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Article

The difference of Zinc And Matrix Levels of Metalloproteinase-9 Serum Between Premature Rupture Of Membrane Aterm And Normal Pregnancy

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A B S T R A C T

Premature rupture of membranes (PROM) is one of the common problems in the obstetric field, ranging from 8-10% of term pregnancies will experience PROM. The increase in MMP-9 also has an impact on the degradation of the extracellular matrix and the apoptotic process of amniotic epithelial cells which ultimately leads to membrane rupture. The purpose of this study was to determine the differences in serum zinc and MMP-9 levels between premature rupture of membranes and normal pregnancies.

This research is quantitative observational with the cross-sectional design carried out in Independent Practice Midwives for research samples of term premature rupture of normal and amniotic pregnancies, and Biomedical Laboratory of the Medical Faculty of Andalas University in March 2018 to March 2019. The research sample amounted to 35 respondents using consecutive sampling. Zinc and MMP-9 levels were examined by the Human ELISA Kit. Data analysis using the unpaired t-test.

The results showed a mean serum zinc level of 13608 ± 1128.976µg / ml in Aterm KPD and 13799,111 ± 1254,335 µg / ml in normal pregnancies with p> 0.05. The mean serum MMP-9 levels were 1682.412 ± 320,398 ng / ml in Aterm PROM and 1807,667 ± 484,735 ng / ml in normal pregnancies with p <0.05.

This study concludes that there is no difference in serum zinc levels in premature rupture of membranes and normal pregnancies; there is a difference in MMP-9 levels in premature rupture of membranes and normal pregnancy.

I. INTRODUCTION

Maternal Mortality Rate (MMR) and Infant Mortality Rate (IMR) is one of the important indicators in assessing the degree of health of the people in a country and a reflection of the opportunity to obtain services in the obstetric and perinatology fields. The Maternal Mortality Rate (MMR) seen from the indicators of Indonesian Reproductive Health in 2013 ranged from 360 per 100,000 live births.

The results of the Indonesian Demographic and Health Survey (SDKI) state that deaths from 1991 to 2007 decreased from 390 to 228 per 100,000 live births. In 2012 the IDHS again noted that AKI had increased again from 228 to 359 per 100,000 live births.[2]

Maternal Mortality Rate (MMR) is associated with various accompanying complications both during pregnancy, labor, and childbirth. Bleeding, preeclampsia and infection accounted for one-third of all maternal deaths. It is estimated that around 20% of pregnancies will experience complications and one of the complications in pregnancy and childbirth is a premature rupture of membranes (KPD).[1]

Early rupture of membranes (KPD) is the rupture of the membranes before the onset of regular contractions at 37 weeks' gestation, while ruptured membranes at gestational age ≤ 37 weeks are called premature rupture of membranes (preterm KPD).[3]

Based on Medical Record data and Midwifery Room RSUP. DR. M. Djamil Padang as a hospital referral center in West Sumatra, in 2013 there were 240 cases of CDD from 1,710 deliveries (14%), in 2014 there were 35 cases of KPD from 942 deliveries (3.7%) and in 2015 35 cases of KPD of 593 deliveries (5.9%). Then the data obtained from the Lubuk Buaya Puskesmas were 29 cases of KPD throughout 2017. RSUD dr. Rasidin Padang as a government hospital in the Padang city, found the incidence of KPD in 2016 was 58 cases, while in 2015 there were 61 cases.

Rupture of the membranes can occur at any gestational age either at the end of pregnancy or long before delivery. If the membranes rupture before the 37-week gestational age is called a Preterm KPD and if after 37 weeks of gestation it is called aterm KPD. Several overseas studies state that the incidence of KPD occurs in 8% in pregnancy. Preterm KPD occurs in about 3% while aterm KPD occurs around 5%.[4]

