ORIGINAL ARTICLE

Vitamin C and IL-6 in women with preterm premature rupture of membranes compared to normal pregnant women — a case-control study

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Abstract

Objective: The study assessed the relationship of plasma ascorbic acid (vitamin C) level and IL-6 with preterm premature rupture of membranes (PPROM) in pregnant women.

Methods: A case-control study was carried out in University Hospital, Baghdad from July 2019 to July 2020. Two groups of pregnant women with a gestational age between 28-36+6 weeks were included. There were 50 PPROM cases, and 50 healthy controls showing uncomplicated pregnancy and intact amniotic membrane. Both groups matched with their body mass index and gestational age. Plasma vitamin C and interleukin-6 (IL-6) were assessed at the time of admission and 48 hours later in the study group while it was measured at the onset of labour in healthy controls. In addition, the culture and sensitivity of the placental membranes after delivery were assessed in both groups.

Results: The mean serum vitamin C value was 2.016±0.15 mg/dl in the PPROM group while it was 5.04±0.22 mg/dl for controls at the time of enrollment. Therefore, women with low vitamin C levels were at a higher risk to have PPROM. The plasma IL-6 mean values were higher in the PPROM group versus healthy controls (18.88±0.31pg/ml vs 5.99±0.12 pg/ml), P <0.0001.

Conclusion: This study highlighted the ability of vitamin C deficiency with the elevated level of IL-6 in pregnant women in the third trimester to predict preterm premature rupture of the membrane.

Keywords: Preterm premature rupture of membranes, Ascorbic acid, Interleukin-6. (JPMA 71: S-45 [Suppl. 8]; 2021)

Introduction

Disruption of the foetal membrane before the beginning of labour and prior to 37 weeks of gestation, is known as preterm premature rupture of membrane (PPROM). The frequency is about 5-10%, with a high perinatal morbidity and mortality due to sepsis and development of post-partum endometritis.[1-4]

PPROM is associated with a high risk of long and short-term complications. The significant morbidity observed as a cause of neonatal sepsis in PPROM are: inherent prematurity, imminent delivery,[5] and intrauterine infection.[6] Several studies have been conducted to decrease the PPROM incidence and increase the survival rate of infants.[7,8] There is marked disruption of the collagen layer, particularly associated with bacterial invasion. The release of the mediators is triggered from intrauterine infection, which causes rupture of the membranes. One of the essential factors responsible for this is the Reactive oxygen species (ROS), which generates an abnormality in the synthesis of collagen structure. Hence the the risk of PPROM increases with its deficiency.

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Ascorbic acid (vitamin C) causes ROS stabilization,[9,10] which is responsible for collagen synthesis, and supports the epithelial layer.[11,12] Macrophages release vitamin C which is accountable for balancing collagen synthesis and degradation, thus participating in PPROM reduction. The normal range of vitamin C level is 0.4-2 mg/dl. Less than 0.4 mg/dl indicates moderate deficiency due to inadequate tissue stores.[13]

Interleukin-6 (IL-6) is a cytokine having a wide variety of biological functions. It is produced by the amnion, chorion, and the macrophages and is regarded as a diagnostic marker for neonatal sepsis in patients with PPROM. The level of IL-6 in plasma can predict infection in patients with PPROM as it is a helpful biomarker for early diagnosis of neonatal sepsis with PROM.[13,14]

Patients and Methods

A case-control study was conducted at the University Hospital, Baghdad from July 2019 to July 2020. The study group included 50 hospitalized pregnant women at 28-36+6 weeks and diagnosed with PPROM. The control group included 50 pregnant women matched in preterm birth history, socioeconomic status, gestational age and body mass index. They were inducted from the antenatal clinic and had an uncomplicated pregnancy. The inclusion criteria were pregnant women aged 18-36 years

with a singleton pregnancy with a gestational age of 28-36+6 weeks.

The exclusion criteria were, pregnant women with hypertension, diabetes mellitus, respiratory tract infection and urinary tract infection, multiple pregnancies and polyhydramnios, history of smoking, weakness of cervical tissue with shortening of cervical length < 25mm in cases of obstetrical laceration, cone biopsy, genetic disorders, and uterine anomalies. The other factors were, short time interval (< 6 months) between pregnancies and presence of placenta previa.

