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RESEARCH ARTICLE

High Level of Uterine Endocervical Interleukin-8, Matrix Metalloproteinase-8, and Interleukin 1B as a Risk Factor for Preterm Labor

Suwardewa TGA[1], Suwiyoga K[1], Mantik Astawa N[2], Sutirta Yasa IWP[3]

[1] *Depart. Obstetrics and Gynecology, Medical Faculty of Udayana University, Sanglah Hospital Bali, Indonesia.*

[2] *Laboratory of Veterinary Medicine, Udayana University Bali, Indonesia.*

[3]*Laboratory of Clinical Pathology Medical Faculty of Udayana University, Sanglah Hospital Bali, Indonesia.*

*Corresponding Author: **Suwardewa TGA**

Abstract

The preterm birth is a birth that takes place on a gestational age between 20–36 weeks and six days. The preterm birth is the leading cause of neonatal mortality and morbidity. Despite the current treatment procedures, the incidence of preterm birth has not changed in the last two decades. Incomplete understanding of the pathophysiological mechanisms underlying preterm labor is the major obstacle to prevent preterm birth. Recently, endocervical inflammation has been thought as a risk factor of a preterm labor. Several important substances such as endocervical IL-8, MMP-8, and IL-1ß, has been considered to play a key role on preterm birth through cervical ripening. With those assumptions, the detection of a higher level of uterine endocervical IL-8, MMP-8, and IL-1ß can be used to predict preterm labor. The design of this study is an observational case control study with a case group, which consisted of pregnant women with 20–36 weeks and six days of gestational age who had signs of labor, and the control group consisted of pregnant women with 20–36 weeks and six days of gestational age who had no signs of labor. The material used for this study is cervical mucous, and the enzyme-linked immunosorbent assay method was used to assess the level of IL-8, MMP-8, and IL-1ß at laboratory of Veterinary Medicine of Udayana University Denpasar. In this study we used 48 samples, among which 24 were case group, and 24 as a control group. The mean age of the women, gestational age, and parity from both group are (27.04 vs 28.62 years old), (31.8 vs 29.50 weeks), and (0.96 vs 1.29) respectively, which are statistically homogenous ($p > 0.05$). Each level of interleukin-8, matrix metalloproteinase, and interleukin-1ß suggests a risk factor of a preterm labor for: 35 times (OR = 35.00; CI 95% = 6.95–176.39; $p = 0.001$); 6.6 times (OR = 6.60; CI 95% = 1.25–34.95; $p = 0.016$), and 8.3 times (OR = 8.3; CI 95% = 2.15–32.3; $p = 0.001$), orderly. Among these cytokines, one that contributes most through a preterm birth is interleukin-8 (61%), followed by matrix metalloproteinase-8 (27%), and interleukin-1ß (12%).Conclusion: Endocervical inflammation with high level of IL-8, MMP-8, and IL-1ß is a risk factor for preterm labor. IL -8 gives the most contribution on preterm labor.

Keywords: *Preterm birth, Endocervical IL-8, MMP-8, and IL-1β.*

Introduction

The preterm labor is a labor that takes place on a gestational age between 20–36 weeks and 6 days [1], and it is still being an obstetric problem since the last decade because of its high risk of perinatal morbidity and mortality. It had been reported on 2005 that 12.9 million (9.6%) births worldwide were a preterm case, where 85% of it took place in Africa and Asia; 0.5 million cases happened at Europe and North America; and 9 million cases occurred at Latin America and Caribbean [2]. In South East Asia, the prevalence of preterm birth is 11.1%[3]. The incidence of preterm births in Indonesia has not been reported nationally;

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however, based on the result of a research done by *Riset Kesehatan Dasar (Riskesdas*) of Indonesian Health Ministry on 2007, the prevalence of a low birth weight (LBW) in Indonesia reached the number of 11.5%, despite the fact that the number of LBW cannot represent the number of preterm birth[4].

The prevalence of preterm labor at Sanglah Hospital Denpasar is 10% and it escalated to 8.19% at 2009, 10.54% at 2010, and 12.70% at 2011[5]. The preterm birth can cause a psychical stress, both to mother and her family, it also can cause a risk of a short term side effects such as respiratory distress syndrome, brain hemorrhage, bronchopulmonary dysplasia, patent ductus arteriosus, and necrotizing enterocolitis.

