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**DENGAN JUDUL**  
**“Differences In Blood Plasma Levels of Vitamin C In Term Pregnancy With Premature Rupture Of Membranes And Blood Plasma Levels of Vitamin C In Term Pregnancy Without Premature Rupture of Membranes”**

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**Differences In Blood Plasma Levels of Vitamin C In Term Pregnancy With Premature Rupture Of Membranes And Blood Plasma Levels of Vitamin C In Term Pregnancy Without Premature Rupture of Membranes**

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***Abstract***

Premature rupture of membranes is the most common complications of pregnancy. The incidence of premature rupture of membranes in pregnancy ranged from 6% to 10% and 20% of these cases occur before 37 weeks gestation. The incidence of premature rupture of membranes in Indonesia ranged from 4.5% to 7.6% of all pregnancies. This research was conducted by cross sectional method to determine the differences in the blood plasma levels of vitamin C in term pregnancy premature rupture of membranes with blood plasma levels of vitamin C in term pregnancy without premature rupture of membranes in M. Jamil Padang hospital, Achmad Muchtar Bukittinggi Hospital, and Pariaman Hospital. There were significant difference in blood plasma levels of vitamin C in term pregnancy with premature rupture of membranes and term pregnancy without premature rupture of membranes (  $P < 0.05$ ). Mean levels of vitamin C in blood plasma at term pregnancy with premature rupture of membranes were lower than in the blood plasma levels of vitamin C in term pregnancy without premature rupture of membranes.

***Keywords:*** *Premature rupture of membrane in term pregnancy, levels of vitamin C in blood plasma*

**INTRODUCTION**

Premature rupture of membrane is the most common complications of pregnancy. The incidence of premature rupture of membranes ranges from 6% to 10% and 20% from this case occurred before 37th weeks of pregnancy. The incidence of premature rupture of membranes in Indonesia ranges from 4.5% to 7.6% of all pregnancies.<sup>1</sup>

Premature rupture of membranes caused higher complication in preterm and aterm pregnancies. Infection risk after occurred premature rupture of membranes affect to the mothers, fetals, or neonates. The incidence of infection in neonates after 24 hours premature

rupture of membranes approximately 1% and increasing clinical risk about 3% to 5% if there are chorioamnionitis.<sup>2</sup>

Chorioamniotic have many layers and complexity and consist of epithelial and supporting tissues which each component hasan important role in the metabolism for phisiological integrity for the development of pregnancy. Amnion gain strength through collagen. The mechanism how fetal membrane weakening by exogenous and endogenous still

in an active investigation. Endogenous factor, such as local variations in thinning membranes or collagen, and exogenous factors such as the effect caused by microbial metabolism, host, or due nicotine that reduces antiprotease activity also caused disruption to local membrane.<sup>3</sup>

The strength and integrity of chorioamniotic are mantained by the balance of intrinsic factors to regulate synthesis and degradation of connective tissues. Degradation of collagen in chorioamniotic controlled by metalloproteinases matrix. The release of metalloproteinase marix regulated by tissue inhibitors of metalloproteinases or TIMPS.<sup>1</sup>

Unstable molecules which are produced continously in body, known as reactive oxygen species (ROS) are said to be able causing the tissue damage that causes premature rupture of membrane (PROM). Chorioamniotic exposure with ROS are said will increase metalloproteinase thus causing PROM. Normally there is a balance between production and elimination of ROS.

Oxidative stress occurs when prooxidant exceeds antioxidants.<sup>4</sup>

Vitamin C (Ascorbid acid) is a water-soluble vitamin that is not synthesized by humans, therefore this essential vitamin must be obtained by food. As we know, vitamin C is one of the antioxidants. The body using many antioxidants to limit tissue damage caused by free radicals. Ascorbic acid stimulate synthesis of collagen directly. Ascorbid acid also acts as a reducing agent that send a hydrogen atom with electron to ROS. Ascorbic acid make strong and stable collagen.<sup>5</sup>

Oxidative stress occurs when prooxidant exceeds antioxidants and lead to PROM. One of the roles of Vitamin C is send a hydrogen atom and the electrons to ROS, then it make collagen stronger and stable. Thus, we want to compare vitamin C level in blood plasma in term pregnancies with PROM and without PROM in three hospitals of West Sumatera: M. Djamil Hospital Padang, Achmad Mochtar Hospital Bukittinggi, and Pariaman Hospital.

## METHODS

This study was conducted by cross sectional method to determine the differences in the blood plasma levels of vitamin C in term pregnancy with premature rupture of membranes and blood plasma levels of vitamin C in term pregnancy without premature rupture of membranes in M. Jamil Padang hospital, Achmad Muchtar Bukittinggi Hospital, and Pariaman Hospital.

