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Elevated Monocyte Serum Levels as a Risk Factor for Preterm Labor

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Abstract: ***Preterm delivery is still an obstetric problem today, because it often causes perinatal morbidity and mortality. Monocytes are non - specific inflammatory cells that respond early to inflammatory reactions, especially in the endometrium, so logically, the levels will be high both in the cervix and in the mother's serum. This study aims to determine that high levels of maternal serum monocytes are a risk factor for preterm labor. This research uses a case control study. Samples were taken on cases that met the inclusion and exclusion criteria, and were divided into two groups. This research was conducted at the Obstetrics - Gynecology Polyclinic and in the Emergency Room of the Sanglah Hospital Denpasar. The time of the research was carried out from 22 June 2020 to 31 October 2020. Based on the distribution of the sample in this study, it can be concluded that the majority (86.4%) were in the 20–35 - year age group and the proportion in the> 35 years and <20 4.5% and 9.1% respectively. The Fisher's Exact Test statistical test was carried out, it was found that p = 0.664 was not significantly different between the age of the mother and the incidence of preterm labor. From this study, it was found that high levels of maternal serum monocytes had a statistically significant relationship with the incidence of preterm labor (p = 0.016, OR 5.714, 95% CI 1.560 - 20.929).***

Keywords: Monocyte's serum, preterm labor

1. Introduction

Preterm delivery is still an obstetric problem today, because it often causes perinatal morbidity and mortality. The incidence rate is still quite high and there is no downward trend, especially in developing countries. Inflammation related to infection is mentioned as one of the risk factors for preterm delivery. Preterm birth is delivery that occurs at gestational age between 20 and less than 37 weeks. This labor is characterized by uterine contractions at least four times in 20 minutes or eight times in 60 minutes accompanied by progressive changes in the cervix and cervical effacement of more than or equal to 80% [1].

Prevention of preterm labor is still very difficult because the cause is unknown, but efforts must be made to prevent the occurrence of preterm labor such as detection of risk factors, examination of cervical length, fetal fibronectin and examination of biological and genetic markers [2]. One of the risk factors that is often associated with preterm labor is inflammation due to lower genital tract infection, which is characterized by the occurrence of leukocytosis (high monocyte and neutrophil levels) [3]. It was also stated that 70 - 80% of preterm deliveries that occur spontaneously have a significant relationship with the incidence of infection in the vagina and cervix, which are often associated with infection in the chorioamnion tissue [4].

Preterm labor associated with infection/inflammation begins with the invasion of microorganisms in the endometrium, originating from the urogenital tract, gastrointestinal tract, periodontitis, and from the vagina [5]. Microorganisms in the vagina spread upward and colonize the endocervix and spread to the endometrium. Microorganisms or their endotoxins in the form of lipopolysaccharide (LPS) will stimulate monocytes in endocervical and choriodecidual cells to produce an inflammatory response, namely interleukins. These cytokines will then stimulate arachidonic acid to produce prostaglandins (PG). Prostaglandin E2 (PGE2) and Prostaglandin F2α (PGF2α) cause myometrial contractions [6]. In addition, PGE can directly activate matrix metalloproteinase (MMP) activity and indirectly affect vasoactive action, resulting in the formation of monocyte and neutrophil cells in the cervicovaginal, which is known that neutrophils and monocytes will produce collagenase, namely matrix metalloproteinase. or MMP which can degrade collagen [7].

This study was conducted based on the theory that preterm labor is associated with infection or inflammation that originates from the spread of microorganisms from the vagina to the endometrium through the uterine endocervix. Microorganisms or their products (LPS) will stimulate the natural immune system in the endocervix and endometrium to secrete monocytes, neutrophils, dendrites, and Tumor Necrosis Factor (TNF) cells. In previous studies, it was said that 70 - 80% of preterm deliveries that occurred spontaneously had a significant relationship with the incidence of vaginal and cervical infections at Sanglah Hospital. Monocytes are non - specific inflammatory cells that respond first to inflammatory reactions, especially in the endometrium, so logically, their levels will be high both in the cervicovaginal and in maternal serum. This study aims to determine serum monocyte levels in preterm labor. In addition to supporting previous research, it is hoped that this research will be able to provide input in diagnostic methods for early detection of preterm labor at Sanglah Hospital.