Besides, the risk of an occurrence of sepsis, apnea or respiratory arrest, and retinopathy of prematurity are increasing. More than a million kids suffer from death annually caused by the complications of preterm births, and those who survive shall face threats of a lifetime disabilities, including learning, eye and hearing disorders[6 and 7]. Complications mentioned above, can create a low quality of human resources in the future. Also, caring for a preterm newborn needs a more sophisticated medical technology and a higher cost.

Lot of efforts have been taken in order to lower the perinatal morbidity and mortality, which is mainly caused by a preterm newborn, such as an intensive care of a neonatus, administration of some medications, thermal therapy, or caring given by healthcare professional, but those efforts have not given a satisfying results yet [8].

The best effort to prevent morbidity and mortality is to avoid the preterm birth itself, but that will be really difficult since the cause of it has not been known yet clearly.

The administration of a tocolytic to eliminate the contractions of the uterus, or the administration of an antibiotics to prevent infection, reducing the volume of amnion to lessen the pressure within the uterus, and an enough bed rest time are all usually done in preterm labor, but none can prevent the perinatal morbidity and mortality[6, 8, and 9].

Preterm birth is a multifactorial syndrome like infections, hemorrhage and ischemia of uterus, uterine over distension, cervical incompetence, abnormal allograft reaction, allergic phenomenon, and endocrine disorder.

These factors are associated with a series of clinical symptoms which cause a synchronization of myometrial contraction, tear of a chorionic and amnion membrane, and cervical ripening[10]. In the last decade, a study about the correlation between infection and preterm labor has been a concern. In Indonesia, the incidence of infection is still somewhat high, including vaginitis, and cervicitis in pregnancy, but the number of infections itself has not been reported nationally.

At Sanglah Hospital Denpasar, multi bacterial infections of vagina and endocervix in preterm birth are significantly higher compared to a multibacterial infections of vagina and endocervix in an aterm birth [11 and 12].

Conventionally, preterm labor that is being related with infections, begin with a bacterial invasion deep into endometrium way long before the time of pregnancy, for example during a menstrual period, and then the bacterias colonize within a layer of chorio-decidua[6]. However, a recent observation found that there were cervical ripening (softening, shortening, and dilatation) without the contraction of the myometrium, and labor will take place subsequently. As known before, cervix has a function to keep the pregnancy within the uterus till the aterm is reached.

To do its role, cervix needs a biomechanical force which comes from an effort of extracellular matrix such as collagen fibers, hyaluronan (HA), elastin, and water[13 and 14]. The cervix has 64.3–72% of collagen [15], which consist of type I collagen (66%), type III collagen (33%), and few amounts of type IV collagen at basal membrane[16].

The otherwise role of uterus happens when the pregnancy reaches aterm, where cervix softens and open, allowing the baby to pass out. The exact mechanism of how cervix adjusts these complicated dynamic changes is really interesting and is one of biomechanical problem.

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Critical problems will emerge as these biomechanical changes happen when the pregnancy is still preterm, thus increasing the risk of a birth with insufficient months or preterm. The background of these biomechanical changes has not been known yet[14]. It has been thought that the inflammation in endocervix is the trigger of a spontaneous preterm labor through collagen degradation.

This research studied about the mechanism of a preterm labor related with an inflammation of uterine endocervix. It is estimated that the infection occurs within a vagina spreading into endocervix and colonizing there.

The microorganism themselves or their products like lipopolysaccharide (LPS) induce the innate immune system to release inflammatory cells like macrophages, dendrite cells, natural killer cells (NK cells), and granulocytes (mastocyte and neutrophile.

The activation of cells come from an innate immune system, which will produce interleukin-1ß (IL-1ß), interleukin—6 (IL-6), and tumor necrosis factor α (TNF α). Besides, macrophage produces interleukin-8 (IL-8). The IL_1ß triggers IL-8 to activate neutrophil, then, neutrophil will produce collagenase, which are neutrophilic collagenase or matrix metalloproteinase 8 (MMP-8).

This MMP will degrade collagen in the cervix into fragments; hence, weaken the cervix and softening them, making it easier to open. With a pressure within a uterus (fetus and amnion), orificium uteri internum will stretch and widen, making it to change its shape like a funnel and the cervical canal will be shortened in length[17].

