events involving matrix metalloproteinase (MMP)-9, tissue inhibitor of metalloproteinases 1 (TIMP1), cytokines and proapoptotic genes, which is initiated by several factors such as infection, genotoxic agents or some unknown etiology. In PROM, there is an increased expression and activation of MMP-2, MMP-3 and MMP-9 and a reduction of TIMP1. p53 and tumor necrosis factor (TNF)- α mediate the major apoptotic pathway of PROM. p53 can transactivate some MMP genes, resulting in the overexpression of MMPs. This leads to apoptosis. MMP-2 and MMP-9 degrades type-IV collagen, which is the major structural component of chorioamnion. Understanding the fundamental pathology at the molecular level, it appears necessary to adjust the biologically protective mechanism to prevent spontaneous preterm labor. Our findings show that the novel combination of arginine, ascorbic acid, folic acid, glutamine, glutathione, thiamine, lactic acid bacillus spores, vitamin E acetate and pyridoxine is safe and effectively prevents PROM and PPRM (97% patients) and prolongs pregnancy term.

Keywords: Amino acids, vitamins, Lactobacillus spp., PROM, PPRM

Prelabor rupture of membranes (PROM) is defined as the rupture of membranes before the onset of labor. When membrane rupture occurs before delivery and 37 weeks of gestation, it is referred to as preterm PROM (PPROM).¹ Rupture of membranes results from various factors that ultimately result in accelerated membrane weakening. The primary causes are increased local cytokines, untimely abnormal expression and activation of metalloproteinase (MMP)-9 that leads to an imbalance between MMPs and tissue inhibitors of metalloproteinases (TIMP), increased collagenase and protease activity. Factors activating MMP also activate the apoptotic pathway mediated by p53 and tumor necrosis factor (TNF). The protein p53 triggers apoptosis by regulating the expression of two genes, Bax and Bcl-2. p53 increases proapoptotic Bax

gene expression and down-regulates the antiapoptotic Bcl-2 gene.² The synergy between MMPs activation and apoptotic change leads to rupture of the fetal membranes and low Lactobacillus spp.³ Hence, termination of a series of cytokine activation followed by reduction of activated MMP with the introduction of specific amino acids, vitamins and Lactobacillus spp. spores may encourage resealing of the ruptured fetal membranes. The previous study suggests that supportive factors like glutathione, folic acid, glutamine, vitamin E, pyridoxine and L-arginine help in membrane resealing.⁴ Hence, a prospective study was conducted among mothers diagnosed with PROM, PPRM, minor or high leakage, twin pregnancy or a history of PROM in the last pregnancy to establish the efficacy and safety of a novel combination of arginine, ascorbic acid, folic acid, glutamine, glutathione, thiamine, lactic acid bacillus spores, vitamin E acetate and pyridoxine.

METHODS

The study was a real-world prospective, questionnaire-based study to assess the efficacy and safety of the novel combination and the occurrence of adverse events in mothers diagnosed with PROM, PPRM, minor or high

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leakage, twin pregnancy or a history of PROM in the last pregnancy. The data was collected anonymously. Formal written consent was obtained from the study participants after they were informed about the study, the usage of the medication for their condition, and the potential adverse effects. The responses were analyzed using a simple percentage calculation.

RESULTS

The study included 210 expecting mothers aged 21 to 41 years diagnosed with PROM, PPROM, minor or high leakage, twin pregnancy or a history of PROM in the last pregnancy. Most patients were diagnosed with PROM, minor leakage or at risk of PROM due to medical history (Fig. 1). Patients were treated as the doctor recommended. Most study participants

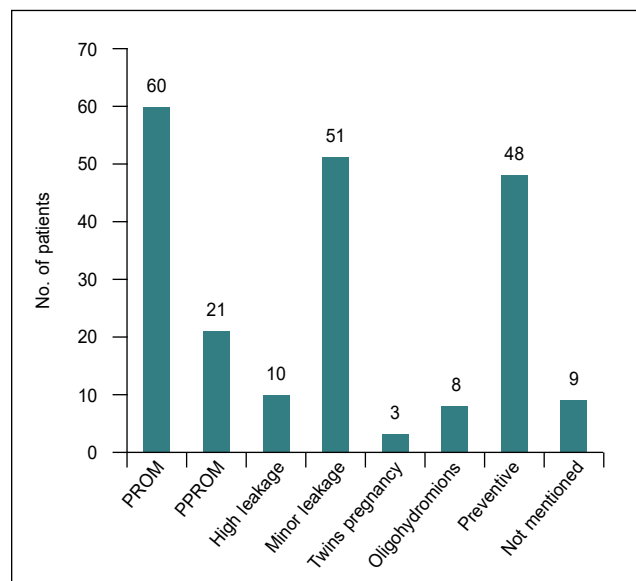


Figure 1. Diagnosis of participating patients.

