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Cervical Length and Matrix Metalloproteinase-8 Level in Endocervix of Spontaneous Preterm Labor

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Preterm labor is one of the critical obstetrics issue until now, due to high risk of respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), sepsis, and even death towards the preterm newborn babies. For those surviving preterm babies, they are facing lifetime disabilities such as blindness, deafness, mental retardation, and motor disabilities. Preterm labor is hard to prevent due to the unknown definite etiology. Early cervical maturation due to inflammation is hypothesized to be the triggering factor of preterm labor mechanism. To investigate the correlation between cervical length and matrix metalloproteinase 8 (MMP-8) in the endocervix of spontaneous preterm labor case. This research was conducted with analytical cross-sectional study. Samples were pregnant women with gestational age between 20 until 36 weeks and 6 days with preterm labor in the Sanglah General Hospital Denpasar, Bali, Indonesia, which were obtained in October, 1st 2014 until January 2015. Samples were collected consecutively. The length of the cervix was measured by transvaginal ultrasonography (TVS), and the level of MMP-8 was evaluated with ELISA in the Veterinary Laboratory of Udayana University. This study shows that age, parity, gestational age, hemoglobin, white blood cell, platelet and neutrophile count were having p value > 0.05 for all parameters, which means that there was no significant difference between two groups. The high level of MMP-8 in the endocervix (e"4.3920 ng/ml) is the risk factor for short cervical length by 4 fold, compared to the low MMP-8 level (PR = 4,00; 95% CI = 1,07-14,90; p=0,006). In the spontaneous preterm labor, the shorter cervical length has higher level of uterine endocervical MMP-8.

Keyword: Cervical length, Matrix metalloproteinase 8, Preterm labor.

Preterm labor occurs in the pregnancy between 20 – 36 weeks and 6 days gestational age, that is calculated from the last menstrual period[1,2]. The highest incidence occurs in the Africa and Asia, which are 85% among all preterm labor incidence throughout the world[3]. In the Southeast Asia, the prevalence of preterm labor was 11.1%[4]. Based on the Riset Kesehatan Dasar (Rikesdas) the incidence of preterm labor in Indonesia was 11.5% in 2007[5], while in Sanglah General Hospital Denpasar was 12.7%[6]. More than one million neonates died throughout the world due to the complication of preterm babies. The most common short-term complication are RDS, IVH, bronchopulmonary dysplasia, patent ductus arteriosus, and NEC[1]. The long-term complication can be mental retardation, cerebral palsy, seizure, blindness, deafness and motor disabilities[1]. Moreover, preterm babies also

need special care and assistance that require high cost.

The most ideal way to prevent the risk of complication is by preventing the baby born at preterm. The prevention of preterm labor is difficult since the etiology is still unknown. Tocolytic and bedrest are not fully proven to prevent preterm labor. Not only that, the intensive care of neonates was proven not fully reduce the morbidity and mortality of preterm neonates. The prevention of preterm labor can be done by evaluating and preventing the risk factor. As the result, the preterm labor can be prevented[7,8]. Preterm labor is a syndrome that correlates with various factors such as excessive uterus distention, placental ischemic or sub-placenta bleeding, abnormal allograft reaction, allergic phenomenon, endocrine disturbance, and infection. These multi factorial nature of preterm labor triggers the myometrial contraction, maturation of cervix, rupture of amniotic membrane, and preterm labor finally occurs[9]. In the last decade, infection/inflammation usually correlates with the mechanism of preterm labor. In the conventional way, the preterm labor is initiated by the infection/inflammation that is due to the invasion of microorganism into the endometrium, which occurs before pregnancy, or in the menstruation period[1]. Recent study shows that the maturation of cervix sometimes even occur without any uterine contraction preceeding. Other facts also show that induction of labor in the unripe cervix often fails, and the success rate will increase if ripening of the cervix is conducted first, by mechanical or using medication such as prostaglandin. Based on this hypothesis, author assumed that labor starts with cervical maturation, and followed by uterine contraction afterwards. Does endocervical inflammation will cause the cervix to ripe, and trigger preterm labor? Then the correlation between cervical maturation and endocervical inflammation needs to be proven first.

The function of cervix is to maintain the conception product to stay within the uterus until term. In a physiologic state, cervix receives steady pressure from the weight of the baby, amniotic fluid, and also the uterus itself[10]. To maintain its strength, then cervix needs adequate mechanical power. The strength of the cervix is supported by the biomechanical power from the collagen fiber, hyaluronic acid (HC), elastin, and water[11]. The content of cervical collagen is about 64.3 – 72%[12], which consists of 66% type I collagen, 33% of type III collagen, and few of type IV collagen on the basal membrane[13]. On the other hand, the role of cervix is important in term, where cervix will be thin (cervical effacement) and dilates until the baby is born. Would be problems if the cervical ripening occur very early, which is in the preterm pregnancy. The etiology of this early change is unknown, but it is hypothesized that the endocervical inflammation caused by vaginal infection as triggers the preterm labor mechanism[10]. Endocervical inflammation cause a process in the nature and adaptive immune system, that triggers the migration of neutrophil cells to the inflammation site. Due to the triggered of Interleukin-8 (IL-8), neutrophil will form the neutrophil collagenase or matrix metalloproteinase-8 (MMP-8) that will degrade the collagen fiber in the uterine cervix and soften the cervix afterwards. The gravitational pressure from the fetus and amniotic fluid, and also the pressure from the uterine wall, will cause the internal uterine orifice to dilate and the cervical canal will do funneling[14,15]. The protrusion of the amniotic membrane towards the uterine endocervix will disperse the inflammation to the chorio-decidual layer, umbilical cord, and the fetus itself. Fetus lungs and kidney will be triggered to produce the Platelet Activating Factor (PAF) in the amniotic fluid to activate the cytokines. These cytokines will trigger arachidonic acid to produce prostaglandin (PG), such as prostaglandin E2 (PGE-2), and prostaglandin F2± (PGF-2±). These prostaglandin will trigger the myometrial contraction and finally preterm labor will occur.