2. Literature Survey

Preterm labor is delivery that occurs at the age of 20 to under 37 weeks from the first day of the last menstrual period [8]. Preterm birth is one of the clinical problems in obstetrics around the world, because babies born preterm (less months) have a greater risk of morbidity and mortality when compared to babies born at term (term). The smaller or

younger the baby is, especially before 28 weeks of gestation, the greater the risk of suffering from brain injury, chronic lung disease, respiratory distress syndrome, necrotizing enterocolitis (NEC), and in their life will be in dire need of more medical and social care [9].

In essence, preterm labor is very difficult to avoid, because the cause is not known with certainty, but every effort must be made to prevent birth. Strategies to prevent preterm births, namely by preventing the occurrence of preterm labor, which will focus on identifying risk factors [10]. Success in the administration of drugs to prevent labor is unlikely to be reliable, and will only have the effect of delaying preterm birth by 48 hours, and not improving neonatal outcome [11]. However, this 48 - hour delay in delivery will provide an opportunity for doctors to administer corticosteroids as fetal lung maturation.

In 2005 it was reported that 12.9 million (9.6%) births worldwide were preterm, of which 11 million (85%) occurred in Africa and Asia [12]. In Southeast Asia, the average preterm delivery rate is 11.1% (CDC, 2005) Beck et al, 2010). Preterm deliveries in Thailand are approximately 15.5%, in the Philippines 25.9%, and in Malaysia 10% [13]. Meanwhile, in Indonesia, the incidence of prematurity has never been reported nationally. However, based on the results of the Basic Health Research (Riskesdas) of the Ministry of Health in 2007, the proportion of low - birth - weight babies (LBW) in Indonesia reached 11.5%, although the LBW rate does not absolutely represent the incidence of preterm birth. In Sanglah Hospital Denpasar, the incidence of preterm delivery was 12.70% in 2011 [14] and in 2017 and 2018 this incident rate increased to 21.6% and 24.11% [15].

The exact mechanism of preterm delivery in most cases is unknown. The increasing number of risk factors is thought to be related to the mechanism related to the occurrence of myometrial contractions that progress to preterm labor [16]. Identifying risk factors will provide important insights into the mechanisms of preterm delivery. There are several maternal and fetal characteristics that are associated with the occurrence of preterm delivery, but none of these risk factors stand alone. The risk factors are categorized into several groups, namely: 1) Based on demographic information; 2) History of previous pregnancies; 3) The risk factors found in this pregnancy; 4) Nutrition (body size, and weight gain); 5) Associated with biophysical markers [3].

Babies born not old enough or preterm, often have risks related to the immaturity of their organ systems. Complications that often arise in infants born very preterm are respiratory distress syndrome (RDS), cerebral hemorrhage or intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC), sepsis, apnea, and retinopathy of prematurity (ROP) [5]. In the long term, babies born preterm have a risk of severe mental retardation, cerebral palsy (CP), seizures, blindness, and deafness. In addition, learning disorders are often encountered, impaired adaptation to the environment, and motor disorders [5].

Monocytes are innate immune cells that participate in various biological processes. In the uterine decidua, monocytes represent the major leukocyte subset throughout pregnancy, representing 20% of the total leukocyte. Due to their high plasticity, monocytes respond to tissue environmental stimuli by acquiring different functional phenotypes [17]. In particular, the M1 phenotype (pro - inflammatory monocytes) is characterized by strong microbicidal and tumoricidal activity, high production of reactive nitrogen and oxygen species, high production of proinflammatory cytokines, and promotion of type 1 (Th1) helper T cell responses. In contrast, the M2 phenotype (anti - inflammatory and tissue repairing) is characterized by efficient phagocytic activity, promotion of tissue remodeling, parasite containment, suppression of tumor development, and immunoregulatory functions. In fact, this M1 - M2 phenotype reflects the polarization of Th1 - Th2 cells [18] [19].