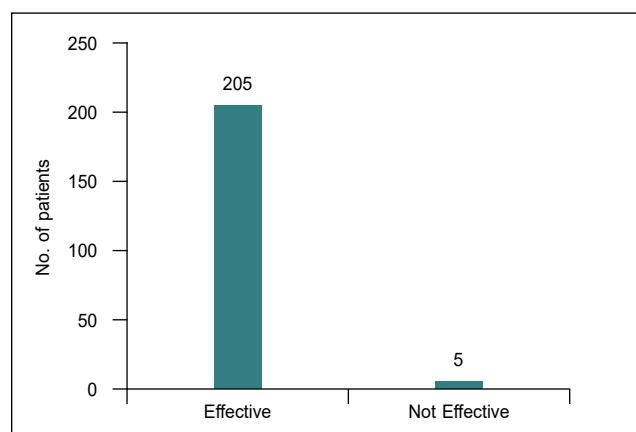


Figure 2. Effect of intervention of the novel combination in preventing and sealing ruptured fetal membranes.

(97%) found it effective in preventing leakage of amniotic fluid from ruptured fetal membranes and in prolonging the gestational age (Fig. 2). According to our survey, it was well tolerated by most patients. Only 3 subjects had adverse effects, although the prevention of leakage was effective. Clinicians found the novel combination of amino acids, vitamins and probiotics to be an excellent remedy for sealing the rupture of fetal membranes in PROM, PPROM and in the prevention of the same.

DISCUSSION

PROM/PPROM continues to be a global health challenge. Assisted by innate immunity, amnion epithelial cells appear vital in healing ruptured fetal membranes. Chorion is a multilayered cytotrophoblastic layer with collagen-rich connective tissue, and amnion comprises a single epithelial layer. The elasticity of membranes depends on chorion, and tensile strength depends on amnion. Increased levels of MMP, p53 and neutrophil elastase contribute to apoptosis. Increased apoptosis resulting in the breakdown of amniochorion has been reported in PROM.⁵

PROM is the result of a cascade of events involving MMP-9, TIMP1, cytokines and proapoptotic genes, which is initiated by several factors such as infection, genotoxic agents or some unknown etiology. In PROM, there is an increased expression and activation of MMP-2 and MMP-9 and a reduction of TIMP1. The imbalance between MMPs and TIMP1 results in extracellular matrix (ECM) degradation. The major apoptotic pathway of PROM is mediated by p53 and TNF- α . p53 can transactivate some MMP genes, resulting in the overexpression of MMPs. p53 triggers apoptosis by down-regulating Bcl-2 (antiapoptotic gene) and overexpressing the apoptotic BAX gene. This leads to apoptosis. p53 also induces MMP-2 expression. MMP-2 degrades type-IV collagen, which is the major structural component of chorioamnion.⁶⁻⁸

Arginine inhibits the Bax-induced cell apoptosis by reducing the amount of Bax mRNA expression. Hence, enhances cell proliferation and reduces apoptosis in human endometrial cells.⁹ This is important for the prevention and healing of ruptured fetal membranes.

Amino acids are also important for the management of secondary infections. For example, arginine deficiency has been linked to an increased risk of secondary infections in sepsis.¹⁰ Glutamine is an essential precursor for the synthesis of not only protein but also other molecules of enormous biological importance. Glutamine

improves TIMP1 protein production, thereby reducing the activity of MMP-2, MMP-9 and helping in the prevention and treatment of PROM. Hence, fetal and placental development depends on the abundance of glutamine.^{10,11} Additionally, pregnant women with high levels of *Lactobacillus* show a relatively low risk of preterm birth.³ The capacity of *Lactobacilli* to adhere and compete for adhesion sites in the vaginal epithelium and the capacity to produce antimicrobial compounds (hydrogen peroxide, lactic acid, bacteriocin-like substances) are important in the impairment of colonization by pathogens. It reduces infection and restores vaginal pH. Lactic acid can also inhibit the production of metalloprotease.¹² Dam et al and Gangwal et al studied the effect of a combination of arginine, glycine, cysteine, glutamate and vitamins along with *Lactobacillus* spores in PROM. Amino acids combinations were proved to have a definite role in PROM as it helps in the prolongation of gestational age by 3.21 ± 6.16 weeks and 3.87 ± 6.00 weeks (24-30 weeks), respectively.^{4,5}

