The low level of plasma vitamin C as a risk factor of preterm premature rupture of membrane

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ABSTRACT

Introduction: Preterm premature rupture of membrane (PPROM) still becomes a problem related with perinatal morbidity and mortality which is caused by multifactorial risk factor; especially strength of foetal membrane. Collagen is the main component of foetal membrane's extracellular matrix whereas plasma vitamin C roles as a collagen biosynthesis enzyme-dependent co-factor, down-regulator activity of MMP-1, MMP-2, and MMP-9 and also as antioxidant in remodelling and preventing structural damage extracellular matrix. The purpose of this study is to prove the low plasma level of vitamin C as a risk factor for PPROM on preterm pregnancy.

Method: This study was an analytical observational unpaired case-control performed from March 23rd until August 23rd 2018 at Polyclinic and Emergency Unit of Obstetrics and Gynaecology Sanglah Central General Hospital, Buleleng General Hospital, Mangusada General Hospital, Sanjiwani General Hospital, Wangaya General Hospital, and Prodia Clinical Laboratory Denpasar. The samples were preterm pregnancy women with premature rupture of membrane (PROM) as a case group and without premature rupture of membrane as a control group. The samples were taken from peripheral venous blood and level of plasma vitamin C is measured by HPLC method. The data were analysed by SPSS for Windows 20.0 version program then presented as table and narrative.

Result: Forty subjects were divided into 2 groups, 20 preterm pregnancy with PROM as a case group and 20 preterm pregnancy without PROM as a control group. Characteristic subject based on maternal age, gestational age, parity of both groups was 27.4 and 26.1 years old, 31 and 31 weeks, and also 1 and 1 time (p>0.05), not significantly different. The mean level of plasma vitamin C on case and control group were 3.90 ± 1.61 dan 9.24 ± 2.31 mg/L (p=0.001). On the case group the low level of plasma vitamin C was 51 times (OR= 51; CI 95% = 7.57–343.73; p=0,001) higher than the control group.

Conclusion: The low level of plasma vitamin C is a risk factor of PPROM.

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INTRODUCTION

Maternal mortality rate (MMR) and infant mortality rate (IMR) are indicators of a country's health status. In Indonesia, the largest contributor of MMR is bleeding, infection and preeclampsia, while the highest contributor of IMR is perinatal mortality related to prematurity, asphyxia, diarrhoea and pneumonia as well as several other infectious diseases. Prematurity itself is mostly related to the occurrence of premature rupture of membranes (PROM) therefore, the role of PROM in preterm pregnancy is wide.

The prevalence of PROM ranges from 10 - 20% of pregnancies and the prevalence of preterm PROM consists of around 3-5% of pregnancy.[1,2] The reported prevalence of PROM at Polyclinic and Emergency Unit of Department Obstetrics and Gynaecology Sanglah Central General Hospital Denpasar is 12.92% which consists of 83.23% of term premature rupture of membranes and 16.77% preterm premature rupture of membranes (PPROM).[3]

Efforts to prevent PPROM is urgently needed to reduce maternal and perinatal morbidity and mortality rates. The cause of PPROM is still not fully known yet.[2-3] The main mechanism of the occurrence of PROM is damage to the integrity of the amniotic membrane, namely increased collagenolytic activity, decreased biosynthesis of the amniotic membrane collagen itself, and increased oxidative stress.[4-6] Decreased collagen concentration occurring too quickly is also thought to be a cause of PPROM.

Vitamin C is a micronutrient that plays a role in biosynthesis and control of collagen degradation, and it also has an antioxidant effect.[6,7] Plasma vitamin C level in pregnancy is thought to be related to the incidence of PROM. Therefore the study of the effect of plasma vitamin C level on the risk of PPROM was created. The aim of study was to determine the average plasma vitamin C level and prove that low vitamin C level was a risk factor for the occurrence of PPROM.

METODE

The design of this study is an observational analytic study with unpaired case-control, hospital-based was conducted at Polyclinic and Emergency Unit of Obstetrics and Gynaecology Sanglah Hospital in Denpasar and affiliated teaching hospitals from March 23rd to August 23rd 2018. The subjects (intended sample) were a pregnant woman with a gestational age of 20 weeks 0 day to 36 weeks and 6 days, who visits to Polyclinic and Emergency Unit of Obstetrics and Gynaecology at Sanglah Hospital Denpasar, Buleleng General Hospital, Mangusada General Hospital, Sanjiwani General Hospital, Wangaya General Hospital that met the inclusion and exclusion criteria. The research sample was 20 samples for the preterm group with PROM and 20 samples for the preterm group without PROM with a total of 40 samples. The subjects filled out questionnaires and their peripheral venous blood was taken to assess plasma vitamin C level. This was done by the Prodia Clinical Laboratory. The data was then processed using the computer program: statistical product and service solution (SPSS) for Windows version 20.0.

RESULTS

In this study a t-independent Test was performed on the variable of maternal age because the data was normally distributed. Variables in gestational age and parity were analysed with the Mann-Whitney Test because the data was not normally distributed. The results of the analysis were presented in Table 1.

The average distribution of plasma vitamin C level was normally distributed (Shapiro Wilk Test for a sample < 50, p> 0.05) so the t-Independent Test was chosen to determine the differences in the mean plasma vitamin C level in the PROM group and without the PROM group. The results of the analysis are presented in Table 2.

Based on the ROC curve, the value of the area under the curve is determined, then plasma vitamin C level is categorised into low and high based on the threshold value determined by calculating the maximum value of 1- (sensitivity + specificity) of each point of the ROC curve. From these calculations, we obtained a threshold value of 6.45 mg/L with a sensitivity of 85% and a specificity of 90%. This means that level below the threshold value is categorized as low plasma vitamin C level and level higher or equal to threshold values is categorised as high plasma vitamin C level.

