COMPARISON OF VITAMIN C LEVELS IN CASES OF PREMATURE RUPTURE OF MEMBRANES (PROM) AND NORMAL PREGNANCY IN GENERAL HOSPITAL H. ADAM MALIK MEDAN AND MEDAN NETWORK HOSPITAL

[1]Rina Sinta Dhanu, [1]Makmur Sitepu, [1]Ichwanul Adenin, [1]Herbert Sihite, [1]Syamsul Arifin Nasution, [1]Roy Yustin Simanjuntak

[1]Departement of Obstetric and Gynecology

[1]Medical Faculty, University of North Sumatra, General Hospital H. Adam Malik Medan

Abstract: This research has been undertaken to determine the ratio of vitamin C levels in cases of premature rupture of membranes (PROM) and normal pregnancy. This research was comparative analytical study with case-control design in 40 women with and without a diagnosis of PROM at H. Adam Malik General Hospital Medan and Medan Network Hospital from January 2019 until the sample size was met by consecutive sampling technique. Univariate analysis was performed to assess the frequency distribution of sample characteristics. Bivariate statistical analysis was performed using independent T-Test with a confidence level of 95%. Mean serum vitamin C levels in the group of pregnant women with PROM is 5.82 ± 1.63 ng / mL lower than the group of normal pregnant women who had a higher mean serum vitamin C levels with 77.57 ± 52.84 ng / mL. : Based on the Mann-Whitney statistical test, there were significant differences in the serum vitamin C levels of the two study groups with p value <0.05. There were differences in the average value of vitamin C levels in the PROM and non-PROM groups, where vitamin C levels were lower in the PROM group than in the non-PROM group in the categories based on age, gestational age, parity and nutritional status.

Keywords - Vitamin C, Premature Rupture of membranes (PROM), Normal Pregnancy

INTRODUCTION

Premature rupture of membranes (PROM) is the main cause of preterm birth. This situation occurs in 1-3% of all pregnancies and remains a major cause of perinatal morbidity and mortality worldwide, especially the occurrence of premature birth where 40% of preterm births are associated with PROM. This is also associated with significant maternal morbidity. Its contribution to perinatal mortality and morbidity is quite high, so it is important to identify the factors that can help prevent this condition.[1]

Micronutrient deficiencies which are predisposing factors for abnormal changes in collagen structure are associated with an increased risk of PROM. Micronutrient vitamin C (ascorbic acid) is an effective water-soluble antioxidant that can suppress several reactive oxygen species thereby reducing oxidative stress. Vitamin C also acts as an enzyme cofactor in collagen biosynthesis where vitamin C is needed for the formation of triple helical structures of collagen. Therefore vitamin C is considered to participate in the balance between synthesis and degradation of collagen. The role of vitamin C in PROM was first studied by Widemen, they showed that women with low serum concentrations of ascorbic acid tended to have higher rates of PROM than those with normal serum concentrations. This finding is confirmed by subsequent studies that reported low collagen content in membranes of patients with premature PROM, while Plessinger found that in vitro treatment of amniotic membranes with vitamin C prevented damage to membranes by hypochlorous acid. More and more literature about the role of vitamin C as a factor in the occurrence of CDD has led to the idea that vitamin supplementation may be an important intervention strategy.[1]

The role of Vitamin C in the incidence of PROM / preterm has been investigated previously. Other studies have examined the role of Vitamin C in maintaining membrane integrity, then intake of vitamin C and also in measuring the concentration of ascorbic acid in plasma, leukocytes and umbilical cord because it affects the occurrence of PROM and preterm birth.[2]

In several studies that have been carried out, there is a tendency decreasement serum vitamin C levels in pregnant women along with the increase in gestational age and also in mothers with PROM. According to a study conducted by Osaikhuwuomwan, et al., The mean levels of plasma vitamin C in pregnant women was 0.60 ± 0.02 mg / dl.[1]

MATERIAL AND METHODS

This research is a comparative analytical study with a case-control design that assesses the comparison of serum vitamin C levels in pregnant women experience PROM and normal pregnancy in General Hospital H. Adam Malik Medan and Medan Network Hospital in January 2019 until 40 samples were fulfilled with consecutive sampling technique. The study sample was women who gave birth with and without a diagnosis of PROM who had fulfilled the inclusion criteria, namely pregnant women with PROM and normal pregnancy who gave birth at General Hospital H. Adam Malik Medan and Medan Network Hospital with gestational age ≥ 20 weeks and single fetal pregnancies, meanwhile exclusion criteria namely blood samples broken, polyhydramnios and gemelli.