In addition to immunological processes, monocytes actively participate in pregnancy because a transient inflammatory phase is required for successful implantation. Notably, during the peri - implantation period, the polarization pattern of decidual monocytes is skewed towards M1. When the trophoblast attaches to the endometrium and invades the uterine stroma, the decidua undergoes a transition to a mixed M1 - M2 profile [20]. These mixed M1 - M2 monocytes persist until mid - gestation and cooperate with trophoblast cells to support the extensive remodeling of uterine blood vessels required for adequate placenta - fetal blood supply [21]. After completion of placental development, decidual monocytes have been shown to undergo M2 polarization, characterized by abundant production of IL - 10 and indoleamine 2, 3 - dioxygenase. This feature of M2 increases maternal immune tolerance to semi allogenic fetuses and fetal growth until birth [22]. Ultimately, decidual monocytes are the primary innate immune cells that contribute to the birth process [23].

Cervical maturation and dilation during labor are known to have a relationship with the inflammatory response. Monocyte levels are known to be higher during the cervical ripening process. There is an increase in cervical and serum monocyte levels on the day before delivery compared to mid or late pregnancy. Large numbers of cervical monocytes are also found in lipopolysaccharide - induced (LPS) preterm labor. This suggests that there is a possible involvement of monocytes in cervical remodeling [24].

The role of decidual monocytes in the regulation of cervical ripening and initiation of labor is primarily dependent on the secretion of the proinflammatory cytokines IL - 1, IL - 6, and Tumor Necrosis Factor (TNF), as well as Matrix Metalloproteinase (MMP) and Nitric Oxide (NO) [24]. Increasing evidence suggests that aberrant activity of uterine monocytes may be involved in the etiology of preterm labor. In fact, idiopathic preterm labor is preceded by selective accumulation of decidual monocytes (Hamilton, et al., 2012). Monocytes also play a role in the rupture of fetal membranes because monocytes are recruited by this tissue and produce MMP - 9. MMP - 9 is elevated at term, preterm, and in PPROM. These data indicate that MMP - 9 is directly related to physiological premature rupture of membranes

and pathological PPROM [24]. Monocytes are also implicated in the etiology of preterm labor because the concentration of CC motif Ligand 2 (CCL2) is increased in the amniotic fluid of women who deliver preterm, both in the presence and absence of intra - amniotic infection, compared with women who deliver at term [24].

In addition, increased levels of chemokines in the amniotic fluid of women undergoing idiopathic preterm labor and associated infection are correlated with increased monocyte recruitment [24]. Furthermore, monocyte depletion protects pregnant mice from LPS - induced preterm labor [25]. As these cells play a central role in the maintenance of fetal tolerance and in the initiation of labour, the regulation of monocyte trafficking and activation/polarization during normal delivery and preterm labor warrants further investigation.

The above statement is supported by the findings of a study in London (61 - 100%) in women with a history of previous preterm delivery, which found that the number of cervical monocytes was not detectable. The absence of detectable monocytes in the cervix may be due to ascending bacterial translocation as evidenced by chorioamnionitis seen in the extraembryonic membrane in 5 of 6 cases.

Macrophage migration inhibitory factor (MIF) is also said to be associated with preterm delivery. MIF is a cytokine that was first described as an inhibitor of the random migration of monocytes and macrophages that has a number of immune and catalytic functions. A study conducted in China [26] stated that there was a significant relationship between an increase in plasma MIF concentrations in the early trimester and preterm delivery (OR=1.09, 95%CI: 1.03 - 1.18). For every 1mg/ml increase in MIF concentration, the adjusted risk of preterm delivery increased by 7% (OR 1.07 [1.02 - 1.15], P=0.002). MIF is said to upregulate toll - like receptor 4 (a recognition molecule for bacterial endotoxins) and induce cyclooxygenase - 2. In addition, the MIF cellular receptor is CD74 (Cluster of Differentiation 74), which is a molecule that is regulated in fetal membranes and is associated with premature rupture of membranes [26].

3. Methods

This study uses a case control study. Samples were taken on cases that had met the inclusion and exclusion criteria, and were divided into two groups. The case group was cases of preterm delivery, while the control group was cases of preterm pregnancy that were not in the labor phase. All samples were examined for maternal serum monocyte levels.