On the subject that corresponded to the inclusion and exclusion criteria, we took 5 cc blood sample, inserted into 5 cc vacutainer tubes, sentrifuged, then 2 cc the serum was separated, and was inserted into microtube and sent to the laboratorium to see the level of vitamin C in the blood plasma. The examination used a special serum Kit ascorbic acid type KT671-100 using chromatography methods. The result was recorded and then conducted data analysis.

## RESULTS

**Table 1.** Characteristics of Samples

	Without PROM	With PROM	Total	P
Age	29,47 ± 5,22 tahun	29,00 ± 5,53 tahun		0,600
Parity	Nullipara 26 (36,1%)	29 (40,3%)	55 (38,19%)	0,153
	Multipara 46 (63,9%)	43 (59,7%)	89 (61,81%)	
Smoking	Yes 1 (1,4%)	4 (5,6%)	5 (3,47%)	0,174
	Tidak 71 (98,6%)	68 (94,4%)	139 (96,53%)	
Infection	Ya 1 (1,4%)	7 (9,8%)	8 (5,55%)	0,002

Study has been performed to compare levels of Vitamin C in blood plasma in term pregnancies with premature rupture of membrane and without premature rupture of membrane in some hospitals in West Sumatera. This study was conducted on pregnancies women who came to maternity room Dr. M. Djamil Hospital Padang, Achmad Mochtar Hospital Bukittinggi, and Pariaman Hospital. Examination was conducted in the Health Laboratory in Padang in period March to September 2014. Total number of women who were included in the statistic calculation after metthe criteria of inclusion and exclusion were 144 patients, divided into two groups, 72 patients on group of term prenanancies with premature rupture of membrane and 72 patients without premature rupture of membrane.

From 144 samples that met the inclusion and exclusion criteria, we obtained characteristics of the sample based on age, parity, smoking, and infection as shown in table 1.

The result of statistical analysis as shown in Table 1. Based on the age of sample, age average nearly the same between pregnant woman with PROM and pregnant woman without PROM. The result of further statistical analysis, the difference between pregnant woman with PROM and pregnant woman without PROM did not have significant differences, this can be seen from p value 0,600 ( $p > 0,05$ ).

In nullipara, the samples of woman in term pregnancy without PROM were less than woman with PROM. In multipara, samples of term pregnancy without PROM were more than term pregnancy with PROM. Further statistical analysis results, parity on term pregnancy without PROM and with PROM did not have significant differences. This can be seen from the p value 0,153 ( $p > 0,05$ ).

Women in term pregnancy with PROM who smoke were greater than women in term pregnancy without PROM who smoke. While women in term pregnancy with PROM who didnot smoke is lower than women in term pregnancy without PROM who smoke. T-test result obtained the distinction of smoking between woman in term pregnancy with PROM and without PROM did not have significant differences, this can be seen from p value 0,174 ( $p > 0,05$ ).

Women in term pregnancy without PROM had less infection than women with PROM. The further statistical result seen that there were significant discrepancies of infection (leukocyte levels) between women in term pregnancies without PROM and women in term pregnancies with PROM. This can be seen from p value 0,002 ( $p < 0,05$ ).

**Table 2.** The differences in blood plasma levels of vitamin C in term pregnancy with PROM and term pregnancy without PROM.

	Without PROM n = 72	PROM n = 72	P
<b>Minimum</b>	0,35 nmol/mL	1,78nmol/mL	
<b>Maximum</b>	306,43nmol/mL	210,71nmol/mL	
<b>Mean Level of vitamin C</b>	97,56 nmol/mL	60,07 nmol/L	<b>0.001</b>
<b>SD</b>	78,28 nmol/mL	50,33 nmol/L	

Vitamin C level in blood plasma in term pregnant without PROM have higher

level compared to term pregnant with PROM. Normality test was performed by

using Kolmogorov Smirnov and the results was normal. This can be seen from P value of vitamin C levels in the blood plasma in term pregnancy without PROM (0,110) and P value of vitamin C levels in the blood plasma in term pregnancy with PROM (0.174) ( $p > 0.005$ ), therefore T-test can be done. The result of the statistical analysis with T-test obtained wide distinction average level of vitamin C blood plasma of term pregnancy with PROM. This can be seen from P-value 0,001 ( $p < 0.05$ ).

The highest level of Vitamin C in blood plasma in term pregnancy without PROM ranged between 80-90 nmol/mL whereas the highest level of vitamin C blood plasma in term pregnancy with PROM ranged between 10-20 nmol/mL.