Zinc in various components of blood changes during pregnancy. Plasma or serum concentrations decrease 15% - 35% at the end of pregnancy compared to pre-pregnancy or early pregnancy concentrations. Zinc needs for pregnant women are zinc accumulation in fetal or maternal tissue. Zinc in pregnant women, 57% is used by the fetus, 6.5% in the placenta, <1% in amniotic fluid, 24% in the uterus, 5% in breast tissue, and 6.5% in maternal blood volume. Zinc also causes anti-amniotic bacteria anti-bacterial properties, so that the decrease in maternal zinc status during pregnancy can also result in decreased availability of zinc and placenta zinc, then affect the extracellular matrix restructuring (ECM) process in the amniocorion membrane, leading to weak membrane strength and integrity finally, it is more likely that KPD will occur.[5]

Matrix Metalloproteinase (MMP-9) is an important intermediary and causes premature rupture of membranes other than zinc. Matrix Metalloproteinase (MMP) 9 is a group of proteins that break down collagen. Collagen provides the main strain strength in the fetal membrane, therefore rupture of the fetal membrane membrane is associated with increased MMP-9 expression and activity and decreased expression and activity of Tissue Inhibitors of Matrix Metalloproteinases (TIMPs).

Matrix etalloproteinase-9 (MMP-9) is the main MMP responsible for amniotic gelatinolytic activity. MP-9 plays a role in the degradation of type IV collagen which is the main type of collagen of the amniotic basement membrane. Prostaglandin production increases prostaglandin production. Prostaglandin increases the ability of decidua to degrade matrix extracellular (ECM) by increasing MMP-9 and decreasing TIMP-1 secretion.[6]

Identification of pregnant women at risk of giving birth with premature rupture of membranes is needed by knowing the causal factors. Among the causes of premature rupture of membranes are biochemical changes in this case zinc levels which play a role in the protection of infection by bacteria and the presence of infection and inflammation. Infection causes an increase in one of the inflammatory cytokines, TNFα, which triggers an increase in MMP-9 which results in weakening of the membranes during labor.

From the background above, the researcher was interested in conducting a study entitled "Differences in serum Zinc and Matrix Metalloproteinase-9 (MMP-9) levels between Early Rupture of Amniotic fluid (KPD) and Normal Pregnancy"

II. METHODS

This type of research is an observational study with a cross-sectional study design.
Research place was conducted in the Independent Practice Midwife in the Lubuk Buaya Padang Health Center area for Normal Pregnancy; and at the RSUD. Dr. Rasidin, Bahayangkara Hospital, RST Reksodiwiryo for Pregnancy with Early Rupture, Unand Biomedical Clinic Laboratories in Unand. Time of research was conducted in March 2018 until March 2019. The population in this study were all pregnant women with gestational age ≥37 weeks, in KPD women as the study group and women who did not experience KPD as a control group. The research sample is part of the population that meets the inclusion and exclusion criteria.

Inclusion criteria, namely; pregnant women with KPD and normal pregnancy with gestational age ≥ 37 weeks; the mother is willing to be the subject of research and sign an informed consent; single pregnancy and normal presentation. Exclusion Criteria namely; the history of diabetes; the history of heart disease; the history of hypertension; the history of cancer; the history of antibiotic administration after the membranes rupture; polyhydramnios
The method of sampling in this study was consecutive sampling. All subjects who came in sequence and fulfilled the inclusion criteria were included in the study until the required number of subjects was met.[7]
The sample size is determined based on the calculation formula of the sample using two independent populations.

Based on the calculation of the sample formula, the minimum number of samples is 18 for each group.
Research materials are respondent's blood serum sample; material for examination to determine maternal serum zinc and MMP-9 levels; explanation sheet before research; approval sheet for informed consent. Research tools are 3 ml disposable syringe; centrifuge tube and tube rack; centrifuge; elicot / ependorf tube; refrigerator; EDTA Reagent Tube and data collection sheet.

How to Take Blood Samples are ask the patient for permission to take blood with evidence of signing the agreed consent form; venous blood is taken before giving antibiotics; blood collection is carried out by a labor officer on duty at that time; using disposable syringes;Taking blood in the mediana cubiti vein; Disinfect the skin area with cotton alcohol first; Install tourniket with a distance of 3-5 cm from the vein; 3 ml of venous blood is taken and put into a vacuum tainer tube; The blood is centrifuged at 3000 rpm for 15 minutes until a serum is formed above the clot; the formed serum is taken with a disposable needle and poured into a serum cup (closed tube) labeled, named and registered and stored in a refrigerator at -30 ° C in an upright position.