Poor folate status in mothers has also been linked to an increased risk of premature birth.¹³ Direct activation of proMMP-2, MMP-9 by homocysteine is one of the established mechanisms involved in ECM deterioration.¹⁴ It has been shown to induce apoptosis in endothelial cells. Folic acid supplementation can lower homocysteine levels and therefore may decrease the risk of apoptosis in endothelial cells.¹⁵ This helps in the prevention and healing of ruptured fetal membranes.

Vitamins C is another important ingredient that modulates collagen metabolism and improves strength through increased helix formation. Vitamin C markedly stimulates collagen synthesis without affecting the synthesis of other proteins.¹⁶

Recent studies proved that vitamin C administration to women with a previous history of PPRM is efficient and safe in preventing such events in the current pregnancy. Another study proved that pretreatment with vitamins C and E prevent this damage in the amniotic epithelial layer.¹⁷ Thiamine inhibits intracellular p53 activity. It also reduces the production of TNF- α and interleukin-6.^{18,19}

Glutathione is an antioxidant that protects against fetal membrane damage and provides strength to the fetal membrane. Along with pyridoxine, it plays an important role in attenuating protective placental inflammatory and oxidative stress.²⁰ Glutathione prevents preterm parturition and fetal death by targeting macrophage-induced reactive oxygen species production in the myometrium.²¹

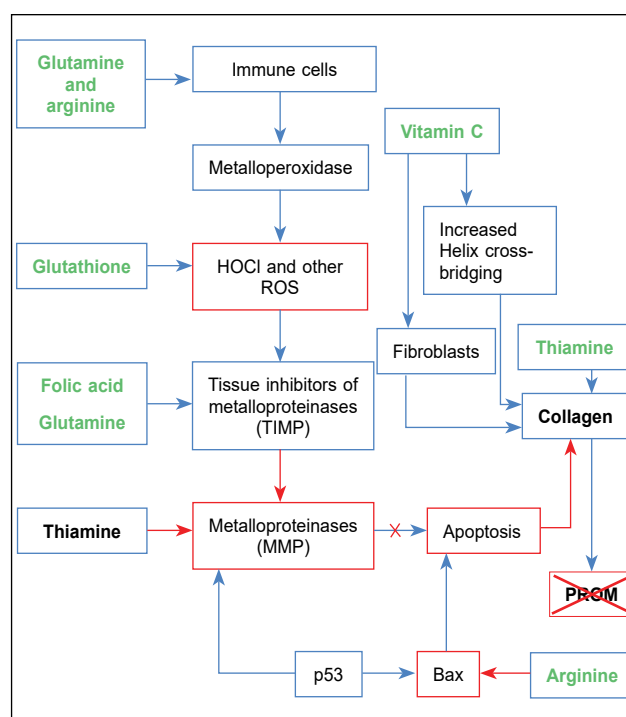


Figure 3. Role of the combination of amino acids and vitamins in PROM and PPRM.

HOCl = Hypochlorous acid; ROS = Reactive oxygen species.

The probable mechanism of action of the ingredients in membrane sealing has been depicted in Figure 3.

In our study, most participants had complained of PROM or minor leakage or a history of PROM in the previous pregnancy. Supplementation of the combination of arginine, ascorbic acid, folic acid, glutamine, glutathione, thiamine, lactic acid bacillus spores, vitamin E acetate, and pyridoxine helped in treating and preventing these conditions through membrane sealing and prolonging the term. Hence, supplementing the combination of these protective factors significantly increases beneficial effects in preventing and treating PROM/PPROM.

CONCLUSION

Based on our research, we propose that a novel combination of arginine, ascorbic acid, folic acid, glutamine, glutathione, thiamine, lactic acid bacillus spores, vitamin E acetate and pyridoxine is able to stop the leaking of amniotic fluid thereby can help prevent preterm labor in pregnancies.

It helps in the prolongation of gestational age. It is an oral medication, so it is simple to provide to the patient and hold up the promise of a healthy and safe pregnancy.

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