## DISCUSSION

Data analysis of characteristics sample in this study resulting that PROM were more frequently occurred in women who smoke, but this result were not meaningful statistically ( $p > 0.05$ ). This might be because smoker women in Eastern countries such as Indonesia are not as much as Western countries and the data that obtained here were passive smokers. Therefore, to assess whether smoking is associated with the occurrence of PROM or not, we should have larger number of samples. Risk of

PROM increased two times in women who smoke during pregnancy. Mothers who smoke were associated with an increased incidence of PROM. Results from a large case control study showed that there were an increased risk of PROM with smoking (OR = 2.2, 95% CI, 1.4-3.5) can be reduced by quitting smoking before conception (OR = 1.4, 95% CI, 0.9-2.0) and in the first trimester (OR = 1.6, 95% CI, 0.8-2.9).<sup>6</sup>

Gosselink et al found no relationship of smoking (>10 cigarette per day) and PROM (OR = 0.94, 95% CI 0.48,-1.8). Pathophysiology mechanism that smoking could caused PROM are nicotine and main metabolite cotinine and carbon monoxide, hydrogen cyanide, nitrogen oxides and other components of tobacco smoke is distributed to tissues and fluids throughout the body. Smoking increases risk of PROM with disruption from cytokines, decreasing function of leukocytes, changes the essential nutritional factors, and through the malfunctioning dan developments of a normal placenta. Smoking may also damage the immune function of systemic and local reproductive tract. Nicotine and cotinine concentrated in cervical mucus compared with serum and the constituents of cigarette smoke also interfere the endogenous activity of

antibacterial and can interfere with approaching macrophages to microbes.

The results of the characteristic samples data analysis in this study was obtained in patients with infection (levels of blood leukocyt $>16,900/\text{mm}^3$ ), occurrence of term pregnant women with PROM occurred more frequently than term pregnant women without PROM. There was a term pregnant woman aterm without PROM with infection in sample number 45 (annex 6) with the levels of vitamin C in blood plasma 13.21 nmol/L, while in term pregnant women with the levels of vitamin C in blood plasma there were 7 people with sample number 83, 87, 105, 106, 107, 124 and 142 with blood plasma levels of vitamin C in a row i.e 3.21 nmol/mL, 159.28 nmol/mL, 22.5 nmol/mL, 13.92 nmol/mL, 35.35 nmol/mL, 46.07 nmol/mL and 57.14 nmol/mL.

Infection was said to be able to decrease the levels of vitamin C. In this study on a sample number 45 were term pregnancy without PROM with infections, we found the levels of vitamin C are fairly low (13.21 nmol/mL) and in term pregnancy with PROM with infections we found the levels of vitamin C blood plasma ranged 60 nmol/mL < but in sample number 87 (on term pregnancy with PROM with infection) we found blood plasma levels of vitamin C 159.28

nmol/mL. Therefore to assess whether the infection levels of vitamin C lowers blood plasma samples, we need more study because only 5.55% case showed that there was an infection. The infection caused a decrease in the levels of vitamin C due to things that can't be explained, Mac fine (1977) said this is due to the effect of the migration of polimorfonuklear which is rich in vitamin C to the site of infection with consequences the replacement with immature leukocyte which have relatively low of vitamin C levels. On the sample number 124 (infections and smoking) we found the blood plasma levels of vitamin C 46.07 nmol/mL.

The results of this study show that there was a difference in the blood plasma of vitamin C level of pregnant term with PROM and pregnant term without PROM that the levels of vitamin C in blood plasma of pregnant term with PROM were lower than pregnant term without PROM. Studies comparing the levels of vitamin C in blood plasma of pregnant term between with PROM and without PROM are not quite performed. But, several previous studies shows the opposite results that the level of vitamin C in blood plasma of pregnant term with PROM have higher levels of vitamin C compared to blood plasma of pregnant term without PROM.<sup>7</sup>

Previous study that have similar result was conducted by E. Casanueva (1998), said the levels of vitamin C  $< 1,8 \mu\text{g}/10^8$  cells increased the risk of occurrence of PROM, and on this level at 28 weeks gestational age showed a high predictive value ( $p < 0.05$ ). Study by Tejero et al. (2003) showed vitamin C level in term pregnant women with PROM were lower than term pregnant women without PROM. Contrary to research Barret et al (1994) found no relationship between low levels of vitamin C with PROM and in the research said the administration supplement of vitamin C cannot prevent the incidence of PROM though on the study said it needed a larger sample amount to assess whether administering vitamin C supplementation was associated with PROM. Study by Rizka (2011) shown the opposite thing on the result that the levels of vitamin C in term pregnancy with PROM was higher compared to term pregnant without PROM.

