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Original Article

Vitamin A, E, and C levels in maternal blood of patients with idiopathic preterm premature rupture of membranes, spontaneous preterm birth, and term birth

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ABSTRACT

Objectives: To compare vitamin A, E, and C levels in cases with idiopathic preterm premature rupture of membranes (pPROM), idiopathic spontaneous preterm birth with intact membrane (sPTB), and term birth (TB).

Material and Methods: There were three groups in this study: pPROM, sPTB, and TB. The sample size was 60 patients in each group (n = 60, Total = 180). Serum vitamin A and E levels and leucocyte vitamin C levels were measured using commercially available research kits.

Results: The mean (SD) vitamin A levels were 49.56 (18.66) µg/dL in the pPROM group, 48.67 (10.28) µg/dL in the sPTB group, and 52.69 (24.39) µg/dL in the TB group. The mean (SD) vitamin E levels were 19.17 (9.23) µg/dL in the pPROM group, 16.94 (10.17) µg/dL in the sPTB group, and 17.47 (11.19) µg/dL in the TB group. The mean (SD) vitamin C levels were 47.89 (9.53) µM in the pPROM group, 45.78 (7.92) µM in the sPTB group, and 42.35 (6.14) µM in the TB group. Vitamin C levels were significantly higher in mothers who developed pPROM ($p<0.05$) when compared with TB and tended toward significance in mothers who developed sPTB compared with TB.

Conclusion: Vitamin A and E levels were comparable in all three groups. Higher leucocyte vitamin C levels, observed in patients with pPROM (vsTB) and sPTB (vsTB), were not able to protect against pPROM and sPTB. Thus, supplementation of these vitamins during pregnancy is questionable and needs further exploration.

Keywords: Vitamin A, Vitamin E, Vitamin C, pPROM, Fetal membranes

INTRODUCTION

Poor nutritional status is linked to poor pregnancy outcomes. Micronutrients like vitamins and minerals are required for the normal development of not only the fetus but also of the extra fetal tissue, which helps support the conceptus throughout pregnancy. Micronutrient deficiency can affect both the growth of the fetus (leading to Intrauterine Growth Restriction (IUGR)) and the integrity of fetal membranes.[1] Preterm premature rupture of membranes (pPROM) is such a condition arising due to premature weakening of fetal membranes prior to completion of gestation. It is defined as a rupture of membranes from the age of viability to 36 weeks plus 6 days of gestation and prior to the start of delivery.[2] One of the most common complications associated with pPROM is preterm birth. Preterm birth is defined as any birth that occurs before the completion of 37 weeks gestation.[3] Established risk factors of pPROM include prior preterm labor, cigarette smoking, polyhydramnios, urinary and sexually transmitted infections, prior PROM, work during pregnancy, low body mass index, bleeding, low socio-economic status, etc. However, in many cases, risk factors still remain unknown.[4]

Vitamin A and E are fat-soluble vitamins mainly obtained from dietary sources. Vitamin A is mainly responsible for vision, but its isoforms also play a role in keratinization and regulation of gene expression. Vitamin E is an important

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lipid-phase antioxidant, a deficiency of which could lead to oxidative stress–induced damage to fetal membranes leading to pPROM.[5]

Vitamin C is a water-soluble vitamin with various physiological roles, most notably increasing the tensile strength of collagen. The health-promoting effects of vitamin C can be attributed to its biological functions as a co-factor for a number of enzymes (hydroxylases involved in collagen synthesis) and as a water-soluble antioxidant. It is involved in the synthesis and degradation of collagen and is important for maintaining the integrity of chorioamniotic membranes.[6] Vitamin C is also recognized as a cofactor for collagen post-transcriptional modification[7] and a down regulator of the gene transcription of type IV collagenase, matrix metalloproteinase 2 in the amnion cells.[8] A study has suggested that altered patterns of collagen synthesis and diminished leucocyte concentration of vitamin C in gestation have been associated with the subsequent occurrence of pPROM.[9] This indicates that vitamin C probably plays a role in the maintenance of fetal membranes. Therefore, the prediction and prevention of pPROM would offer the best opportunity to prevent its complications. Keeping this in mind, this study was designed to estimate the levels of vitamin A, E, and C in patients of pPROM, sPTB, and healthy term birth (TB).

MATERIAL AND METHODS

This study was conducted in the Department of Biochemistry and Department of Obstetrics and Gynecology of a tertiary care center from March 2017 to September 2019 in accordance with guidelines laid down in the Declaration of Helsinki. Ethical clearance was obtained from the Institutional Ethics Committee – Human Research (IEC-HR). Written informed consent was obtained from all the patients.

Sample size calculation

The sample size was first determined by using the values of vitamin C. From our extensive literature search, we did not find any study on vitamins A and E in the groups we were considering. The mean ± SD in term delivery for vitamin C was 0.58±0.05mg/dl. Considering 1 SD as significant difference, a minimum sample size of 22 subjects with 80% power and 2% level of significance was required. Since we have three groups, type I error was considered as per Bonferroni correction (0.05/3+0.017). Keeping in mind the frequency of patients and fixed time period, a sample size of 60 subjects per group was selected.