This fact explains that the osteum uteri internum is actually where the maximum softening of a cervix takes place [18]. It can be concluded that preterm birth began with an inflammation of uterine endocervix. Inflammation can be caused by lot of mechanisms, and one that mostly causes it is the presence of an infection that is spreading from a vagina into endocervix. The mechanism mentioned by the latter needs a further research.

Study Design

We conducted an observational case-control study at Sanglah Hospital Denpasar to normal pregnant women with 20–36 weeks and six days of gestational age, single live fetus, intact of fetal membrane, and cephalic presentation. The case group consisted of pregnant women who had signs of labor, whilst the control group consisted of pregnant women with no signs of labor.

The material for this study was endocervical mucous, and we assessed the level of IL-8, MMP-8, and IL-1ß at Laboratory of Veterinary Medicine of Udayana University. This study got an approval from Ethic Committee of Research of Medical Faculty of Udayana University/Sanglah Hospital Denpasar.

Result and Discussion

A case control study had been done to 24 normal pregnant women with 20–36 weeks and six days of gestational age with signs of labor as case group and 24 normal pregnant women with 20–36 weeks and six days with no signs of labor as a control group at Obstetrics and Gynecology Department of Sanglah Hospital Denpasar.

Normality Test Data

Normality test of mother's age, gestational age and parity was done with Shapiro–Wilk test. The result showed that the data were normally distributed ($p > 0.05$) and was presented on table 1.

Table 1: Result of normality test data of mother's age, gestational age, and parity for each groups

Groups	P	Interpretation
Mother's Age Case	0.276	Normal
Mother's Age Control	0.412	Normal
Parity Case	0.102	Normal
Parity Control	0.234	Normal
Gestational Age Case	0.261	Normal
Gestational Age Control	0.092	Normal

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Data Homogenous Test

Homogenous test of mother's age, gestational age, and parity was done using Levene's test. Results showed that the data was homogenous ($p > 0.05$) and was presented on table 2.

Table 2: Homogeneity of mother's age, gestational age, and parity between two groups

Variable	F	P	Interpretation
Mother's Age	0.797	0.377	Homogenous
Parity	2.945	0.093	Homogenous
Gestational Age	2.270	0.139	Homogenous

Distribution of Mother's Age, Gestational Age, Parity between Two Groups

In this case control study, we did an independent t-test as a comparability test of mother's age, gestational age, and parity. The result of the analysis was presented on table 3

Table 3: Distribution of mother's age, gestational age, and parity between two groups

Variable	Case Group (n=24)		Control Group (n = 24)		P
	Mean	SD	Mean	SD	
Mother's Age (year)	27.04	6.55	28.62	5.84	0.382
Gestational Age	31.38	4.15	29.50	4.73	0.181
Parity	0.96	0.86	1.29	1.04	0.257

As shown on table 3, it was found that each variables: mother's age, gestational age, and parity of both groups are statistically indifferent or comparable ($p > 0.05$)

Risk of Preterm Labor with a High Level of Uterine Endocervical IL-8

In order to know the odd ratio of whether the high level of uterine endocervical IL-8 can be a risk factor of preterm labor, we did a chi-square test. The result of the test was presented on table 4.

Table 4: Risk of preterm labor with a high level of uterine endocervical IL-1β

		Group		OR	CI 95%	P
		Case	Control			
IL-8	High	21	4	35.00	6.95–176.39	0.001
	Low	3	20			

Table 4 showed that the high level of uterine endocervical IL-8 was 35 times as risk factor of preterm labor (OR = 35.00 CI 95% = 6.95–176.39; p = 0.001)

Risk of Preterm Labor with High Level of Uterine Endocervical MMP-8

In order to know the odd ratio of whether the high level of uterine endocervical MMP-8 can be a risk factor of preterm labor, chi-square test was used. The result was presented on table 5.

