



Maternal Vitamin C and Preterm Pre-labour Rupture of Membranes

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ABSTRACT

Background: Preterm pre-labor rupture of membranes (PPROM) is the main reason for premature labor. **Aim of the study:** The main goal of that study was to determine whether there was a relationship between maternal vitamin C level and occurrence of PPRM in pregnant females. **Study design and setting:** A case-control study which was conducted in Babylon Teaching Hospital for Gynecology and pediatrics from 1 March 2016 to 30 November 2016. **Patients and methods:** The study included 68 women: 30 women as patients group diagnosed as cases of preterm pre-labor rupture of membranes who meet the inclusions and exclusions criteria, and 38 women as control groups. Vitamin C concentration was measured for all included women. **Results:** The two groups were matched in terms of women's age, gestational age, occupation, address, their parity and level of education ($p > 0.05$). Maternal vitamin C levels were measured in the patient's group and found to be lower than the control group. (Mean \pm SD) ($15.25 \pm 4.11 \mu\text{g/ml}$) Vs. ($16.6 \pm 4.83 \mu\text{g/ml}$) respectively, however, no statistical significance was found ($p = 0.127$). Low maternal vitamin C level is not correlating with the risk of PPRM, odds ratio (1.0182), 95% CI (0.344-3.011), and ($p = 0.97$). Vitamin C levels were significantly higher in women with no PPH ($15.08 \pm 3.80 \mu\text{g/ml}$) in comparison to those who had PPH ($10.54 \pm 3.38 \mu\text{g/ml}$) ($p = 0.022$). On the other hand, a non-significant negative correlation has been noted between low maternal vitamin C level and latency period ($r = 0.35$, $p = 0.067$). **Conclusions:** Lower maternal serum level of vitamin C levels has been found in patients with PPRM compared to the control group, but there was no statistical significance. Maternal vitamin C deficiency is not a contributing factor in the development of PPRM.

Keywords: Maternal, Perinatal, Mortality, Gestational ages

INTRODUCTION

Preterm pre-labor rupture of membranes (PPROM) is stated as a spontaneous rupture of membranes from age of viability to 36 weeks plus 6 days of gestation and prior to the start of delivery [1]. PPRM complicates 2% of all pregnancies. It is associated with nearly 30% of all perinatal deaths and is associated with 40% of deliveries before 37 weeks gestation, and contributes to significant levels of mortality and morbidity in neonates. Prematurity, sepsis and pulmonary hypoplasia are the most important complications of PPRM that can lead to neonatal death. Women with chorioamnionitis deliver prematurely [2].

Risk Factors and Pathophysiology

Multiple factors are incorporated to an increased risk of PPRM, these include black race, low socioeconomic status, smoking, history of antepartum hemorrhage [3], cervical incompetence, previous operations involving the uterine cervix, uterine risk of PPRM being 16% to 32% [4,5]. Collagen anomalies (decreased collagen content of the membranes anomalies, uterine overdistension (polyhydramnios, multiple pregnancies), previous PPRM is a major risk factor, the recurrence (excessive collagen degradation), amniocentesis, chorionic villus sampling, fetoscopy, and cervical cerclage are rare causes of PPRM [6].

Many factors appear to contribute to the pathophysiology of PPRM. One or more pathophysiologic process may play a role in any given patient. Choriodecidual infection found to have an important action in the etiology of premature

PROM. In a normal pregnancy, there is a balance between synthesis of collagen by fibroblasts and collagenolysis depending on the controlled response of enzymes released in the fetal membranes or derived from inflammatory cells to maintain the chorioamniotic sac [7,8].

The layer nearer to foetus contains amnion epithelial cells resting on the basement membrane contain type IV collagen. Compact layer and composed of types I, III, and V collagens, lies beneath the basement membrane. The fibroblast layer contains mesenchymal cells which secrete these collagens. Below the fibroblast layer, lies the spongy layer, it consists of glycoproteins, proteoglycans and type III collagen. This layer intervenes between amnion from chorion allowing sliding of amnion on the underlying chorion. Cytotrophoblasts are entrenched in a matrix consisting of type IV and V collagens within the chorion. It adheres cruelly to the uterine decidual layer [9].

The major fibrous integrity of the amniotic membrane is composed of a compact layer of stromal matrix. Mesenchymal cells in fibroblast layer secrete collagen that ensures the mechanical integrity of amniotic membrane by interstitial collagen (types I and III) which dominates and organize in parallel bunches while collagen type V and VI create filamentous connections between interstitial collagens and epithelial basement membrane [10].