Researchers conducted a study of pregnant women who experienced PROM. Then anamnesis about maternal age, gravida, physical examination, and documentation of UAC (Upper Arm Circumference) measurements were carried out. Then an assessment of serum vitamin C was carried out from pregnant women who had PROM and normal pregnancy woman. To examine serum vitamin C, a blood sample of 3 cc was carried out, put in a vacutainer 3cc tube and then sent to a USU integrated laboratory and centrifuged for 10 minutes at 3,600 revolutions per minute (rpm). Then the 2 cc separated serum is inserted into the microtube to be examined for vitamin C levels in blood serum. Examination using a special serum ascorbic acid kit with chromatography method. The results are documented and then analyzed. Data collected then analyzed statistically.

The results of this research are presented in the frequency distribution table. To assess the frequency distribution of the characteristics of the study sample based on age, parity, gestational age, and nutritional status, univariate statistical analyzes were carried out. To assess the comparison of serum vitamin C levels between women with PROM and normal pregnancy, bivariate statistical analysis was performed using an independent T-Test. This study uses a 95% confidence level.

RESULTS

Characteristics of Research Subjects

The characteristics of the subjects following the research can be seen in the table below.

Table 1. Characteristics of Research Subjects

Characteristics	Pregnant Mother Status			
	PROM		Normal Pregnancy	
	N	%	N	%
Parity				
Primigravida	13	65	3	15
Multigravida	7	35	17	85
Gestasional Age				
< 24 weeks	1	5	1	5
24-28 weeks	1	5	1	5
29-32 weeks	1	5	1	5
33-36 weeks	7	35	7	35
37-40 weeks	10	50	10	50
Maternal Age				
< 20 years old	1	5	0	0
20-25 years old	8	40	2	10
26-31 years old	7	35	8	40
32-37 years old	3	15	9	45
>37 years old	1	5	1	5

Nutritional Status				
Normal/Good	12	60	20	100
Malnutrition	8	40	0	0
Total	20	100	20	100

Based on table 4.1 the characteristic of pregnant women with PROM shows that mostly primigravida (65%) while in normal pregnant women generally was multigravida (85%). Both are mostly with gestational age of 37-40 weeks (50%). Based on the maternal age category, in PROM group, there were more subjects research aged 20-25 years (40%) while in the normal pregnancy group there were more subjects research aged 32-37 years (45%). Based on the nutritional status category, in the group PROM found that mostly (60%) with good nutrition, while in the normal pregnancy group all had good nutritional status (100%).

The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy

Table 2. The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy

VIT C levels (ng/mL)	N	Mean	SD	Min	Max	p value*
PROM	20	5.82	1,63	1,99	9,53	0,000
Normal Pregnancy	20	77,57	52,84	30,4	237,0	

**Mann-Whitney Test*

Table 2. explains that mean serum vitamin C levels in the group of pregnant women with PROM was 5.82 ± 1.63 ng / mL lower than the group of normal pregnant women which having a higher mean vitamin C levels with 77.57 ± 52.84 ng / mL. Based on the Mann-Whitney statistical test, there were significant differences in the serum vitamin C levels of the two research groups ($p < 0.05$).

The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy based on maternal age

Table 3. The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy based on maternal age

Maternal Age (years old)	Vitamin C	
	PROM	Normal Pregnancy
<20	5.07	-
20-25	5,26 ±1,90	107 ±83,7
26-31	5.74 ±1,45	64,12 ±32,05
32-37	5,96 ±0,92	65,08 ±33,08
>37	9,53	237

From table 3. there was a difference in the average level of vitamin C based on the age group of mothers in the KPD group which was highest in the age group of mothers> 37 years (9.53 ng / mL) and lower at the age of mothers <20 years ie 5.07 ng / mL . But in the non-KPD group, where vitamin C levels were much higher also in the maternal age group> 37 years (237 ng / mL) followed by maternal age 20-25 years (107 ± 83.7 ng / mL) and the lowest at maternal age 26 -31 years, namely 64.12 ± 32.05 ng / mL.