Table 5: Risk of preterm labor with a high level of uterine endocervical MMP-8

		Group		OR	CI 95%	P
		Case	Control			
MMP-8	High	22	15	6.60	1.25–34.95	0.016
	Low	2	9			

Table 5 showed that the high level of uterine endocervical MMP-8 was 6.6 times as risk factor of preterm labor (OR = 6.60; CI 95% = 1.25–34.95; p = 0.016)

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Risk of Preterm Labor with High Level of Uterine Endocervical IL-1β

In order to know the odd ratio of whether the high level of uterine endocervical IL-1ß can be a risk factor of preterm labor, we did chi-square test. The result of the analysis was presented on table 6.

Table 6: Risk of preterm labor with a high level of uterine endocervical IL-1β

		Group		OR	CI 95%	P
		Case	Control			
IL-1ß	High	15	4	8.30	2.15–32.3	0.001
	Low	9	20			

Table 6 showed that the high level of uterine endocervical IL-1ß was 8.3 times as risk factor of preterm labor (OR = 8.3; CI 95% = 2.15–32.3; p = 0.001).

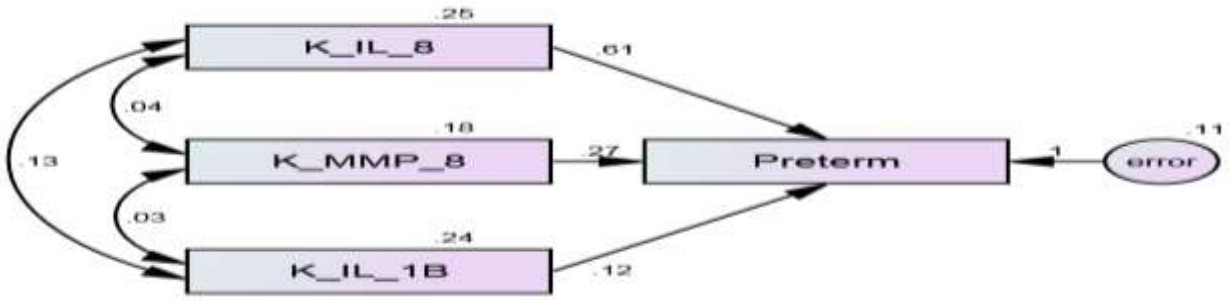

Figure 1: Pathway analysis between the level of IL-1β, IL-8, and MMP-8 with preterm labor
Fig. 1 Path Analysis between the Level of Endocervical IL-1ß, IL-8, and MMP-8

Based on a model of goodness of fit, the three variables have a role as a risk factor of preterm labor and they are able to contribute 56.6%, which means that 43.4% of the rest of the risk factor is contributed by another factor which had not been studied. From the description of a path analysis above, we can assume that all those three factors are related to the occurrence of preterm labor, and IL-8 gives the most contribution of 61% followed by MMP-8 (27%) and IL-1ß (12%).

Conclusion

Endocervical inflammation with the high level of IL-8, MMP-8, and IL 1ß is a risk factor of a preterm labor mechanism. The IL-8 gives the largest contribution on this mechanism.

Discussion

Endocervical IL-8 as a Risk Factor of Preterm Labor Mechanism

The IL-8 is one of the member of chemotactic cytokine or a family of chemokine[19]. This chemokine was first found during 1986–1987 as a solvent in supernatant. The IL-8 is also called as neutrophil-activating factor (NAF), but then the term neutrophil-activating peptide is used by some group and the molecule is finally called as IL-8[19]. The interleukin-8 shows a biological activity through two glycosylate receptors which has a high affinity to interleukin-8 receptor A (IL-8RA) and interleukin-8 receptor B (IL-8RB). Those that induce the production of IL-8 are pro-inflammation cytokines such as IL-1α or IL-1ß, tumor necrosis factor α (TNF-α), and also lipopolysaccharide (LPS) of bacteria.

Besides its role in triggering the activation of neutrophil, the IL-8 also has a role in regulating the expression of adhesion molecule on the surface of neutrophil and inducing the vital changes for in vivo cells' migration. The IL-8 holds a key role in the regulation of reproductive organs, its level is high within amniotic and other reproductive tissues before the delivery.

There is a significant correlation between the high level of endocervical IL-8 and the inflammation of cervicovaginal region, which has been considered as a risk factor for preterm labor[20]. In the study by Holst (2009) and Kacerovsky (2014), they revealed that the level of uterine endocervical IL-8 could be used to determine whether there were microbacteria within the amnion or histological

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chorioamnionitis (HCA). It was also reported that the assessment of the level of uterine endocervical IL-8 could be used as a non-invasive examination with the presence of an intra-amnion inflammation[21 and 22].