Deficiencies of micronutrients will affect collagen formation and its structure leading to increased risk of preterm PROM [8].

Vitamin C

Vitamin C, also known as ascorbic acid (Figure 1), is a donor of electron for some enzymes of human that take part in collagen synthesis, hormones, and neurotransmitters, also it works as antioxidant scavenging reactive oxygen species and nitrogen species in water-soluble milieu [11]. Ascorbic acid is an essential white crystalline sugar compound that is needed by the body for performing a lot of biochemical and physiological processes. Vitamin C is an unstable compound that is oxidized easily and can be destructed by alkali, oxygen and high temperatures [12]. Ascorbic acid is found mainly in vegetables and fruits, especially tomato, citrus fruits, strawberry, and potato. Vitamin C concentration in vegetables and fruits changes according to growth conditions, maturity stage, geographical location, time of the year, time of storing and food preparation methods [13].

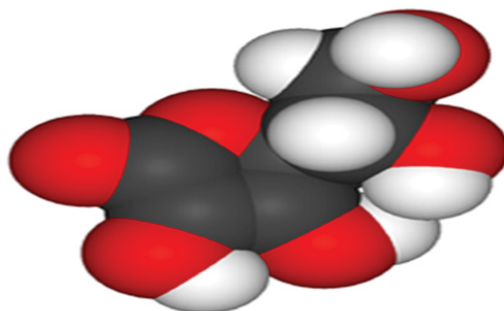


Figure 1 Model of a vitamin C molecule, black is carbon; red is oxygen and white is hydrogen [11]

Nearly 70% to 90% of vitamin C ingestion from diet or dietary supplements (30-180 mg/day) is absorbed. Absorption occurs mainly by a sodium-dependent active transport. Absorption efficiency decreases to less than 50% in case of increased intake to more than 1 gram per day and it will be mainly by simple diffusion [14]. Vitamin C decreases the action of metalloproteinase-2 (MMP-2) transcriptional factor, thus decrease the expression of MMP-2 and block its destructive action on tissues [15].

Absorption and metabolism of vitamin C: In human, vitamin C absorption takes place in the buccal mucosa, stomach and small intestine by passive diffusion and active transport system.

Recommended safe dose vary. Recommendation of the American Pregnancy Association is to take 80-85 mg daily, while the National Institutes for Health and Care Excellence recommend that consumption in pregnancy is about 120 milligrams daily. The adverse effect of vitamin C is likely to occur at a dose above 2,000 milligrams. If intake rises above this amount, the excretion in the urine rapidly rises. As the absorption capacity in the intestine and kidney can accomplish a saturation level, it is advised to ingest smaller and numerous dosages of vitamin C during the day rather than taking a single large dose [12].

Some researchers reveal that a large daily intake of vitamin C in pregnant women can result in vitamin C deficiency in the new-born baby, termed scurvy, according to the American Pregnancy Association. This occurs because the baby may develop an intolerance or resistance. Woman's kidneys also contribute by an increase in the amount of vitamin C excretion, even when she stops ingesting high doses; therefore, we need to assess the safety of supplementation of vitamin C during pregnancy [12].

Vitamin C deficiency: Deficiency of ascorbic acid in the body occurs due to lack of adequate amounts of vitamin C in dietary intake. Deficiency of vitamin C implies that new collagen could not be synthesized. That leads to damage to various tissues of the body and the integrity and renovation of the body become influenced. Prolonged vitamin C deficiency, for the duration of 3-months or more, can cause a disease known as scurvy [16].

Vitamin C in pregnancy: Maternal serum vitamin C level falls over pregnancy, mostly because of increased plasma volume in addition to active transport to the fetus [17]. The placenta takes the oxidized type of vitamin C from the maternal blood and secretes it in a reduced form to fetal circulation. In pregnancy, deficiency of vitamin C is considered a risk factor for infection, preeclampsia, preterm rupture of membranes (PPROM) and preterm birth [18].

Vitamin C and preterm pre-labor rupture of the membrane: Ascorbic acid is implicated in the composition of collagen, its secretion and in collagen degradation processes [19] Manifestation of PPRM is found to be linked to the abnormal mode of collagen formation and deficiency in ascorbic acid at 26 weeks of gestation. It is hypothesized that PPRM can be a useful practical check of vitamin C condition in pregnant women; this idea is obtained from the function of vitamin C in collagen synthesis and degradation and the role of collagen in keeping the mechanical intensity of the chorioamniotic membranes all over pregnancy [20].