The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy based on parity

Table 4. The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy based on parity

Parity	Vitamin C	
	PROM	Normal Pregnancy
Primigravida	5,61 ± 1,66	48,3 ±12,5
Multigravida	6,29 ±1,60	82,7 ± 55,74

From table 4. based on the parity category, it was seen that the mean difference in vitamin C levels in the PROM primigravida group was lower (5.61 ± 1.66 ng / mL) than normal pregnany group (48.3 ± 12.5 ng / mL), as well Vitamin C levels in the multigravida PROM group were lower (6.29 ± 1.60 ng / mL) than in normal pregnancy group (82.7 ± 55.74 ng / mL).

The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy based on gestational age

Table 5. The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy based on gestational age

Variabel	< 24	25-28	29-32	33-36	37-40
PROM	5.07	5.60	9.53	5,57±1.83	5.7±1,38
Normal	167	237	132	67,68±34,69	54,23±19,1

From table 5. there are differences of mean vitamin C levels in the PROM and normal pregnancy groups, where vitamin C levels in the PROM group are lower than in normal pregnancy groups based on the gestational age category.

The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy based on nutritional status

Table 6. The difference of mean levels vitamin C in preganant women with PROM and normal pregnancy based on nutritional status

	Vitamin C	
	PROM	**Normal Pregnancy**
Normal/Good	5.79 ±1,14	77,57 ±52,8
Malnutrition	6.04 ± 2,24	-

From table 6. it can be seen that the mean vitamin C levels in the good nutrition PROM group is 5.79 ± 1.14 ng / mL and malnutrition is 6.04 ± 2.24 ng / mL, whereas in the normal pregnancy group all the nutrients are good with a mean vitamin C level which is higher at 77.57 ± 52.8 ng / mL.

DISCUSSIONS

Age

Age greatly influences the mother's readiness during pregnancy and facing labor. The age for optimal reproduction for a mother is between 20-35 years old. Under or above this age will increase the risk of pregnancy and childbirth. The age of a person will greatly affect the reproductive system, because her reproductive organs have begun to diminish its ability and elasticity in accepting pregnancy.[3,4]

Parity

Parity has been used as a marker of pregnancy risk in nulliparous and grande multipara women. Nulliparous women are considered to have a risk of hypertensive disorders, operative labor and delivery of low-weight babies. Whereas women with high parity especially women with grand multipara have a risk of antepartum bleeding, gestational diabetes mellitus, pregnancy related to hypertension, premature rupture of membranes, premature labor and postpartum hemorrhage.[5,6]

The parity factor is divided into primiparas and multiparas. Primipara is a woman who was once pregnant with a fetus reaching a point of being able to survive. Primipara mothers who experience premature ruptured membranes are associated with psychological conditions, including pain during pregnancy, physiological disorders such as emotions and include anxiety about pregnancy.[7]

In mothers who experience anxiety, emotions during pregnancy will interfere the condition of the mother, because the adrenal gland will produce hormone cortisol. So that when the mother experiences anxiety, the part of the brain called the amygdala will send a signal to the hypothalamus, then hypothalamus will produce hormone CRH which is associated with ACTH (adenocorticotropic hormone), then ACTH will send a signal to the adrenal gland to release cortisol. But if excess cortisol production suppress the immune system, it is possible for the mother to be susceptible to infection or inflammation which can cause increased activity of iL-1 and prostaglandin, produce tissue collagenase, resulting in collagen depolymeration in the chorion or amnion membrane. Then this condition causing thin, weak and easily break spontaneously membranes so that premature rupture of the membranes occurs.[8]