The amount of 10.0 ng/mL of endocervical IL-8 is a strong predictor of histological chorio-amnionitis (HCA), which is associated with preterm labor with 100% sensitivity and 67% of specificity, a positive predictive value of 63% and a negative predictive value of 100%[21]. A case control study with an aim to differ the average level of cervico-vaginal IL-8 on preterm and aterm birth found that approximately the level of uterine endocervical IL-8 on preterm labor was significantly higher than on aterm.

This result suggested that IL-8 was useful for predicting preterm labor[23]. The approximate level of endocervical IL-8 on an early preterm labor is 4.8 times higher compared to an aterm, and IL-8 is sensitive enough to predict preterm labor[24]. Down reported that the concentration of IL-8 on cervical mucous of women who had a preterm labor was higher than women who did not have a labor ($p < 0.01$)[25]. However, the production of IL-8 by vaginal epitel and endocervix due to pathogen can be inhibited with the administration of progesterone (P_4)[26].

Endocervical cells produce some IL-8, in which there is on preterm labor, this IL-8 only acts locally and does not extend into amnion[27], or spreads through blood vessels [28].

Serum level of IL-8 on preterm labor, both with cervicitis or not, statistically does not show the significant differences; which means that the difference in the level of IL-8 is occurs only in uterine endocervix[29]. Another researcher found that endocervical cytokines' profile did not show any changes in women who experienced a miscarriage except for IL-8 level, which is in a high level significantly[30].

The explanation above enlightens about the activity of IL-8, which solely acts depending on its production. If it is produced in a less amount on an early phase, they will only act locally before their level can be detected on the periphery. Author opines that before inducing a trigger for a preterm labor, IL-8 will act locally first on the uterine endocervix, making them soft and moist. The effect of gravitation force from the fetus, placenta, and amniotic fluid on (as the weakest defense) causes the shape of the cervical canal wider and to resemble like a funnel, and with time, the length of the cervix will be shortened. The Il-8 holds a key role on cervical ripening indirectly by triggering the neutrophil to produce collagenase (neutrophil collagenase or MMP-8)[31].

In this study we found that the average amount of uterine endocervical IL-8 on a case group was significantly higher than the average level of IL-8 on a control group (1.1284^3 pg/mL: 1.1838^2 pg/mL). Based on table 5.4 above, the high level of uterine endocervical IL-8 is 35 times more as risk factor for preterm labor (OR = 35.00; CI 95% = 6.95–176.39; p = 0.001).

The result of this study is similar with another study, where a high level of IL-8 was found on a case of genital tract infection/inflammation from the vagina to uterus. Nenadic and Kacerovsky, on their studies, found that there was a correlation between the high level of uterine endocervical IL-8 and the infection/inflammation of cervico-vaginal and chorioamnionitis. Its high level triggered IL-1ß in response to an infection/inflammation caused by bacterias and their lipopolysaccharides[20 and 22].

The approximate level of uterine endocervical IL-8 is higher on early preterm labor (pathological pathway) compared to aterm (physiological pathway)[24]. The IL-8, at first, acts locally by ripen the cervix through collagenase production. So, the high approximate level of IL-8 on uterine endocervix can be a risk factor for preterm labor, therefore, the IL-8 is useful to predict the occurrence of preterm labor [23 and 24].

Matrix Metalloproteinase-8 of Uterine Endocervix as a Risk Factor of Preterm Labor Mechanism

Cervix has a function to keep the result of a conception during pregnancy, and as a passage for the baby during delivery. The mechanical characteristics of cervix come from forces of extracellular matrix such as

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collagen fibers, proteoglycans, hyaluronan (HA), elastin, and water[13]. Collagen fibers are made up of type I collagen (66%), type III collagen (33%), and less amount of type IV collagen on basal membrane[16]. During delivery, these collagen components will degrade due to action of lytic enzymes such as collagenase/MMP-1, MMP-8, and MMP-13, which are produced by fibroblast and leukocyte. And leukocyte elastase is produced by macrophage, neutrophil, and eosinophil. In a normal condition, the degradation of collagen is always followed by production of new collagens along with its base components, where this process called as remodelling.