The literature said the normal vitamin C in blood plasma is  $26.1-84,6 \mu\text{mol}/\text{L}$  ( $>0,6 \text{ mg}/\text{dl}$ ,  $>20 \mu\text{g}/10^8$ ,  $>114 \text{ nmol}/10^8$  cells) and have a deficiency if  $<11 \mu\text{mol}/\text{L}$  ( $0, 2 \text{ mg}/\text{dl}$ ,  $<10 \mu\text{g}/10^8$  cells,  $<57 \text{ nmol}/10^8$  cells). From the results of this study are obtained wide range that the mean value of vitamin C level in

blood plasma of term pregnancy with PROM  $60.07 \pm 50.33 \text{ nmol}/\text{mL}$ , with the smallest value  $1.78 \text{ nmol}/\text{mL}$  and the greatest value  $210.71 \text{ nmol}/\text{mL}$  and mean value of vitamin C levels in blood plasma of term pregnancy without PROM  $\pm 101.01 78.28 \text{ nmol}/\text{mL}$ , with the smallest value  $0.35 \text{ nmol}/\text{mL}$  and the greatest value  $306.43 \text{ nmol}/\text{mL}$ . But if we see from appendix 7, there were  $58,73\%$  ( $<90 \text{ nmp}/\text{mL}$ ) of vitamin C levels in blood plasma of term pregnant without PROM, and  $58.33\%$  levels of vitamin C in blood plasma of term pregnancy without PROM ( $< 60 \text{ nmol}/\text{mL}$ ).

Vitamin C (Ascorbic acid) is a water-soluble vitamin that is not synthesized by human. Therefore this essential vitamin must be obtained from food. As we know that vitamin C is one of the anti oxidants. Ascorbic acid directly stimulate synthesis of collagen. The function of Ascorbic acid is reducing agent to send a hydrogen atom with the electrons to the ROS with single-pair electron on the outer ring. ROS that paired with electrons in the outer rings become stabilized. Ascorbic acid makes a strong and stable collagen.<sup>6</sup> ascorbic acid also affects the expression of metalloproteinase 2 (MMP-2). In the analysis on medium culture, MMP-2



mRNA is decreasing with the addition of Ascorbic acid. This experiment show an increase in the production of collagen I as a response from ascorbic acid exposure. In addition, the function of vitamin C that is its involvement in lysine and proline hydroxylation at procollagen residue. This reaction is catalyzed by prolylhydroxylase such as lysylhydroxylase which require ascorbic and Fe. This step occurs on fibroblasts and has been observed on cell culture amniotic membrane. Ascorbic also modulate mRNA expression in the synthesis of collagen as well as gene expression of collagen types I, III and X.<sup>8</sup>

## CONCLUSIONS

The mean levels of vitamin C in the blood plasma in term pregnancy without PROM were 97.56 nmol/mL ( $\pm$  78.28 nmol/mL) and in PROM were 60.07 nmol/mL ( $\pm$  50.33 nmol/mL). The mean levels of vitamin C in the blood plasma in term pregnancy without PROM were higher compared to the levels of vitamin C in the blood plasma in term pregnancy without PROM in DR. M. Djamil Hospital Padang, Achmad Mochtar Hospital Bukittinggi and Pariaman Hospital.

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*Cerijanya*



This is to certify that  
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**THE ROLE OF MASTER OF MIDWIFERY IN DEVELOPING  
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1	Dr. dr. Yusrawati, SpOG (K)	Differences Anthropometry Of The Newborn According To Nutritional Status Of Women Before Pregnant	Rabu, 20 April 2016 jam 11.00 s/d 13.00	Sidang A Wing Timur Lantai 4 Gedung Eijkman
2	Dr. dr. Joserizal Serudji, SpOG (K)	Correlation between macronutrient composition of breast milk and weight gain of neonates	Rabu, 20 April 2016 jam 11.00 s/d 13.00	Kelas B Pasca Sarjana Wing Barat Lantai 4 Gedung Eijkman
3	Dr. dr. Ariadi, SpOG (K)	Effect Of Suturing Intrauterine Device For Continuity In Postpartum Contraception Family Planning tran caesarean Method	Rabu, 20 April 2016 jam 11.00 s/d 13.00	Sidang A Wing Timur Lantai 4 Gedung Eijkman
4	Dr. dr. Defrin, SpOG (K)	Differences In Blood Plasma Levels Of Vitamin C In Term Pregnancy With Premature Rupture Of Membranes And Blood Plasma Levels Of Vitamin C In Term Pregnancy Without Premature Rupture Of Membranes	Rabu, 20 April 2016 jam 11.00 s/d 13.00	Kelas B Pasca Sarjana Wing Barat Lantai 4 Gedung Eijkman
5	Mary Denita Wati MZ, M.Keb	Difference of activin a serum in preeclampsia And normal pregnancy	Rabu, 20 April 2016 jam 11.00 s/d 13.00	Kelas C Pascasarjana Wing Barat Lantai 4 Gedung Eijkman