Determination of Research Subjects in this study is pregnant women who come to the research site and have been diagnosed by a doctor, or midwives responsible for fulfilling the inclusion criteria are used as research samples. Next, the mother is given an explanation before approval.
Serum Retrieval and Storage in this study were 1) blood samples taken in the mediana cubital vein of ± 3 cc by labor officers, nurses or midwives at the research site who were on duty at that time,

2) Blood was inserted into the yellow lid EDTA tube, then placed on a shelf tube to avoid shocks and remain in position. Then the blood is distributed using a cool box containing ice gel while maintaining the position of the sample to remain upright and not shaken to the Unand Biomedical Laboratory, 3) Arriving at the blood laboratory is allowed to be left and then centrifuged at a speed of 2000-3000 rotations per minute (rpm) for 20 minutes, 4) The centrifuge serum is taken using a micropipet which is then inserted into a microtube coded according to the research subject's identity, 5) Serum is stored and put in a laboratory refrigerator Biomedical Unand, 6) Serum samples are stored in a refrigerator with a temperature of -80 ° C in the Unand Biomedical laboratory until the examination is carried out, 7) After all serum samples have been met the measurements will be carried out using Human ELISA Zinc and MMP-9 kits at the Biomedical Laboratory of the UNAND Faculty of Medicine.

Examination of Zinc Serum levels in this study are as follows: a) Before serum separation is carried out, blood can be placed at room temperature or overnight at 4 ° C and b) Reagent preparations include: 1) Place all reagents at room temperature (18-25 ° C) before use, 2) Wash Buffer, dilute 30 ml of wash buffer concentrate to 750 ml wash buffer with deionized or water distilled water. Place unused liquid at 4 ° C. If crystals and concentrates are formed, heat them in a water bath at 40 ° C (not more than 50 ° C) and mix lightly until the crystals dissolve at room temperature before use, 3) Standard, standard preparation in 15 minutes before use. Centrifuge at 10,000 xg for 1 minute and rearrange the standard with a 1.0 ml standard reference and sample diluent. Tighten the lid, leave the standard for 10 minutes and turn it over for a while.

After dissolving completely, mix it thoroughly with a pipette. Mixing procedure in 100 microgram/ml solution. Then make the thinner series needed. Recommended concentrations of 100, 50,25, 12,5,6,25, 3, 12,1, 563,0 µg / ml. If you want to make a standard solution with a concentration of 500 uL. Place the standard 500 uL at 100 µg / ml, add to the EP tube with a standard 0.5 ml reference and sample diluent and stir. The undiluted standard provides a high standard (10 ng / ml). Reference standards and diluents of the sample results are blank (0 ng/ml), 4) Biotinylated Detection Ab, add the inventory before the experiment (100 µl / well). Prepare more than 100-200 µl. Centrifuge tube before use. Dilute the Biotinylated Detection Ab concentrate into the work concentration using a Biotinylated Detetion Ab thinner (1: 100), 5) HRP conjugate concentrate, add the inventory before the experiment (100µl / well). In actual preparation, prepare more than 100-200 µl.

Dilute the concentrated conjugate HRP into the working concentration using conjugate HRP thinners, 6) Reagent substraatee, reagent substrate very sensitive to light and contamination, do not open the vial until it will be used. The reagent dose needed can be aspirated with sterilization tips and unused residual reagents should not be put back into the vial. Contamination while preparing reagents can affect results.

Research washing procedures include: 1) Automatic washing, add 350 µl wash buffer into each well, injection distance and suction for about 60 seconds, 2) Manual washing, add 350 µl of wash buffer into each well, soak for 1-2 minutes. After washing, pour the remaining wash buffer by flipping the plate and dry stain by scrubbing it thoroughly and drying it with a rough surface of absorbent paper.