Some researchers have found that vitamin C levels were lower in the serum, leucocytes and the amniotic fluid in patients with PPRM when compared to the control group. However, little information is supplied about the association between intake of vitamin C and its effect on the premature rupture of membranes [21].

Aim of the Study

The goal of the study was to determine the relation of maternal vitamin C serum concentration with development of PPRM in pregnant women.

PATIENTS AND METHODS

A case-control study was conducted in the Obstetrics Department of Babylon Teaching Hospital for Gynaecology and Paediatrics. Period of the study was from 1 March 2016 to 30 November 2016 after maintaining approval by Iraqi Board of Medical Specialization. The study included 68 women: 30 women as patients group with a diagnosis of PPRM who meet the inclusion and exclusion criteria and 38 women as control groups who were normal pregnant women came for an antenatal check-up. Gestational ages were ranging from 24 to 36 weeks plus 6 weeks for both groups and all participated women were followed until labor occurred.

The study objective was clarified to all included women; verbal consent was taken from each participant. Inclusion criteria include singleton pregnancy of apparently normal fetus with a diagnosis of PPRM. Exclusion criteria include any medical disease including infections, polyhydramnios, smoking, history of preterm pre-labor rupture of membranes in previous pregnancies and evidence of chorioamnionitis in the current pregnancy.

History taken from the patients was according to a questionnaire which included: patient's name, age, parity, LMP, GA, occupation, time and duration of rupture membrane, colour, odour of fluid leaking per vagina, associated symptoms which include: abdominal pain, fever and urinary symptoms, past obstetrical history (history of previous rupture membranes in previous pregnancies), past medical history (any history of diabetes or hypertension), past surgical history, drug history and social history including the educational level and address. A general and abdominal examination was done; temperature blood pressure and pulse rate were checked (to exclude signs of chorioamnionitis).

Sterile per vagina speculum examination was carried out to confirm PPRM (made the patients rested supine for 20-30 minutes and pooling of fluid noticed). A blood sample was taken for complete blood count for measuring WBC, PCV, and CRP. Total 5 ml of maternal venous blood were taken in gel tubes to measure the vitamin C level from both patients and the control groups.

Patients group were followed for latency period (which is the time interval between ROM and onset of labor), development of chorioamnionitis, mode of delivery, and development of PPH, admission to the neonatal care unit and birth weight.

Summary of the Procedure of Measuring Maternal Vitamin C Levels

The sample was allowed to be overnight at 4°C before centrifugation for 15 minutes at 1000Xg. Collection of the serum and storage was done immediately.

Serum was collected in a disposable, non-pyrogenic, and non-endotoxin at a degree of -20°C and after the collections of samples had been completed, results were obtained by ELISA test using VIT. C ELISA kit (catalog No. E-EL-0011) was used for measuring serum maternal vitamin C level in (µg/ml).

The procedure was done as the following:

- Add 50µl standard or sample to each well
- Add 50 µl Biotinylated Detection Ab to each well immediately
- Incubation for 45 minutes at 37°C
- Aspiration and washing 3 times
- Addition of 100 µl HRP conjugate to each well then incubates for 30 minutes at 37°C
- Aspiration and washing 5 times
- Addition of 90 µl of substrate reagent then incubation for 15 minutes at 37°C
- Addition of 50 µl of stop solution. Reading at 450 nm immediately
- Calculation of results

Statistical Analysis

Statistical analysis was done in this study using SPSS (Statistical Package for Social Science) version 17 program. In continuous variables, independent t-test was utilized to assess the significance of differences between 2 groups and ANOVA to estimate differences between more than 2 groups. We utilize a Chi-square test to estimate differences between groups regarding categorical variables. Pearson's correlation coefficient was to compare between parameters. Results are documented as mean ± SD) unless $p < 0.05$ was assigned as statistically significant.

RESULTS

Table 1 shows a summary of the demographic characteristics of patients and control groups. Difference between the 2 groups was not significant in terms of maternal age, weeks of gestation, parity, occupation, address, and education ($p > 0.05$).