The results of this research are in accordance with Defrin et al (2014) 's study of the characteristics of pregnant women with PROM which was the majority of parity in multiparous category as much as 84% and normal pregnancy majority in multiparity category of 59%.[9]

The second and third parity is a relatively safer condition during pregnancy and childbirth during the reproductive period, because in these conditions the uterine wall has not undergone much change, and the cervix has not been exposed too often so that it can properly support the membranes. Mothers who have given birth several times are more at risk experiencing PROM, because vascularity in the uterus has a disorder which results in the membranous connective tissue being easily fragile and eventually spontaneous rupture.[7]

Pregnancy that is too frequent, multiparous or grandemultipara affects the embryogenesis process, the membranes are thinner so they break easily prematurely. The theoretical statement stating that there is more parity, the more easily amnionic infections occur because of damage to the cervical structure in previous labor. PROM is more common in multiparas, due to decreased reproductive function, reduced connective, vascular and cervical tissue that has opened one cm due to past labor.[10]

Gestational Age

The age of preterm pregnancy is 28-36 (<37 weeks) in the third trimester, the membranes are easily broken, the weakening of the strength of the membranes has to do with enlargement of the uterus, uterine contractions and fetal movements. This is because the rupture of the membranes is related to changes in the biochemical processes that occur in the collagen amnion extracellular matrix and fetal membrane apoptosis. Membranes and decidua react to stimuli such as infections and the role of the membranes by producing mediators such as prostaglandins, cytokines, and protein hormones that stimulate the activity of enzyme degrading matrixes. PROM in premature pregnancy is caused by the presence of external factors such as vaginal infections, polyhydramnios, incompetent cervix and placental abruption.[9]

At term pregnancy which is 37-42 weeks' gestation. In accordance with the results of this research, it was also supported by another study which said that 50% of mothers who experienced PROM after term (aterm) pregnancy would begin labor within 12 hours, 70% within 24 hours, 85% within 84 hours. , 95% within 72 hours. Towards a term gestational age, focal weakness occurs in the fetal membrane above the ostium internal cervical will triggers the rupture in this location.

Nutritional Status

WHO states that malnutrition in pregnancy can eventually cause several diseases and complications in pregnancy, including malnutrition, low birth weight, premature rupture of membranes, bleeding and IUGR. Food deficiency plays a very important role in the emergence of malnutrition, so it can be understood that the frequency of occurrence of malnutrition in developing countries is higher compared to developed countries. In Indonesia, of the total number of pregnant women, 40% of them experience malnutrition and 34% of chronic malnutrition.

Vitamin C levels of pregnant women with PROM and normal pregnancy.

The results of this research in accordance with the study of Defrin et al. (2014) which is found a significant difference in the blood plasma vitamin C levels of aterm pregnancy with premature rupture of membranes and term pregnancy without premature rupture of membranes (P <0.05). The mean blood plasma vitamin C level at term pregnancy with premature ruptured of membranes is lower than blood plasma vitamin C levels at term pregnancy without premature rupture of membranes in the RS.DR.M. Djamil Padang, Achmad Mochtar Bukit Tinggi Hospital and Pariaman Hospital.[9]

Premature rupture of membranes is one of the most common complications of pregnancy. The incidence of premature rupture of membranes in pregnancy ranges from 6% to 10% and 20% of these cases occur before 37 weeks' gestation. The incidence of premature rupture of membranes in Indonesia ranges from 4.5% to 7.6% of all pregnancies. Premature rupture of the membranes causes an increasement in complications in pregnancy both at term and preterm gestational age. The risk of infection after an rupture of the membranes affects the mother, fetus or neonate. The incidence of neonatal infection after premature rupture of the membranes more than 24 hours is approximately 1% and if there is clinical chorioamnionitis the risk increases to 3% to 5%.[11,12]

Chorioamnion is a large and complex layer consisting of epithelial and supporting tissue elements where each component has an important role in metabolism which is important for physiological integrity for the development of pregnancy. Amnion gains its strength through collagen. How can fetal membranes weaken with exogenous and endogenous mechanisms still under active investigation. Endogenous factors such as local variations in membrane or collagen depletion and exogenous factors such as the effects of microbial, host metabolism or due to nicotine which reduce antriprotease activity also cause local membrane disruption.[13]