Examination procedures include: 1) Place reagents and samples at room temperature before use. Centrifuge after dilution before the inspection. All reagents must be stirred with a soft spin before suctioning. All samples and standards are examined in duplication, 2) Add sample and Biotinylated Detection Ab, add 50µl standard, blank or sample per well. Empty wells are added with reference standards and sample diluents. Immediately add 50µl of Biotinylated Detection Ab working solution into the well. Cover with a sample plate. Press the plate gently to ensure complete mixing of incubation for 45 minutes at 37 ° C, 3) Washing, aspirating each well and washing, repeat the procedure 3 times. Wash by filling each well with wash buffer (approximately 350µl), use spray bottles, multi-channel manifold dispenser pipettes or automatic washing.

Transfer of complete fluid at each step is very important. After washing, remove wash buffer with aspiration. Flip the plate and pat it with thick and clean absorbent paper, 4) HRP conjugate, add 100 µl HRP conjugate work solutions for each well. Cover with a new plate seal. 30 minutes incubation at 37 ° C, 5) Washing, repeat step 3 as much as 5 times, 6) Substrate, add 90µl of the solution substrate to each well. Cover with a new plate seal. Incubate 15 minutes at 37 ° C, avoid light. The reaction time can be shorter or exceed the actual time, but not more than 30 minutes. If a clear gradient appears from the standard well the reaction has just ended, 7) Stopping, add 50µl stop solution to each well. The color immediately turns yellow. Addition of stop solution must be the same as substrate solution, 8) Measurement of OD, determination of optical density (OD) in each well 1 time. Using a set microplate reader at 450 nm. Microplate reader is opened first, preheat the instrument and set the inspection parameters, 9) Calculation of results, average duplicate readings for each standard and sample, then subtract the standard OD blank standard.

The examination procedure based on the Human MMP-9 ELISA Kit consists of 1) All reagents, work standards, and serum samples are prepared according to work procedures, 2) All samples and Kits are left at room temperature (18-25 ° C) before use, 3) Microwell strips needed are released and the remaining unused strips are stored, 4) standard 50µl is added to each well, 5) After 40 µl of sample is added into the well the sample is then added 10µl of anti-MMP-9 antibody into the good sample, after it was added 50µl streptavidin-HRP to the sample well (not added to the control well), 6) The plate was closed with a seal and incubated for 60 minutes at 37 ° C, 7) After finishing incubation, the seal was opened and the plate was washed 5 times with wash buffer. Well, soak in the wash buffer for 30 seconds to 1 minute at each wash. This procedure is repeated 5 times and the plate is dried with a clean towel/paper, 8) Then 50µl of Solution A substrate is added to each well then 50µl of the solution substrate B is also each well. The plate is covered with a new wrapper and incubated for 10 minutes at 37 ° C in a dark room. Next 50µl stop solution is added to each well, the color will turn yellow. Then the optical density value (OD) of each well using a microplate reader with a wavelength of 450 nm is carried out in 30 minutes after the addition of stop solution, 9) Data analysis is carried out, 10) All examination costs are borne by the researcher.

The data that has been obtained is recorded in the format of research data collection and after all data has been collected then data processing is carried out through the following processes: 1) Editting is an attempt to re-examine the truth of the data obtained or collected from the results of research, 2) Coding is an activity of numeric codes (numbers) for data consisting of several categories. Change data from letter form to numeric data to facilitate interpretation of research results, 3) Entering data is an activity of entering collected data into a master table or computer database, 4) Cleaning by checking again so that it is completely clean from errors.

Data analysis in this study included: 1) Univariate Analysis;

categorical data is presented in the form of frequency distribution while numeric data is presented in the form of a mean and standard deviation, 2) Bivariate Analysis; the value of mean difference is done by using the Independent t-test on data that is normally distributed while the data that is not normally distributed is the Mann-Whitney test. Testing is done with confident interval (CI) 95% and $\alpha = 0.05$. The conclusion of the test results if the p-value is 50.05 then H0 is rejected, meaning that there is a mean difference between the independent variables and the dependent variable, namely the difference in mean zinc levels and MMP-9 between the two groups.