Plasma IL-6 and vitamin C were measured after collecting blood from both groups at the time of enrollment before starting any medication. Another measurement was made 48 hours after onset of labour in the PPROM cases and at the onset of labour for the healthy women selected as controls. Verbal and formal consent was taken from all participants. Confirmation of PPROM in the study group was done by inspecting the amniotic fluid in the vagina via a speculum examination. Ultrasound scanning evaluated the foetal wellbeing and confirmed the gestational age, which had been calculated from the day of the last menstrual period. An enzyme-linked assay was used to measure the level of plasma IL-6 after taking 2 ml of maternal venous blood. The spectrophotometer was used to measure the plasma vitamin C level. The placental membranes were taken from all study participants after delivery and sent for culture. The culture was regarded as negative if there was no growth of bacteria 72 hours after the culture. The number and percentage (%) were used to represent the categorical variable, and the mean ±standard deviation and median represent the continuous variables. For all diagnostic factors, the research data were analyzed using a descriptive statistical tool represented by the mean. The test tool (T-test / Mann Whitney) was used for the related variables and the chi-square test tool for discrete variables. The odds ratio (OR), the confidence interval of the mean (95% confidence interval (CI) for the mean), and P-value for OR found by using the chi-square test for two independent samples or more. Statistical analysis was performed by SPSS version 22.0. P-value of <0.05 was considered significant.

Results

The study included 50 pregnant women with PPROM and 50 age and BMI matched healthy controls. Table-1 shows the features of both groups with the plasma levels of vitamin C and IL-6 having no differences in age, gravidity, smoking, and the frequency of Caesarean section among groups, as P-value (0.489, 0.802, 0.941, and 0.815) respectively. Still, there were statistically significant differences regarding the preterm birth history, low socioeconomic status, gestational age, and birth weight between the two groups with P-value <0.0001 for all. The patient with PPROM appeared to have higher gravida, lower socioeconomic status and had a history of preterm labour more than the control, as 33 (66%) women from the PPROM group had a history of preterm birth, and this indicates that the odds ratio (OR) is higher by (46.59) times compared to control. The mean plasma vitamin C values were 2.016±0.15 mg/dL in the PPROM cases, while it was 5.04±0.22 mg/dl for control at the time of enrollment, so patients with low vitamin C level showed a higher risk to have PPROM.

Plasma IL-6 mean value was18.88±0.31 pg/ml vs 5.99±0.12 pg/dl in PPROM and healthy controls, respectively (p< 0.0001). There was a considerable rise in the repeated plasma level of IL-6 48 hours after admission, 35.50±0.35 mg/dl in PPROM, and 6.78±0.14 mg/dl in controls. The cut-off level for vitamin C was <0.3 mg/dL, as for IL-6, it was ≥8pg/mL, which can predict microbial invasion in PPROM proved by placental membrane culture. The number of patients with a positive placental membrane culture was 36(72%) in the study group and 3(6%) patients from the second group.

Table-2 shows a significant rise in the risk of PPROM among pregnant women with high plasma IL-6 level and preterm birth history. The binary logistic regression estimated the

Table-1: Descriptive features for 50 patients in each PPROM and control groups.

Parameters	PPROM group (N.=50)	Control group (N.=50)	OR/(95% CI) for mean	P-value for OR	P-value
Preterm birth history	33(66%)	2(4%)	46.59 (-)	0.0001	<0.0001
Low socio-economic status	39(78%)	6(12%)	26 (-)	0.0001	0.0001
Gestational age at birth (weeks)	32.99±0.38	38.20±0.11	0.000 (4.43-5.10)	0.967	0.0001
Birth weight kilogram (KG)	2.10±0.004	3.53±0.06	0.00 (1.32-1.54)	0.994	0.0001
Plasma ascorbic acid at time of enrolment mg/dL	0.2.016±0.15	5.04±0.22	0.27 (2.35-3.41)	0.0001	0.0001
Plasma level of IL 6 pg/mL	18.88±0.31	5.99±0.12	2.621 (12.22-13.55)	0.014	0.0001
Repeat plasma IL 6pg/mL 48 hours later	35.50±0.35	6.78±0.14	5.449 (28.08-29.56)	0.995	0.0001
No. of patients with low plasma ascorbic acid(c)	50(100%)	5(10%)	1.37 (-)	0.638	0.0001
No. of placental membrane culture (+)(c)	36(72%)	3(6%)	32.54 (-)	0.0001	0.0001

Table-2: Significant predictive factors for preterm premature rupture of membranes based on logistic regression test results for all study participants (N=100).