This research was carried out at the Obstetrics - Gynecology Polyclinic and in the IGD Maternity Room at Sanglah Hospital Denpasar. The time of the study was carried out from June 22, 2020 to October 31, 2020. The study sample was mothers with preterm pregnancies with and not in the labor phase who came to visit the IRD delivery room or Obstetrics - Gynecology Polyclinic, Sanglah Hospital Denpasar, who met the inclusion and exclusion criteria., willing to participate as research subjects and have signed an informed consent. The inclusion criteria for cases and controls were pregnant women between 20 weeks to 36 weeks 6 days, experiencing uterine contractions at least 2 times in 10 minutes, cervical dilatation of about 2 cm (for cases) and no uterine contractions or cervical dilatation (for controls), the first day of the last menstrual period was clear or had an ultrasound before 20 weeks so that the estimated delivery was clear, single fetus and live fetus. Meanwhile, the exclusion criteria were placental abruption, premature rupture of membranes, uterine myoma, receiving antibiotic therapy in the past week, ante partum bleeding, known congenital abnormalities in the fetus by ultrasound, hypertension in pregnancy and polyhydramnios.

Samples of preterm delivery cases were taken by consecutive sampling. Cases were caught directly at the Obstetrics - Gynecology Polyclinic and in the Maternity Room in the Emergency Installation of Sanglah Hospital Denpasar according to the inclusion and exclusion criteria. After signing the informed consent, general obstetric examination and specimen collection were carried out. Preterm pregnancies that did not undergo labor were used as controls for each case, so that the number of controls was equal to the number of cases with a ratio of 1: 1. The independent variable in this study was maternal serum monocyte levels, while the dependent variable was preterm delivery.

Preterm pregnant women with and without preterm labor who came to the delivery room and Obstetrics - Gynecology Polyclinic Sanglah Hospital, were sampled according to the inclusion and exclusion criteria, recruited, and then signed the informed consent. All samples were managed according to the Guidelines for Diagnosis and Therapy of the Department of Obstetrics and Gynecology at Sanglah Hospital Denpasar, both cases and controls were treated the same. All samples were taken cubital vein blood, and collected in a tube containing purple Ethylenediaminetetraacetic (EDTA), the process was carried out by a certified laboratory officer and then sent to the Clinical Pathology Laboratory of Sanglah Hospital Denpasar for processing and counting monocyte levels. The data obtained were recorded in the data collection form.

The research instrument consisted of a 5 - cc syringe, a tube containing EDTA, alcohol swab, data collection forms, and equipment for documentation. The sampling method was carried out in several steps where the patient was initially lying on the examining bed, then asepsis was performed in the cubital fossa area, then blood was drawn and inserted into the EDTA tube. The sample was then sent to the Clinical Pathology Laboratory of Sanglah Hospital Denpasar to calculate the serum monocyte level. The results obtained are then recorded on the data collection form. After the data is sufficient, then statistical analysis is carried out using the Windows version of the IBM SPSS 26.0 program. All data obtained in this study were analyzed descriptively. The data were tested for normality of the data using the Shapiro - Wilk test. Then the homogeneity test for Maternal Age and Parity data was carried out using Levene's Test. Independent t - test was used for comparability of maternal age and parity data. The calculation of the odd ratio of maternal serum monocyte levels as a risk factor for preterm delivery used the Chi - Square test based on a two - by - two table.

4. Result

Based on research that has been carried out at the Obstetrics - Gynecology Polyclinic and in the IGD Maternity Room, Sanglah Hospital, Denpasar, regarding high maternal serum monocyte levels as a risk factor for preterm labor since June 22, 2020. The data on the characteristics of the research sample can be seen in table 1.