Table 1 Demographic characteristics of patients with PPROM compared to control group

Demographic Characteristics		Patients (N=30)	Control (N=30)	p-value
Age (Years)		25.55 ± 6.21	26.21 ± 6.71	0.67
Gestational age (Weeks)		30.78 ± 3.43	31.76 ± 2.54	0.75
Address	Urban	(16) 48.9%	(22) 60%	0.71
	Rural	(14) 51.1%	(16) 40%	
Education	Illiterate	(9) 33.3%	(10) 31.1%	0.66
	Primary Education	(8) 22.2%	(8) 17.8%	
	Secondary Education	(10) 26.7%	(12) 22.2%	
	Higher Education	(3) 17.8%	(8) 28.9%	
Occupation	Housewives	(21) 56.9%	(25) 59.6%	0.71
	Employers	(9) 43.1%	(13) 40.4%	

Parity	Primigravida	(10) 21.7%	(9) 27.7%	0.67
	P1-P3	(12) 73.3%	(18) 43.1%	
	≥ 4	(8) 26.7%	(11) 29.2%	

Maternal serum vitamin C levels: A comparison of vitamin C level was done between patients with PPRM and control group. The level was insignificantly lower in the patient's group ($p>0.05$) as shown in Table 2.

Table 2 Serum vitamin C level in patients with PROM and control groups

Parameter	Vitamin C		p-value
	Patients (N=30)	Control (N=38)	
	(Mean ± SD)	(Mean ± SD)	
Vitamin C (µg/ml)	15.25 ± 4.11	16.6 ± 4.83	0.127

In the patient group, 8 out of 30 patients have vitamin C below 25 Centile compared with 10 out of 38 women in control group ($p>0.05$), the odds ratio equal to 1.0182 as shown in Tables 3 and 4.

Table 3 The distribution of women according to the serum value of Vitamin C

Number of Women	Study Group	Control Group
Women with Vit C < 25 Centile	8	10
Women with Vit C ≥ 25 Centile	22	28

Table 4 Shows the ODD ratio, 95% CI and p-value for the correlation between vitamin C and preterm PROM among pregnant

Criteria	ODD ratio	95%CI	p-value
Women with serum vitamin C < 25 Centile in the study group and control (8, 10 patients)	1.0182	0.344-3.011	p=0.97
Women with serum vitamin C > 25 Centile in the study and control (22, 28 Patients)	Reference Group		

Correlation of maternal vitamin C levels with the demographic characteristic of patients group: Insignificant correlation was noted in terms of the maternal age, address, occupation, education, parity and latency period ($p>0.05$). Women lived in an urban area had a lower level of vitamin C when compared with women who lived in a rural area ($p=0.002$) (Table 5).

Table 5 Correlation of vitamin C level with the demographic characteristic of patients group

Demographic Characteristics	Vitamin C (µg/ml) (N=30)	p-value
Age (years)	18-29 years	15.65 ± 4.20
	30-39 years	14.52 ± 4.08
Address	Urban	13.36 ± 4.02
	Rural	16.73 ± 3.30
Occupation	Housewives	14.61 ± 4.24
	Employers	16.55 ± 3.49
Education	Illiterate	15.27 ± 3.78
	Primary Education	14.19 ± 4.62
	High Education	15.65 ± 4.11
Parity	Primigravida	15.32 ± 4.22
	P1-P3	14.34 ± 3.79
	≥ 4	16.26 ± 4.32
Latency period	<1 week	15.55 ± 3.71
	>1 week	14.34 ± 3.21

**Highly significant differences at $p<0.01$.

Maternal vitamin C levels and parameters of follow up in patients group: Maternal vitamin C levels were higher in patients who delivered normally in comparison to those who delivered by c/s but the difference was not statistically significant ($p=0.56$). However, a significantly higher level was noted in women who did not develop PPH in comparison to those who had PPH ($p=0.022$) (Table 6).

Vitamin C levels were higher in women with no chorioamnionitis in comparison to those who developed chorioamnionitis but the difference was not found to be statistically significant ($p=0.33$).

Table 6 Maternal vitamin C levels and parameters of follow up in patients group

Parameters		Vitamin C ($\mu\text{g/ml}$) (N=30)	p-value
Mode of delivery	CS	14.51 \pm 4.18	0.560
	NVD	15.56 \pm 3.96	
PPH	Yes	10.54 \pm 3.38	0.022*
	No	15.08 \pm 3.80	
Chorioamnionitis	Yes	14.28 \pm 5.02	0.330
	No	16.59 \pm 4.56	

*Significant differences at $p < 0.05$

Correlation of maternal vitamin C levels with latency period: Correlation analyses between maternal vitamin C level with latency period (11.63 \pm 7.09 days) showed insignificant negative correlation which was $r = -0.35$, $p = 0.067$ (Figure 2).