The strength and integrity of chorioamnion is maintained by the balance of intrinsic factors that regulate the synthesis and degradation of connective tissue. The degradation of collagen in chorioamnion is controlled by matrix metalloproteinases. The release of matrix metalloproteinases is regulated by Tissue Inhibitors of Metalloproteinases or TIMPS. Unstable molecules produced continuously in the body known as reactive oxygen species (ROS) are said to produce tissue damage that causes Premature Rupture of Membrane (PROM).[13]

Chorioamnion exposure with ROS is said to increase matrix metalloproteinase, causing premature rupture of the membranes. Normally there is a balance between production and elimination from ROS. Oxidative stress occurs when the antioxidant exceeds antioxidants. Vitamin C (ascorbic acid) is a water-soluble vitamin that is not synthesized by humans, so this essential vitamin must be obtained from food. As we know vitamin C is one of the antioxidants. The body uses various antioxidants to limit tissue damage caused by free radicals. Ascorbic acid directly stimulates collagen synthesis.[14]

Ascorbic acid also functions as an reducing agent by sending hydrogen atoms with a single electron to ROS. Ascorbic acid makes collagen strong and stable. Oxidative stress occurs when the antioxidant exceeds the antioxidant so that it can cause premature rupture of the membranes and one of the roles of vitamin C sends its single hydrogen atom to ROS to make collagen stronger and more stable.[15]

There have not been many studies comparing vitamin C levels of aterm pregnant blood plasma with premature rupture of membranes and aterm pregnancies without premature rupture of membranes but from the results of this research in line with several studies that have previously been carried out.In some other studies it shows the opposite where exactly blood plasma vitamin C levels of aterm pregnancy with premature ruptured membranes higher than the blood plasma vitamin C levels of aterm pregnancy without premature rupture of membranes.[16]

Research that shows similar results is a study conducted by E. Casanueva (1998) in which the study said vitamin C levels <1.8 ug / 108 cells increased the risk of premature rupture of membranes even said vitamin C levels <1.8 ug / 108 cells at 28 weeks gestation showed a high predictive value ($p < 0.05$).[17]

The Tejero et al (2003) study showed a concentration of vitamin C in women with premature rupture of the membranes at term lower than normal pregnancy women (without premature rupture of membranes). Contrary to the research of Barret et al (1994) no association was found between low levels of vitamin C and the incidence of premature rupture of membranes and in the study it was said that vitamin C supplementation could not prevent the occurrence of premature rupture of membranes even though the study said a larger sample was needed to assess whether vitamin C supplementation is associated with the incidence of premature rupture of membranes.[18,19]

Likewise, the research conducted by Rizka (2011) resulted in the opposite in which the results showed that vitamin C levels in pregnant women with premature rupture of membranes were higher than those at term without premature rupture of membranes. Literature says the normal levels of blood plasma vitamin C are 26.1-84.6 umol / L (> 0.6 mg / dl,> 20 ug / 108 cells,> 114 nmol / 108 cells) and are said to be deficiencies if <11 umol / L (0.2 mg / dl, <10 ug / 108 cells, <57 nmol / 108 cells).[11]

CONCLUSION

There were differences in the mean value vitamin C levels in the PROM and normal pregnancy groups, where vitamin C levels were lower in the PROM group than in the normal pregnancy group in the categories based on age, gestational age, parity, and nutritional status.

ACKNOWLEDGEMENT

The researcher thanked the supervisor of the obstetrics and gynecology department for his guidance and contribution in this research. In addition, the researcher also thanked all parties from the staff to the research samples involved in conducting this research. Researchers found that vitamin C supplements should be given to pregnant women since first week of pregnancy to increase vit C levels in the blood and reduce the risk of premature rupture of membranes.

CONFLICT OF INTEREST

The researcher ensures that there is no conflict of interest in this research

ETHICAL CLEARANCE

For research permission, research approval was obtained from the research subject and the Ethics Committee of the Faculty of Medicine, University of North Sumatra who would conduct an assessment of the feasibility of the research proposal.

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