The Chi-Square test was used to determine the role of plasma vitamin C and the risk of preterm PROM. The results of the analysis are presented in Table 3.

Table 1 **Characteristics of Subjects in the Preterm Pregnancy with PROM and without PROM Groups**

Variable	Case (n = 20) mean + SD	Control (n = 20) mean + SD	p
Mother's age (years)	27.4+5.6	26.1+5.4	0.444a
Gestational age (weeks)	31 (22-34)	32 (21-34)	0.848b
Parity (children)	1 (0-2)	1 (0-3)	0.667b

Description: a). t-Independent Test, b). Mann-Whitney Test.

Table 2 **Mean Result of Vitamin C Plasma Level Between the PROM Group and Those without PROM In Preterm pregnancy**

Variable	Groups		p
	Case (n = 20) mean ± SD	Control (n = 20) mean ± SD	
Vitamin C Plasma Level	3.90 ± 1.61	9.24 ± 2.31	0.001

Table 3 **Plasma Vitamin C Content Relationship with the Occurrence of PPROM**

		Groups				
		Case (n=20)	Control (n=20)	OR	CI 95%	P
Vitamin C Plasma Level	Low	17	2	51	7.57-343.73	0.001
	High	3	18			

DISCUSSION

The age of the mother influences the incidence of PPROM. According to the research of Okeke et al., it was stated that the incidence of PPROM was most common in the 21-30 years age group and the lowest in the 16-20 years and 41-45 years age group. However, in this study, no differences in maternal age were found between the PROM group and without the PROM group.[8] In the Negara et al. study, it was found that the mean age of expectant mothers was younger than in this study, where the age of women with PROM averaged at 26.59 years, and the average age of women without PROM was 28.72 years.[9] The results of this study was also different from the research by Patil et al. which was done with 100 mothers giving birth with PROM and 100 mothers giving birth without PROM, the average age was younger, that is 23.0 ± 3.5 years (with PROM) and 23.4 ± 2.9 years (without PROM) respectively, which is statistically not significant.[10]

Gestational age affects the incidence of PPROM. In the research of Okeke et al., it states that the incidence of PPROM is most common at 35-37 weeks and rarely occurs at 26-30 weeks. However, in this study there were no differences in the gestational age between the group with PROM and without PROM.[8] This is consistent with the research of Negara et al., which shows the significance of the

level of difference in gestational age between the PROM group and without PROM of 0.898 (p> 0.05) with the conclusion that the difference of the mean gestational age in the groups with PROM and without PROM is not statistically significant.[9]

Research by Okeke et al. mentions that PPROM most often occurs in primigravids. However, the results of this study indicate that the number of group parity with PROM is not significantly different from the number of group parity without the KPD.[8] The results of this study are consistent with the research findings by Patil et al., where the occurrence of primigravids with PROM was 53 cases out of 100 births, compared to primigravids without PROM; 52 cases out of 100 cases - which is not significantly different.[10] This shows that the characteristics of subjects are based on maternal age, gestational age, and parity is a risk factor that causes rupture of the membranes in this study.

Vitamin C is a micronutrient that plays a role in the synthesis and control of collagen degradation and has antioxidant effects.[5,11-13] Vitamin C is an enzyme cofactor for collagen biosynthesis.[14-16] Decrease in plasma vitamin C level will cause the structure of collagen to be formed imperfectly, to be weaker, and easily destroyed in the amniotic membranes.[17] Several studies have been conducted to determine the effect of vitamin C on PPROM. However the results have been varied.[18-20] Therefore, a threshold value is needed to differentiate pregnant women with or without low plasma vitamin C level and to prove whether there is a relationship between low plasma vitamin C level and an increased risk of premature rupture of membranes in preterm pregnancy. This study shows that a plasma vitamin C level of 6.45 mg/L can be used as a threshold value to determine the status of a high plasma vitamin C level in preterm pregnancy. This study also proves that low plasma vitamin C level increases the risk of the incidence of PPROM.

Research on the differences in plasma vitamin C level has been done before, but show different results.[21] According to a research that was conducted in India, measured serum vitamin C level was found to be lower in PPROM compared to normal preterm pregnancies, 0.41 ± 0.08 mg/dL versus 0.84 ± 0.19 mg/dL. They also found a significant linear decrease with increasing gestational age.[22] The same was reported by Osaikhuwuomwen et al., where plasma vitamin C level decrease with increasing gestational age and term pregnancies, and a lower plasma vitamin C level is found in PPROM compared to preterm pregnancies without PROM, at 0.53 ± 0.05 mg/dL and 0.58 ± 0.05 mg/dL, respectively.[23]

Threshold level of vitamin C varies in their effects toward those with PROM and without PROM. The difference in vitamin C level in the occurrence of PROM varies greatly from various studies. This is also influenced by race, diet, and dietary intake of high vitamin C content. The results of this study reinforce recommendations that vitamin C supplementation is considered important for pregnant women to prevent PPROM.

CONCLUSION

Based on the results of the research that have been carried out, it can be concluded that there is a significant difference in the average level of plasma vitamin C between those with PROM and without PROM in preterm pregnancies, with a difference of 3.9 ± 1.61 mg/L and 9.24 ± 2.31 mg/L respectively. Low plasma vitamin C level increases the risk of developing PROM by 51 times compared to those without PROM in preterm pregnancy. Low plasma vitamin C level is a high-risk factor for PROM in preterm pregnancies after accounting for maternal age, gestational age, and parity.

CONFLICT OF INTEREST

The author declares there is no conflict of interest regarding publication of current study.

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