On preterm labor, there is a synchronization process between myometrium contraction, tear of amniotic membrane, and the ripening of uterine cervix. The ripening process of cervix itself (softening, thinning, and dilation) is a result of the weakening of cervix due to the degranulation of cervical collagen by matrix metalloproteinase enzyme and gravitation force (fetus and amnion) by pressing the cervix continually[33].

A radiolabel study shows that the degradation of collagen is due to the migration of neutrophil from blood vessel to cervix because of the trigger from bacterial LPS, as result of which neutrophil produces elastase and collagenase (MMP-8)[16].

The MMP-8 is a human neutrophil collagenase or type 2 collagenase, which is recently known to be expressed by epithelial cells. The MMP-8 is released from cells during chemotactic stimulation of infection and inflammation. The neutrophil activation is induced by inflammatory factors and microbe is a key phase in controlling the concentration of MMP-8. The MMP-8 is activated by another protease and oxidizing agents on extracellular milieu or on the surface of cells. The escalating concentration of MMP-8 reflects an increasing rate of its production and release[33].

The MMP has two different effects on pregnancy, first, it is responsible for protein degradation of tissue on extracellular matrix; thus, it will induce the ripening of cervix and tearing of amniotic membrane; second, it activates variable cytokines, and intra-amnion infection/inflammation. As known before that the largest components of cervix are type I and type III collagen[16 and 34]. An Inflammation of uterine endocervix will induce migration of neutrophil from blood vessels into endocervix and this neutrophil will release elastase of protease and neutrophil collagenase, or MMP-8[16]. Elastase and collagenase act synergically to degrade collagen tissues. The higher the concentration of neutrophil in endocervix, the higher the collagenase will be produced; thus, increasing the rate of degradation of collagen as connective tissue of cervix[33].

Rahkonen in one of her cohort study on 2009 found that the concentration of MMP-8 in endocervical mucous is somewhat higher compared to vaginal fluid. The high level of MMP-8 is also reported in a case of bacterial vaginosis (BV). In her study, she also stated that MMP-8 is a physiological constituent of lower genital tract, whereas MMP-8 may correlate with a host response to inflammation and infection[35].

However, the increasing concentration of endocervical MMP-8 (>90 percentile) on first or the middle of second trimester, is associated with a process of spontaneous preterm labor[33]. The average level of MMP-8 in the cervical mucus plug (CMP) on preterm labor is 2–5 times higher than on aterm. The MMP-8 is associated with a defense mechanism against ascending infection, which is primarily located distally from CMP. The presence of MMP-8 in all part of CMP on preterm labor shows that there is an infection extended into uterus[36].

In a previous study of Yoon, it is found that if the level of MMP-8 in amniotic fluid is more than 23 ng/mL, the MMP-8 can be a strong predictor for an occurrence of preterm labor before 32 weeks of gestational age[37]. This study found that the approximate level of uterine endocervical MMP-8 in case group is higher compared to a control group (79.1360 ng/mL: 70.9798 ng/mL). Table 5 shows that the high approximate level of MMP-8 in endocervical uterus is 6.6 times more as a risk factor of preterm labor (OR = 6.60; CI 95% = 1.25–34.95; p = 0.016).

The high level of uterine endocervical MMP-8 is actually a product of neutrophil as a result of inflammation process within the endocervix and its surrounding. It has been

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known that MMP-8 is a proteolytic enzyme which is able to degrade collagen in the cervix and is believed to cause cervical ripening (softening, thinning, and dilation) during the labor. Besides, MMP-8 has a role in preventing the ascending infection from lower genital tract into proximal part. Therefore, the MMP-8 is found in the CMP. In a primary process of ascending infection, MMP-8 is located only on distal CMP, and if MMP-8 fills the entire length of CMP on cervical canal, it means that the inflammation has already reached the proximal part of genital tract (such as amnio-chorio-decidua)[36]. In order to predict the inflammation within the endocervix and the process of its ascent into the proximal part, the assessment of MMP-8 in endocervix is really useful.