Table 1: Characteristics of the sample

Variable		*Variable*		*p*
		Preterm Delivery N (%)	*Preterm Pregnancy N (%)*	
Mother's age	<20 years	2 (50%)	2 (50%)	0.664[a]
	20 - 35 years	18 (47, 4%)	20 (52, 6%)	
	>35 years	2 (100%)	0 (0%)	
Parity	Primipara	1 (33, 3%)	2 (66, 7%)	0.240[a]
	Multipara	20 (55, 6%)	16 (44, 4%)	
	Grande multipara	1 (20%)	4 (80%)	
Gestational age	20 - <28 weeks	2 (100%)	0 (0%)	0.366[b]
	28 - <32 weeks	11 (55%)	9 (45%)	
	32 - <37 weeks	9 (40, 9%)	13 (59, 1%)	
Body Mass Index	Underweight (<17, 0 - 18, 4)	3 (42, 85%)	4 (57, 15%)	0, 132[b]
	Normal (18, 5 – 25, 0)	14 (63, 6%)	8 (36, 4%)	
	Overweight (25, 1 - >27, 0)	5 (33, 3%)	10 (66, 7%)	

a: *Fisher's Exact Test*
b: Chi Square

After the data is collected, categorized and then the data is analyzed for normality test to determine whether the distribution of this research data is normal or not, because the number of samples <50, normality test was carried out using the Saphiro - Wilk test. The distribution of the data can be seen in Figure 1, then after analyzing the significance (p) of the two groups, namely the high maternal serum monocyte group and the normal maternal serum monocyte group, the results were 0.114 and 0.340 respectively (can be seen in table2). Thus, based on the normality test of the Saphiro - Wilk data, the data is normally distributed because p>0.05.

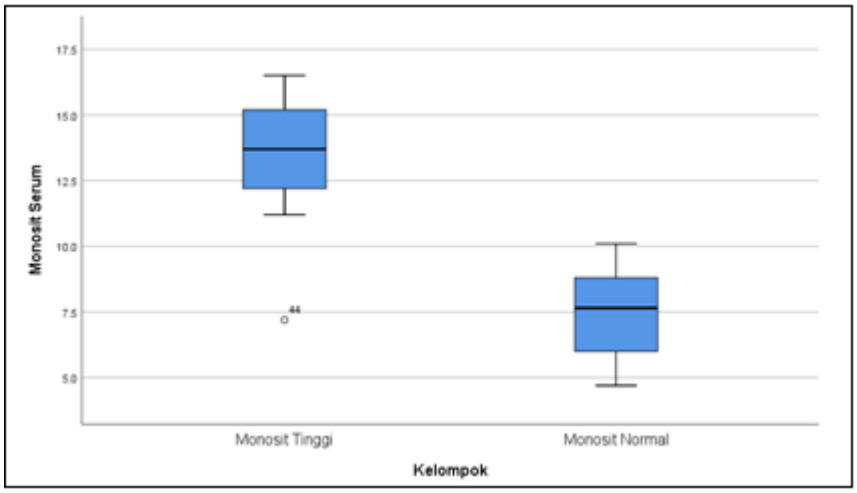

Figure 1: Histogram of maternal serum monocyte data distribution

Table 2: Saphiro - Wilk test for data normality test

Group	Statistic	df	Sig.
High Mother Serum Monocytes	0.929	22	0.114
Low Mother Serum Monocytes	0.952	22	0.340

After the data is tested for normality, then the data is analyzed using Levene's Test to test whether the variation in the data from the research sample has the same variance. The results of the Levene's Test in this study sample obtained a significance of (p) 0.165, where a significance (p) > 0.05 which can be interpreted as a variant of maternal serum monocyte data in the high and normal groups is homogeneous.

Bivariate analysis is intended to determine the significance of the relationship between the independent variable and the dependent variable.

Table 3: Homogeneity Test with Levene's Test

Variable	F	Sig.
Mother Serum Monocytes	1.999	0.165

This analysis uses the Chi Square test (x2) with a 95% confidence level using SPSS 26.0. The results of the bivariate analysis in this study can be seen in table 4, obtained p = 0.016, where p <0.05 which can be interpreted that high maternal serum monocyte levels are statistically significant as a risk factor for preterm delivery. There was

also an odd ratio of 5.714, which can be interpreted as pregnant women with high serum monocyte levels and having a 5.7 - fold risk of preterm delivery when compared to pregnant women with normal serum monocyte levels.