This research was conducted after obtaining ethical clearance (ethical clearance) from the research ethics committee. The Certificate of Ethics Assessment is from the Research Ethics Committee of the Faculty of Medicine, Andalas University, Padang with a statement No: 364 / KEP / FK / 2018. During the research, subjects who met the research inclusion criteria were given an explanation of the objectives and treatment to be given. After the subject understood it and agreed, he was asked to fill out and sign an informed consent statement. Research subjects are free to refuse to

participate in research if they do not agree. All research costs and other costs arising from research are borne by the researcher.

III. RESULT

Research has been conducted to determine the differences in serum levels of Zinc and MMP-9 between Early Ruptured Amniotic and Nomal Pregnancy performed on 36 aterm pregnant women patients based on a cross-sectional comparative study approach. The study subjects consisted of 18 respondents each in early rupture of membranes and normal pregnancies taken at Bhayangkara Hospital, RSUD DR. Rasidin, Lubuk Buaya Health Center, BPM Armiati, BPM Rika Hardi, S.SiT Padang City.

Table 1. Differences in Respondent Characteristics in Early Rupture of Amniotic and Normal Pregnancy

Characteristic	KPD (n=17)	Normal Pregnancy (n=18)	P Value
Age (Year)	27,47±4,71	28,00±4,99	0,32
Parity			
-Nullipara	3 (17,6)	5 (27,8)	0,48
-Multipara	14 (82,4)	18 (72,2)	
IMT (kg/m^2)	26,61±3,29	26,76±3,84	0,13

Table 1 shows that the mean age of respondents in patients with premature rupture of membranes is 27.47 ± 4.71 years and patients with normal pregnancies 28.00 ± 4.99 years. The statistical test results show that there is no difference in age at premature rupture of membranes and normal pregnancy 0.32 (p> 0.05).

Parity distribution of respondents in patients with premature rupture of membranes was multiparous as many as 14 people (82.4%) and in patients with multiparous normal pregnancies as many as 18 people (72.4%). The results of statistical tests are known to have no relationship between parity in premature rupture of membranes and normal pregnancy p = 0.48 (p> 0.05).

The mean body mass index (BMI) of respondents in patients with premature rupture of membranes was 26.61 ± 3.29 and in patients with normal pregnancies 26.77 ± 3.84, this indicates that the two groups were in the same nutritional status namely obesity /fat. The results of statistical tests showed that there was no difference in BMI in premature rupture of membranes and normal pregnancy p = 0.13 (p> 0.05).

Table 2. Mean Serum Zinc and MMP-9 Levels in Early Ruptured Amniotic and Normal Pregnancy

Variable	Group		P-value
	KPD) (Mean±SD)	Normal Pregnancy (Mean±SD)	
Zinc (µg/l)	13608±1128,98	13799,11±1254,34	0,37
MMP-9 (ng/l)	1682,41±320,40	1807,67±484,74	0,03

The mean level of zinc in premature rupture of membranes is 13608 ± 1128.98, while the mean zinc level in normal pregnancy is 13799.11 ± 1254.34. The mean MMP-9 levels in early ruptured membranes were 1682.41 ± 320.40, while the mean MMP-9 levels in normal pregnancies were 1807.67 ± 484.74. The results of statistical tests obtained p = 0.37 (p value> 0.05). It can be concluded that there is no difference in serum Zinc levels in premature rupture of membranes and normal pregnancies. While the statistical test for MMP-9 obtained p = 0.03 (p-value <0.05), it can be concluded that there are differences in MMP-9 levels in premature rupture of membranes and normal pregnancies.

IV. DISCUSSION

I. Characteristics of Research Respondents

In this study, there were several characteristics of respondents taken in accordance with the format of collecting sample data including age, parity, and BMI. The results of this study indicate that the majority of the samples in this study were multiparous mothers. The parity distribution of the Aterm KPD group was 14 (82.4%) multiparous mothers, while in the normal pregnancy group, there were 18 (72.2%) respondents. The results of the statistical test show that there is no relationship between parity in At term KPD and Normal pregnancy with a value of p = 0.48 (p> 0.05). The results of this study are a similar study which to the results where there were no significant differences in the average number of pregnancies between the KPD group and normal pregnancies with p = 0.11.[8]

The age characteristics showed that the mean age in the term KPD group was 27.470 ± 4.71 years and patients with normal pregnancies were 28 ± 4.99 years. The statistical test results show that there is no age difference in aterm KPD and normal pregnancy 0.32 (p> 0.05). The results of this study are the same as the results of research conducted by Yolanda et al. (2014) and Yulinda (2017) that the mean age characteristics of respondents showed no significant difference between the aterm KPD group and normal pregnancy (p = 0.32).