A research that examine the role of MMP-8 as a risk factor of preterm labor, which is associated with endocervical infection or inflammation is not done yet. Based on our study result and what has been proven before, that the high level of MMP-8 in endocervix is a risk factor of preterm labor, we suggest to run a test for MMP-8 routinely to predict the possibility of the occurrence of preterm labor.

Uterine Endocervical IL 1β as a Risk Factor of Preterm Labor Mechanism

Inflammation/infection in maternal–fetal interface, which is mediated by an inflammatory cytokines is estimated as a main component of preterm labor. During the last two decades, a study about the correlation between infection and preterm labor has been a concern. The presence of infection/inflammation within uterus is a first stage of preterm labor process.

The detection of intrauterine inflammation can be done through an invasive method such as amniocentesis, in which this method is highly risky for a leakage of chorioamniotic membrane, even, this method can induce an intrauterine infection. Another researcher detected an infection within the uterus with a risk scores based on mother's age, the length of the cervix measured by ultrasonography, and the total amount of leukocyte[38]. According to some literatures, approximately 50% of spontaneous preterm labor is associated with an ascending infection from lower genital tract[39].

Some prospective case-control study found that a urogenital tract infection has a role on preterm labor mechanism, either with a complete amniotic membrane or the tear one. That study found that the infection of urogenital tract on preterm labor is 36.54% compared to 17.3% on preterm birth with no signs of labor ($p = 0.027$)[40]. Preterm labor begins with an infection/inflammation within endocervix and it will extend into the layers of chorio-decidua.

In a developing country, the incidence of infection, particularly genital tract infection on pregnant women, is still high, and this fact is related with a non-optimal antenatal care, because the screening and management of an infection is not done routinely. Ideally, every woman who have a risk for preterm labor, should have done a screening and management for BV[41]. The BV is an infection of lower genital tract, which is very common amongst women on their reproductive age.

This condition is believed as a clinical syndrome that is marked with changes of normal flora, and not an infection that is caused by a certain microorganism, and this condition is related with a decreasing concentration of *lactobacillus* and the high concentration of Gram negative and anaerobic bacteria. Intrauterine inflammation that is caused mainly by the ascent of inflammation from genital tract BV can trigger the contraction of myometrium as a first sign of labor[42].

The genital tract infection on pregnant women in Indonesia has not been reported nationally. In a case-control study done by Abdi at Sanglah Hospital Denpasar, it is revealed that the incidence of multifactorial infection in the vagina of pregnant women with preterm labor is significantly higher than on pregnant women with aterm labor[11]. It is reported by Konsita, based on a case-control study at Sanglah Hospital Denpasar, that the amount of neutrophil in vagina of preterm labor is significantly higher than the amount of neutrophil in vagina of aterm labor[13].

The infection of genital tract of pregnant women happened frequently because of a non-optimal antenatal care in Indonesia. Always the antenatal care is only focused on a close monitoring of the growth of the fetus,

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and is not covering the health of the pregnant women themselves holistically. Doing a screening for a sign of an infection and a vaginal swab on pregnant women during early visits are extremely important. This is mainly to detect the infection/inflammation in genital tract, like cervico-vaginitis, and BV, because the infection from these sites is the origin of chorio-decidua inflammation, and acts as a trigger for preterm labor.

Endocervix is a main passage for the spreading and colonization of microbes, which are ascending from vagina into uterus. Knowing the inflammation process on endocervix is one of the effort to prevent the extension of the inflammation into uterus through endocervix. Goldenberg found that relative risk (RR) for preterm labor in Trichomonas infection is 1.3; and the relative risk for Syphilis and Gonorrhea is 2.0[43]. Viral infection can also cause a decreasing ability of women's reproductive tract to prevent bacterial infection in uterus[44].

Microorganism from vagina will ascend through cervical canal and colonize there. From endocervical of the uterus, microorganism or their products such as lipopolysaccharide (LPS), will spread into the layers of chorio-decidua and deeply extend into chorio-amniotic layers, umbilical cord even into the fetus. Either mother or fetus will respond by producing an innate immune system like NK-Cells, monocyte/macrophage, Dendrite cells, and granulocyte[45, 46, and 47].