Participant selection and sampling

This study consisted of three groups. Group 1 included patients with idiopathic pPROM. pPROM was defined as the rupture of membranes prior to $37^{0/7}$ weeks' gestation and was confirmed by tests including amniotic fluid pooling, ultrasound for amniotic volume, and positive Amnisure® dipstick test. Group 2 included idiopathic spontaneous preterm birth with intact membrane (sPTB) and Group 3 included TB. Idiopathic sPTBs were defined as the presence of regular uterine contractions at ≤ $37^{0/7}$ weeks' gestation (2 contractions/10 minutes with documented cervical change) followed by delivery. Normal TBs were age-matched women with normal labor and delivery at term (≥$37^{0/7}$ weeks) without medical or obstetrical complications and no prior history of pPROM or sPTB or previous cesarean section.

In all the three groups, gestational age was determined by the last menstrual period and corroborated by the first-trimester ultrasound. Exclusion criteria were women with preeclampsia, multiple gestations, placental previa, poly- or oligohydramnios, gestational diabetes, and a history of surgical intervention (surgeries like cerclage, laparotomy, and diagnostic interventions like amniocentesis, chorionic villus sampling, etc.) in the present pregnancy. Patients with clinical evidence of chorioamnionitis and those with uterine abnormalities, systemic morbidities, fetal growth restrictions, and fetal anomalies were also excluded. Baseline variables, including age, mean gestational age, socioeconomic status, previous obstetric history, blood pressure, and routine investigations, were recorded. Maternal blood was collected for pPROM (at the time of diagnosis), sPTB (early labor, i.e., cervical dilatation < 3 cm), and normal TBs (early labor, i.e., cervical dilatation < 3 cm).

Estimation of vitamin A, E, & C levels

Vitamin A and E levels were estimated in maternal serum using commercially available research kits (Elabsciences, USA) following manufacturer protocol after appropriate dilution. The detection range of vitamin A ELISA kit was 0.16 –10 ng/mL with a sensitivity of 0.09 ng/mL. The detection range of vitamin E ELISA kit was 1.563–100 µg/mL with a sensitivity of 0.938 µg/mL.

Leucocyte levels of vitamin C were estimated using Ascorbate Assay Research Kit (Cayman Chemical Co., USA), following manufacturer protocol with a detection range of 0–150µM.

Statistical analysis

Data were analyzed with Microsoft Excel as well as SPSS version 20. Quantitative variables were analyzed as mean and standard deviation (SD). Detection of difference was done by one-way ANOVA test. $p < 0.05$ indicated a statistically significant difference.

RESULTS

General baseline characteristics of study group:

All participants belonged to the lower or lower middle socioeconomic status according to the Kuppuswamy's scale. There was no history of low birth weight or abortions and all were housewives.

The general baseline variables and biochemical characteristics of the participants are summarized in Table 1. The mean levels of vitamins A, E, and C in the three groups are depicted in Tables 2a–2c. There was no significant difference in the serum levels of vitamins A and E in all three groups. The leucocyte levels of vitamin C are significantly higher in pPROM than TB [$p<0.05$], while leucocyte levels of vitamin C tended toward

Table 1: Patient parameters (Demographic profile, serum total calcium and serum vitamin D levels) in TB, sPTB and pPROM.

	TB Mean (SD) (n=60)	sPTB Mean (SD) (n=60)	pPROM Mean (SD) (n=60)	p value
Age (years)	23.98 (3.05)	23.77 (2.44)	24.42 (3.43)	TB: sPTB=0.924 TB: pPROM=0.730 sPTB: pPROM=0.494
Mean gestational age in weeks	39.00 (1.15)	35.34 (1.63)	32.73 (2.11)	TB: sPTB =0.000* TB: pPROM=0.000* sPTB :pPROM=0.000*
Total calcium (mg/dL)	8.69 (0.54)	8.65 (0.52)	8.79 (0.60)	TB: sPTB =0.912 TB: pPROM=0.640 sPTB: pPROM=0.390
Serum vitamin D (ng/ml)	17.38 (6.65)	18.83 (6.07)	13.75 (7.21)	TB: sPTB =0.462 TB: pPROM=0.009* sPTB: pPROM=0.000*

*Statistically significant ($p<0.05$); Patients' parameters (group wise, n = 60 in each, total = 180) are shown as mean (SD). pPROM: Idiopathic preterm prelabor rupture of membranes, sPTB: idiopathic spontaneous preterm birth with intact membrane, and TB: term birth, SD: Standard deviation.

Table 2a: Vitamin A levels (µg/dL) in pPROM, TB and sPTB.

Vitamin A levels (µg/dL)	No. of subjects (n=180)	Mean (SD)	p value
TB	60	52.69(24.39)	TB-sPTB=0.467 TB-pPROM=0.964 sPTB-pPROM = 0.628
sPTB	60	48.67(10.28)	
pPROM	60	49.56(18.66)	

Statistically significant ($p<0.05$); pPROM: Preterm prelabor rupture of membranes, sPTB: Spontaneous preterm birth with intact membrane, and TB: term birth. SD: Standard deviation.