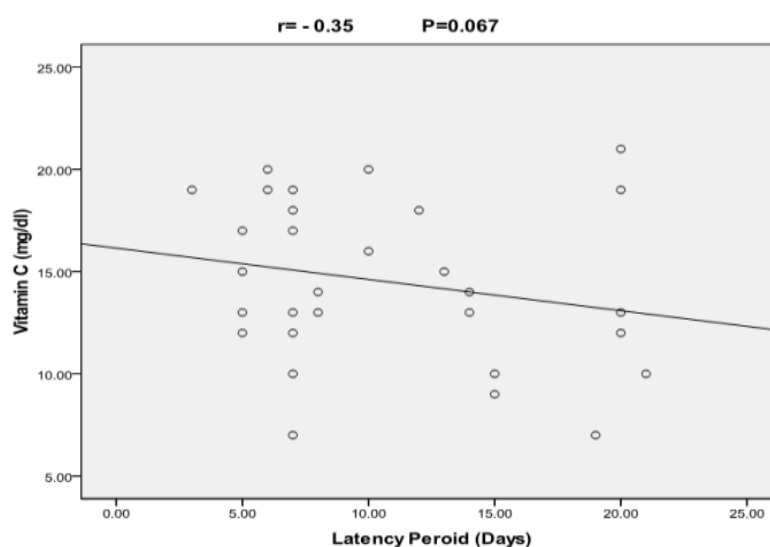


Figure 2 Correlation between vitamin C level and latency period

DISCUSSION

The main reason for PPRM is abnormal metabolism of collagen. Decrease collagen constituent of membranes affects their integrity and lead to rupture of membranes. Many studies were performed about the association of vitamin C with PPRM; these studies showed different results which may be due to the different populations, methods, and assays that were being used. In our study maternal vitamin C levels was insignificantly lower in patients group (Mean \pm SD) (15.25 \pm 4.11) when compared with control groups (16.6 \pm 4.83), ($p = 0.127$).

About 8 out of 30 patients with vitamin C below 25 Centile were compared with 10 out of 38 women in control group, while 22 of patients group were compared to 28 of control groups with vitamin C \geq 25 centile, the ODD ratio was (1.0182), 95% CI (0.344-3.011) and $p = 0.97$.

This goes with a study done by Ansori, et al., (involved 52 subjects separated into 2 groups, the 1st group was with PPRM and the other groups was a control) which concluded that there was no significantly apparent difference of vitamin C levels between patients with PROM and those without [22]. Another study that does not go with ours was done by Tejero, et al., [23] who found an increased risk for PROM associated with a lower concentration of vitamin C (odds ratio: 10.99; 95% confidence interval, 2.40-49.91).

Study done by Sharma, et al., [24] involved taking 40 women (20 patients and 20 control group) all were singleton pregnancies with gestational ages ranging from 28-37 weeks, noticed a diminishing level of vitamin C as the pregnancy progresses and its concentration was low in pregnant with preterm pre-labor rupture of membranes, in this regard; this disagree with our study.

The same disagreement with our study was performed by Osaikhuwuomwen, et al., [25], they showed that vitamin C level in plasma is decreasing with increase in weeks of gestation, and the levels found to be low in patients with PPRM in comparison to pregnant women without PPRM.

Women lived in urban area had significantly lower levels of vitamin C when compared with women who lived in a rural area ($p=0.002$), this may be related to variable dietary intake that is rich with vitamin C.

Correlations of maternal vitamin C level with latency period showed an insignificant negative correlation ($r=0.35$, $p=0.067$).

Simhan, et al., in their study reported vitamin C supplement significantly increase gestational age at time birth, birth-weight, Apgar score of the neonate, and latency period in case PPRM occurs, but the assessment of vitamin C supplement was not part of our study [26].

CONCLUSION

Although no significant correlation of vitamin C deficiency with PPRM was found in our study, higher levels may be attributable to a better outcome and fewer complications.

Recommendations

- More studies with higher sample number are needed to assess the association of maternal vitamin C level with the risk of PPRM
- Evaluation of vitamin C levels in relation to different gestational ages and their effects on the outcome of the pregnancy and associated complications
- More advanced studies needed to be performed about the effect of other micronutrients deficiency on the risk of PPRM
- Improve dietary habits and nutritional state of pregnant women may help in reducing the risk of PPRM

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