The results of calculating the BMI in the Aterm KPD group were 26.61 ± 3.30 and in patients with normal pregnancies 26.76 ± 3.84, this indicates that the two groups were in the same nutritional status ie obesity/fat. The results of the statistical test show that there is no difference in BMI in Atherm KPD and normal pregnancy p = 0.13 (p> 0.05). Overall the characteristics of the respondents in the form of parity, age, and maternal BMI were not statistically different between the Aterm KPD group and normal pregnancy.

II. Differences in Zinc Serum Levels in Early Rupture of Amniotic and Normal Pregnancy

The results of the study revealed that the mean zinc level in premature rupture of membranes was 13608 ± 1128.98, while the mean zinc level in normal pregnancies was 13799.11 ± 1254.34. p-value = 0.37 (p value> 0.05), it can be concluded that there is no difference in serum zinc levels in premature rupture of membranes and normal pregnancies.

Sikorsi proved the difference in the level of premature rupture of membranes between mothers with low zinc status compared to the control group (p = 0.05). Abdullah concluded that low zinc levels will cause preterm birth.[9]

The exact mechanism of action for proper zinc supplementation in pregnancy outcome is not yet fully known. Zinc is very important for growth, the beneficial effects of zinc on growth may be due to the direct role of zinc in protein synthesis and nucleic acid metabolism. In a previous study on subjects in Iran, serum zinc concentrations were lower in preterm mothers compared with mothers who delivered aterm. However, a causal relationship between low maternal zinc concentration and preterm birth was not fully understood. Meanwhile, although several studies have reported an association between maternal zinc levels and pretermity or gestational age

duration, several other studies failed to find this relationship. (Danesh, 2010).[11] Zinc is an important element in the metabolic process of the mother and fetus during pregnancy. Elements of zinc are needed for the preparation of the structure and function of various enzymes, including the enzymes MMP (Matrix Metalloproteinase), DNA and RNA polymerase, and have an important role to stabilize biomembrane.[9]

Zinc also causes antibacterial properties of amniotic fluid, so that the decrease in maternal zinc status during pregnancy can also cause a decrease in zinc availability for the fetus and placenta, then affect the ongoing extracellular matrix restructuring process in the chorionic amnion membrane and cause weakening of membrane strength and integrity and ultimately increase risk premature rupture of membranes.

The matrix metalloproteinase is a zinc-dependent enzyme that has the ability to degrade extracellular matrix components such as collagen and proteoglycans in normal embryogenesis and remodeling and plays an important role in a number of disease processes.

This enzyme is secreted in the form of a pro-enzyme. The latent period of this enzyme occurs because it binds the zinc cysteine to the "active center" which makes the enzyme stable in the form of "inactive". Activation of this enzyme by various mediators must be preceded by the separation of the zinc cysteine chain (sitein Switch)

The activation of MMP is regulated by an endogenous regulator of matrix metalloproteinase (TIMMP) tissue inhibitors. The strength and integrity of the amniotic membrane are determined by the balance between degradation and remodeling by MMP. In a state of zinc deficiency, the balance shifts towards the degradation of the extracellular matrix and causes a weakening of the strength and integrity of the membrane.

Decreasing maternal zinc status will cause a decrease in zinc levels in tissues. The role of zinc is involved in the etiopathogenesis of premature rupture of membranes, suggesting that low zinc levels will cause important protein production, induction of cell death, impaired zinc-mediated response (where zinc plays an important role as antimicrobial and anti-viral in amniotic fluid), causing contractions that do not normal, reducing the number of Gab-Junction in experimental animals, impaired prostaglandin synthesis and increasing sensitivity to female genital tract infections.