Table 4: Bivariate analysis of maternal serum monocyte levels with delivery/preterm pregnancy

Variable	Effect	Total	P		Ho	Odd Ratio
Mother serum monocytes	Preterm delivery	Preterm pregnancy				
High	16	6	22	0.016	Rejected	5, 714
Low	7	15	22	Df: 1		(95% CI 1, 560 – 20, 929)

From this study, it was found that high maternal serum monocyte levels had a statistically significant relationship with the incidence of preterm delivery (p=0.016, OR 5, 714, 95% CI 1, 560 - 20, 929). A study related to the results of this study is a study by Paquette, et al (2017) in Toronto, Canada with a sample of 50 samples (20 mothers with preterm delivery and 30 with normal delivery) using the RNA sequencing method to detect the expression of 262 genes in monocytes and 184 gene in whole blood (leukocytes) in preterm delivery, for which RNA sequencing is known to have high sensitivity. This study found that changes in gene expression in monocytes and whole blood were found to be increased in women with preterm delivery.

A study that had similar results was also found in Malawi by Abrams et al (2004), in this study an increase in inflammatory markers such as MIP - 1α, MCP - 1, IL - 8, IL - 6, TNF - , TGF - ß was found to be increased. It can be concluded that monocytes also increased in the findings of this study, because monocytes when exposed to pathogens will release cytokines. Research conducted by Curry et al (2007) in Denmark, with a sample of >100, 000 women from 1997 - 2002, measured inflammatory markers such as TNF - alpha, IFN - , granulocyte - macrophage colony - stimulating factor (GM - CSF) at age 25 weeks of gestation by multiplex flow cytometry, and found similar results to this study, namely that inflammatory markers were found to be elevated in women who had preterm delivery [27].

A similar study conducted by Taylor, et al (2013), involving 1115 women from 1998 - 2004, looking for inflammatory biomarkers from vaginal fluid samples that can be useful in predicting preterm labor, found that an increase in IL - 6 in the second trimester is at risk for preterm labor, although an increase in TNF - , GM - CSF, IL - 1ß, and IL - 6 was found, but it was not proven to be statistically significant, this study is similar to the study conducted by taking vaginal fluid samples, although the study by Taylor, et al (2013) was more specifically looked for which inflammatory biomarkers play a role in increasing the risk of preterm delivery [28].

The findings of a study by Holst, et al (2009) concluded that the combination of proteins taken from amniotic fluid and cervical fluid samples can predict preterm labor, also found an increase in cytokines and neuropeptides in women who experienced preterm labor, in contrast to this study, research by Holst, et al (2009) took samples of cervical fluid and amniotic fluid [29].

Research in Korea by Kim, et al (2020) on inflammatory proteins of amniotic fluid, plasma and cervicovaginal fluid to predict inflammation and preterm labor concluded that indeed cytokine/chemokine levels were elevated in amniotic fluid and cervicovaginal fluid of mothers with preterm delivery, but found no significant difference between the effectiveness of the samples tested, so this study concludes that non - invasive tests such as protein measurement in cervicovaginal fluid or CRP measurements are effective enough to predict IAI (Intra - amniotic infection and/or inflammation) and preterm labor, however, measurement of other inflammatory mediators will improve the ability to predict the incidence of inflammation / inflammation which is a risk factor for preterm labor [30].

5. Conclusion

In the study of high maternal serum monocyte levels as a risk factor for preterm labor, it was found that pregnant women with high serum monocyte levels had a 5.7 - fold risk of having preterm delivery when compared to pregnant women with normal serum monocyte levels. The weakness of this study is that there is no dental and oral health examination to rule out risk factors for infection or inflammation.

6. Future Scope

Based on the results of this study, there is a recent view regarding the use of maternal serum monocyte levels as a risk factor for preterm delivery. This research can be the basis for conducting a multicenter study in order to obtain results that are more representative of pregnant women in Indonesia. Other research that can be done is to take other samples such as cervical fluid and inflammatory markers thought to be associated with preterm labor such as IL - 6, IL - 8, IL - 10 and others to better predict the risk of preterm labor.

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