Table 2b: Vitamin E levels (µg/dL) in pPROM, TB, and sPTB.

Vitamin E levels (µg/dL)	No. of Subjects (n=180)	Mean (SD)	p value
TB	60	17.47(11.19)	TB-sPTB=0.957 TB-pPROM=0.634 sPTB-pPROM = 0.459
sPTB	60	16.94(10.17)	
pPROM	60	19.17(9.23)	

Statistically significant ($p<0.05$); pPROM: Preterm prelabor rupture of membranes, sPTB: Spontaneous preterm birth with intact membrane, and TB: term birth. SD: Standard deviation.

Table 2c: Vitamin C levels (µM) in pPROM, TB, and sPTB.

Vitamin C levels (µM)	No. of subjects (n=180)	Mean (SD)	p value
TB	60	42.35(6.14)	TB-sPTB=0.051 **TB-pPROM=0.001*** sPTB-pPROM = 0.322
sPTB	60	45.78(7.92)	
pPROM	60	47.89(9.53)	

*Statistically significant ($p<0.05$); pPROM: Preterm prelabor rupture of membranes, sPTB: Spontaneous preterm birth with intact membrane, and TB: term birth. SD: Standard deviation, Bold: signifies a highly statistically significant difference.

significance in mothers who developed sPTB compared with TB [p = 0.051].

DISCUSSION

pPROM is a multifactorial disease characterized by defects in collagen synthesis causing loss in tensile strength, resulting in a weak chorioamniotic membrane.[10]

In this study, we did not find any significant difference between vitamin A & E levels between the three groups.A study[11] has reported that vitamin A supplementation has no effect on the development of preterm birth while another study[12] demonstrated no difference in the levels of retinol in amniotic fluid and serum between pPROM and healthy pregnant controls. In our study, it has been further shown that there is no difference in the vitamin A levels between pPROM, sPTB, and TB. Thus, supplementing vitamin A for the prevention of pPROM and, in addition, sPTB may not be beneficial.

In our study, the levels of vitamin E were comparable in all three groups. The findings are similar to the previous studies done[12, 13, 14] Thus, supplementing vitamin E for the prevention of pPROM and sPTB may not be beneficial.

Vitamin C is known to play a key role in the synthesis of collagen and a deficiency could lead to premature weakening of membranes. In our study, we report a higher leucocyte level of vitamin C in cases of pPROM compared to sPTB and TB groups and in sPTB compared to TB. There was no statistically significant difference between sPTB and pPROM.

The exact role of vitamin C in pPROM is still a gray area, with some studies[15-17] reporting lower serum vitamin C levels in cases of pPROM and others[18-20] reporting no difference in serum vitamin C levels in cases when compared to healthy controls. These studies have also not reported the nutritional intake of patients and have measured vitamin C levels in serum, which are considered to be an inaccurate test when compared to leucocyte vitamin C levels as they are easily affected by acute intake of the vitamin via diet.[21] A study[22] has also suggested that measuring vitamin C levels isn't reliable due to high instability. Our finding is contrary to the findings of studies done till date. A possible explanation for our finding is the effect of gestational age. It was observed that the mean gestational age (weeks) for pPROM was significantly lower ($p<0.000$), i.e., 32.73 (2.11), and that of sPTB was 35.34 (1.63), and for TB it was 39.00 (1.15). Vitamin C levels fall as pregnancy advances; higher levels in the pPROM group could be explained by the early gestational age in these patients.

Reference values of vitamins A, E, and C in adults are 30–80 μg/dL, 0.5–1.8 mg/dl, and 0.4-1.5mg/dL, respectively.[23] Their values are known to change as pregnancy advances, and no clear deficiency cut-offs exist. We, therefore cannot compare our values with the reference value in addition to the fact that research-based kits were used in this study.

CONCLUSION

Vitamin A and E levels were comparable in all three groups. Higher leucocyte vitamin C levels, observed in patients with pPROM (vsTB) and sPTB (vsTB), were not able to protect against pPROM and sPTB. More detailed studies should be performed before recommendations on supplementation of these vitamins in patients of pPROM.

The following were the limitations of our study: Single sample was obtained and analyzed at the time of delivery, while vitamin C levels in pre-pregnant, pregnant, and post-pregnant states and fetal levels were not measured. It is also worthwhile to measure and compare vitamin C levels in gestational age-matched controls.

Implications and future prospects: Probably vitamin A and E supplementation is not required nor will it be of any benefit in patients of pPROM. Our findings suggest that high vitamin C levels were not able to protect against pPROM or sPTB. Supplementation of vitamins A, E, and C is hence questionable and must be done with caution. Vitamins A & E being fat-soluble are stored in the body and therefore excess could lead to hypervitaminosis and vitamin C is known to be toxic at higher doses. Therefore, supplementation should be scientific.

Ethical approval

The research/study is approved by the Institutional Ethics Committee at Human Research of University College of Medical Sciences, number IEC-HR071015R1, dated 08th November 2024.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent

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Conflicts of interest

Dr. Rajarshi Kar and Dr. Amita Suneja are on the Editorial Board of the Journal.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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