Low maternal zinc levels will affect the level of susceptibility to infection or the inflammatory process, it will also cause a decrease in the availability of zinc to the fetus and placenta. This can interfere with the ongoing reconstruction and renovation process. The degradation of the extracellular matrix in the fetal membrane will weaken and the strength and integrity of the amniotic membrane will also decrease.

From the analysis of the researchers, although statistically there were no differences in serum zinc levels in premature rupture of membranes the results of both were not significantly different, this might be caused by imperfect zinc absorption. Where zinc is the most trace found in the human body besides iron. Iron inhibits absorbing zinc when both are given in inorganic forms. The interaction of zinc and iron first occurs in the intestine. Zinc competes with iron to be absorbed in the intestine.

Differences in Serum MMP-9 Levels in Early Rupture of Amniotic and Normal Pregnancy

The results showed that the mean serum MMP-9 levels at term KPD were 1682.41 ± 320.40 and 1807.67 ± 484.74 in normal pregnancies. From the results of this study, it can be seen that the average serum MMP-9 level in normal pregnancy is higher than the aterm KPD, the statistical test obtained $p = 0.03$ ($p < 0.05$). This shows a significant difference in serum MMP-9 levels between Atherm KPD and normal pregnancy.

The metalloproteinase-9 (MMP-9) matrix is one of the metalloproteinase enzymes from the gelatinase group which is also the main enzyme associated with the process of extracellular matrix

degradation (ECM). Increased levels of MMP-9 cause fetal membranes to be susceptible to the occurrence of KPD both aterm and preterm. Significantly the expression of this enzyme will increase in the fetal membrane after the onset of contraction, but it is almost undetectable before labor. Other factors that can increase MMP-9 levels include hypertension, atherosclerosis and cardiovascular disease. Systemic infections can also result in increased MMP-9 levels in maternal pneumonia, typhoid, hepatitis and other systemic infectious diseases [11]

Inflammatory cytokines that can trigger an increase in MMP-9 are also involved in the initiation of normal labor and preterm labor. Increased levels of prostaglandins and contractions that occur can also stimulate an increase in MMP-9 levels and reduce TIMP-1, which means there is an imbalance between proteases and inhibiting factors, this process is the trigger for the occurrence of KPD.

The results of this study are different from those of Ge, Z. et al. (2017) which showed that an increase in serum MMP-9 in pregnant women with aterm KPD. Ge, Z. et al. (2017) mentioned that the results of his study showed that an increase in serum MMP-9 caused degradation of type IV collagen and caused an increase in KPD. Researches concluded that MMP-9 and collagen type IV collaborate in causing the occurrence of KPD which is likely a potential mechanism for the occurrence of KPD in term pregnant women.

The results of the study showed that the expression of amniotic membrane MMP-9 was higher in term labor with KPD compared to its expression in normal labor, with the results of statistical tests showing significant differences in the two study groups with a value of $p = 0.013$ (<0.05). This study was conducted using an immunohistochemical method, with a study group at term maternity with a KPD and a group of normal maternity women.[13]

The results of this study similar to the results of Yulinda's (2017) study showed that the mean serum MMP-9 levels in normal pregnancies were higher than those at aterm KPD. She stated that there were significant differences in serum MMP-9 levels in term KPDs and normal pregnancies.[9]

Based on some of the results of the above research it can be seen that the results obtained in this study are different from the results in previous studies. Other factors such as the disease that has been or is being suffered by a mother that is not detected by the researcher are likely to affect the levels of MPMP-9 in this study, which results in higher MMP-9 levels in normal pregnant women compared to aterm KPD mothers. Thus it can be concluded that the differences in the number of samples and the criteria of the sample used in this study are the supporting factors that cause differences in the results obtained with some of these studies also allegedly influence the results of the examination of MMP-9 levels studied.

In this study, other risk factors associated with the occurrence of KPD were not all included in the exclusion criteria of the study sample such as a history of systemic infectious diseases, history of CDD and a history of preterm labor. This study only conducted a matching process on only 1 type of sample characteristic, so it was still difficult to ensure that the two groups of research subjects were comparable. In the normal pregnancy sample group, this study did not pay attention to the sampling time span and did not see any signs of labor, because some of these things could affect the results of MMP-9 levels.