Parameter	Odd ratio	95% C.I for odd ratio	P- value
Gravidity	1.07	0.65-1.76	0.800
Low socioeconomic status	26	8.79-76.88	0.000(a)
Preterm birth history	46.59	10.08-215.31	0.000(a)
Plasma ascorbic acid at time of enrolment mg/dL	0.27	0.16-0.43	0.000(a)
Plasma level of IL 6 pg/mL	2.621	1.21-5.66	0.014(a)
No. of patients with low serum ascorbic acid at the time of enrolment	1.37	0.37-4.99	0.638

(a) Significant P-value.

odds ratio OR, associated 95% CI and respective P-value to predict the chance of PPROM occurring depending on the study parameters. History of preterm birth scored highest Odd ratio 46.59 for PPROM (10.08-215.31 95% C.I), p<0001, followed by low socioeconomic status, plasma level of IL 6 pg/mL, and plasma ascorbic acid at the time of enrolment being 2.016±0.15 mg/dl made all parameters score a significant p-value < 0.05.

As for the independent variables ,gravidity,patients with low serum ascorbic acid and patients with high IL-6 at the time of enrolment, had no influence on the development (p<0.05) of PPROM.

Discussion

Preterm premature rupture of the membrane is the responsible cause of perinatal mortality and morbidity. Thus, there is a significant elevation in the associated complications of PPROM which increases the associated morbidity and mortality. Earlier studies discussed serum biomarkers and ultrasonic parameters to improve therapeutic and preventative strategies.[15,16] Vitamin C has a role in oxidative stress status as it acts as an antioxidant. In infection, vitamin C stabilizes the ROS released by the phagocytes to control viral and bacterial infection. Hence, a deficiency of vitamin C increases the risk of infection and the risk of PPROM.[17] In most cases of PPROM, low vitamin C levels was the cause and trigger. On the other side, the elevated level of IL-6 with the low vitamin C in the study group at admission was regarded as a marker for microbial invasion and predictor for PPROM. This study found a significantly low level of plasma vitamin C among the patients diagnosed with PPROM.

Nevertheless, just the low level of vitamin C alone is a poor predictor for PPROM. The high levels of IL-6 were found to compliment the development of PPROM as was observed at the time of enrollment and 48 hours later in these pregnant women Interestingly, only a moderate increase in the level of IL-6 was observed among the healthy controls.

A similar study by Sumedha G et al.[18] showed a significant difference in vitamin C levels among the PPROM cases and the healthy control groups with the mean serum levels being (0.60±0.35 mg/dL and 1.18±0.43 mg/dL) respectively. In this study, the 40 samples of placental membranes found culture positive, had 37 from the study group which is similar to our results. Sharma and Mehta et al.[10] reported a significantly low concentration of vitamin C in PPROM patients and the negative correlation with the duration of rupture of membranes. They did not assess the cut-off value of markers which is a point of strength to our study.

Ghomian N et al.[19] evaluated the advantage of adding vitamin C as PPROM prevention in high-risk women. Their study included 170 pregnant women with 14 weeks gestation and a history of PPROM. The patients were divided into two groups. Group-1 was supplemented with daily 100 mg of vitamin C for 14 weeks. The investigators reported the occurrence of PPROM in 44.7% among controls and 31.8% in pregnant women with vitamin C supplement. They confirmed the role of vitamin C in lowering the frequency and prevention of membrane rupture. Further studies are needed to demonstrate powerful biological markers to prevent and assist in the prognosis of PPROM.

Conclusion

The deficiency of vitamin C with higher levels of IL-6 in the third trimester of pregnancy is a strong predictor of preterm premature rupture of the membranes. Females with a past history of PROM and micronutrient deficiency should be treated with replacement therapy to prevent this complication.

Limitation

The sample size for the study was not calculated which could influence the power of analysis.

Acknowledgement: The support provided by the University, Al Mustansiriyah is gratefully acknowledged.

Disclaimer: none.

Conflict of Interest: none to declare by the authors.

Funding Disclosure: none

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