The activation of this innate immune system will form cytokine and chemokine[21], of which the latter will release TNFα and IL-1ß[43]. Interleukin-1ß (IL-1ß) was first found by Igal Gery on 1972, and it is named as lymphocyte activating factor (LAF) because it was actually a lymphocyte mitogen. The IL-1ß is a member of IL-1 family from cytokine, which is produced by macrophage as proprotein. This cytokine is an important mediator on acute inflammation response and covers a lot of cellular activities such as proliferation, differentiation, and cellular apoptosis[48]. The largest source of Il-1ß is phagocytic cells. Like mentioned above, the production of IL-1ß by mononuclear phagocyte is triggered by bacterial products such as LPS. The biological effect of IL-1ß depends on the amount of the production of cytokines. If it is secreted in small amount, IL-1ß will function as a mediator of local inflammation, meanwhile, if it is secreted in large amount, IL-1ß will enter blood vessels and acts as an endocrine effector[49]. In cohort study, Vogel (2007) found that the concentration of IL-1ß in cervico-vaginal fluid is significantly higher compared to its concentration on pregnant women with a repeated preterm births history[28]. The role of IL-1ß on endothelial cells is to increase the expression of surface molecules that mediates the adhesion of leukocyte like ligands for integrins[49].

In this study, the approximate level of endocervical IL-1ß in case group is significantly higher than in the control group (1.6681^2 pg/L: 54.188^2 pg/mL). Table 5.6 shows that the high level of endocervical IL-1ß is 8.3 times as risk factor for preterm labor (OR = 8.3; CI 95% = 2.15–32.3; p = 0.001). It can be concluded that the high level of endocervical IL-1ß is a risk factor for preterm labor.

This statement is supported by other researchers. Sadowsky who examined the effect of IL-1ß by administrating 10 µg of IL-1ß into a pregnant Rhesus monkey's amniotic fluid and the monkey experienced a uterus contraction and give birth subsequently[50]. Another researcher reported that the level of IL-1ß in vaginal and cervical fluid of pregnant women with pathogenic infection is significantly higher compared to a non-pathogenic infection[51]. Preventing an infection of lower genital tract with an administration of antibiotics can reduce the incidence of preterm labor and child birth with weight less than 2500 g[52].

Administration of betamethasone 12 mg/kg intramuscularly (as an anti-inflammation) on preterm labor, reduces the level of IL-1ß within 48 hours ($p < 0.005$) and a delivery within seven days can be avoided[53]. Kazardoust found that the level of proinflammatory interleukin in endocervix is reduce after an administration of betamethasone[54]. In a patient with BV, the level of IL-1ß in vaginal fluid is significantly higher than normal women[55]. The management of BV with douching the vagina before the pregnancy is otherwise a risk factor of the occurrence of preterm labor, either early preterm labor (<34 weeks) or a very early preterm labor with ($p < 0.001$)[56].

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Treatment of BV with antibiotics seems ineffective to prevent preterm labor. It must be noted that antibiotics are only used for the treatment of BV and are not effective to Ureaplasma and Mycoplasma spp, in which both of these organism are frequently related with preterm labor[57]. This can explain why the medications for BV to lessen the incidence of preterm labor on clinical trials are always unsuccessful[56].

From the explanation above, it can be concluded that an inflammation of genital tract, from vagina to uterus, can trigger a preterm labor. This inflammation, through a series of processes involves both an innate (non-specific) and adaptive (specific) immune system, will finally form a cytokines or chemokines, in which one of it is IL-1ß.

The high level of IL-1ß in endocervix gives a sign that there is actually an inflammation, either in endocervix or in the layers of chorio-decidua, and IL-1ß can be used to detect the possibility of a preterm labor. Detecting the labor on 24–34 weeks of gestational age by assessing the level of IL-1ß can be useful to prevent preterm birth. A long term adverse effect on fetus can be lessened and the healthcare for preterm babies will cost somehow cheaper.

An examination of IL-8, matrix MMP-8, and IL-1ß in endocervix takes a high cost because the reagent used for this examination is only for research purposes; however, the efforts to make this examination be a routine assessment is still needed.

We suggest to make a laboratory study focusing on the assessment of the level of IL-8, MMP-8, and IL 1ß in endocervix in semi-quantitative form using a dipstick. It is necessary to research about pattern of microorganism in the vagina and uterine endocervix and its relation with endocervical inflammation and preterm labor.

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