This study did not pay attention to the timing of the KPD. This increase does not observe disease factors that can affect MMP-9 levels such as hypertension, atherosclerosis, cardiovascular and systemic infections (pneumonia, typhoid, hepatitis). The amount of research on serum MMP-9 levels in aterm KPD is still very limited because research on MMP-9 levels in KPDs uses more direct amniotic membrane samples, and KPDs at preterm, so researchers find it difficult to compare research results optimally.

V. CONCLUSION

There was no difference in the mean levels of zinc serum between premature rupture of membranes and normal pregnancies and there was a mean difference in serum MMP-9 levels between premature rupture of membranes and normal pregnancy. Based on the results of this study, it is necessary to have an examination using serum zinc and MMP-9 which can be used as a parameter to help diagnose premature rupture of membranes and can more comprehensively explore information about the medical history of pregnant women which has implications for the occurrence of complications during pregnancy such as the occurrence of KPD it can be prevented early.

REFERENCES

Banaem, LM. Mohamadi, B. Jaafarabi, MA. And Moghadam, NA. Maternal Serum C-Reaktive Protein in Early Pregnancy and Occurrence of Preterm Premature Rupture of Membrane and Preterm Birth. *The Journal of Obstetrics and Gynecology Research*, 38(5).2012,pp.780-786.

Cunningham, FG. Leveno, KJ. Bloom, SL. Spong, CY. Dashe, JS. Hoffman, BL. et al. Williams Obstetrics 24th Edition. New York: McGraw-Hill Education.2014

Ge, Z. Jian-Xin, D. Fang-Fei, Z. And Yan, C. Expression of Matrix Metalloproteinase-9 and Its Substrate Level in Patients With Premature Rupture of Membranes. Journal of Obstetrics and Gynecology.2017, 37 (4), pp. 441-445.

Ministry of Health. 2014 Indonesia Health Profile, Jakarta. Available at: http: //www.kemenkes.go.id.2015

Nguyen, TTN. Matrix Metalloproteinase Expression and Regulation During Pregnancy, Term Labour, and Postpartum. Tesis. University of Toronto.2015

Sastroasmoro dan Ismael.Dasar-Dasar Metodologi Penelitian Klinis, Edisi Ke Empat, Jakarta: Sagung Seto.2011

Subagiyo, M., (2016). Perbedaan Ekspresi Matrix Metalloproteinase-9 (MMP-9), Tumor Necrosis Factor-α (TNF- α) Pada Persalinan Aterm Ketuban Pecah Dini dan Normal. Universitas Sebelas Maret Surakarta.2016

Svigos, JM. Dodd, JM and Robinson, JS. Prelabor Rupture of the Membranes. In D. James et l., eds. High-Risk PregnancyManagement Options. UK:Elsevier Saunders. 2011,pp. 1091-1100.

Weiss, A. Goldman, S. And Shalev, E. The Matrix Metalloproteinases (MMPs) in The Decidua and Fetal Membranes. Frontiers in Bioscience.2007,12, pp.649-659.

Weiss, A. Goldman, S. And Shalev, E.The Matrix Metalloproteinases (MMPs) in The Decidua and Fetal Membranes. Frontiers in Bioscience.2007,12, pp.649-659.

Yabluchanskiy, A. Ma, Y. Iyer, RP. Hall, ME. And Lindsey, ML. Matrix Metalloproteinase-9: Many Shades function in cardiovascular disease. Physiology (Bethesda).2013, 28(6), pp.39-403.

Yolanda, D, Ariadi. And Lipoeto, NI. Perbedaan kadar Protein Serum Ibu Pada Kehamilan Aterm Ketuban Pecah Dini dan Kehamilan Normal. Journal Kesehatan Andalas.2014, 4(3), pp.936-942.

Zitka, O. Kukacka, J. Krizkova, S. Huska, D. Adam, V. Masarik, M., *et al*. Matrix Metalloproteinase. Current Medicinal Chemistry, 17(31).2010, pp.3751-3768.

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