Study Design

This study was conducted with analytical cross sectional study. The samples were pregnant women with 20 – 36 weeks and 6 days gestational age, who came to Sanglah General Hospital Denpasar in labor phase. The specimen for the MMP-8 level examination was from the endocervical fluid that was obtained using the "Sterile Cutiplast Swab", and stored in the -40^{0}C temperature of the Clinical Pathology Laboratory in Sanglah General Hospital Denpasar. The evaluation of MMP-8 level (ELISA method) was conducted in the Veterinary Laboratorium of Udayana University. After the specimen was obtained, then the length of cervix was measured

using the transvaginal ultrasound (TVS). In order to evaluate the prevalence ratio of the high level of MMP-8 in the endocervix as the risk factor for short cervical length, the Chi-square calculation was performed. This study was approved by the Ethical Committee of Medical Faculty of Udayana University / Sanglah General Hospital Denpasar.

RESULT AND DISCUSSION

A cross-sectional study was performed towards 49 preterm pregnant women who were in labor phase, who were collected between October 1st, 2014 until January 2015. Among the 49 samples, there were 22 samples with cervical length less than 2.5cm and 27 samples with cervical length 2.5cm or more.

Characteristics of Pregnant Women with Preterm Labor in this Study

In this cross-sectional study, the characteristic data were regarding to the mother age, gestational age, parity, hemoglobin, white blood cell (WBC), platelet and neutrophil count. The analysis was performed in Table1.

In the Table 1 above, the t-independent test shows that the p-value for all parameters was > 0.05, which means that there were no differences between the two groups.

Risk of Short Cervical Length Regarding to the High Level of Endocervical MMP-8

In order to evaluate the prevalence risk of high level of MMP-8 in the endocervix towards the risk of short cervical length, Chi-Square test was performed. The data analysis was shown in the Table 2.

Table 2 shows that the high level of endocervical MMP-8 had the risk of short cervical length by 4 fold compared to the low level of MMP-8 (PR = 4.00; CI 95% = 1.07-14.90; p =0.006).

DISCUSSION

Cervix is the most distal organ of the uterus, that looks like a cone with the tip facing towards vagina. In the central part, there is a canal that connects the uterine cavity and the vagina, which is called the cervical canal. The function of cervix is to maintain the conception products throughout the pregnancy[13]. The mechanical nature of cervix comes from the extracellular matrix such as collagen fiber, and other materials such as proteoglicans, hyaluronic acid (HA), elastin, and

Table 1. Characteristic Distribution of Maternal Age, Gestational Age, Parity, Hemoglobin, WBC, Platelet and Neutrophil Count

	Cervical Length				P
	Short (n=22)		Normal (n=27)		
	Mean	SD	Mean	SD	
Age (year)	30.23	5.62	26.37	5.60	0.061
Parity	1.32	0.99	1.00	0.92	0.251
Gestational age	31.18	4.66	30.00	4.39	0.366
Hemoglobin	11.89	1.63	11.57	1.14	0.424
WBC	12.12	3.34	11.27	2.41	0.308
Platelet	277.86	93.55	247.85	86.79	0.251
Neutrophil	0.75	0.14	0.72	0.15	0.374

Table 2. Risk of Short Cervical Length Regarding to the High Level of Endocervical MMP-8

		Cervical Length		PR	CI 95%	P
		Short	Normal			
MMP-8	High	20	15	4.00	1.07-14.90	0.006
	Low	2	12			

water[11]. The collagen in the cervix is about 64.3 – 72%[12], which consists of 66% type I collagen, 33% of type III collagen, and few of type IV collagen on the basal membrane[13]. In the labor phase, these collagen will degrade due to the lytic enzymes such as collagenase or matrix metalloproteinase (MMP)-1, MMP-8, and MMP-13, that is produced by fibroblast and leukocyte cells, and leukocyte elastase from the macrophage, neutrophyl, and eosinophil. Matrix metalloproteinase 8, which is also called as the neutrophil collagenase, is the product of the endocervical neutrophil, that is produced due to the inflammation in the cervix or its surroundings. It is also already known that MMP-8 is one of the proteinolytic enzyme that is able to degrade collagen in the cervix and causing the cervix to be ripe (soft, thin and dilated)[13]. In the normal condition, this phase of collagen degradation by MMP-8 will occurs in the term pregnancy. Problem happens when the cervical maturation occurs during preterm pregnancy, where the preterm babies are not able to adapt in the environment yet, and will cause the morbidity and mortality of neonates to increase.

Recent studies show that preterm labor starts after the cervix matures, and the myometrial contraction occurs afterwards. Endocervical inflammation occurs due to the transmission of inflammation from the vagina to the uterine cavity, by passing the cervical canal. Inflammation is a process that involves the nature and adaptive immune systems, that finally triggers the movement of neutrophil from the blood vessels into the inflammation site, which in this situation occurs in the endocervix. Neutrophil will produce elastase and collagenase (MMP-8). Radiolabel study shows that the collagen degradation occurs due to the migration of neutrophil from the blood vessel to the cervix after the trigger from the bacterial lipopolysaccharides (LPS)[13]. By the increasing level of neutrophil in the cervix, MMP-8 will also increase, the degradation of collagen will increase, and with the addition of gravitation pressure from the fetus and amniotic fluid, then the cervix will be effaced and dilates[16,17]. Rahkonen (2009) shows that the concentration of MMP-8 in the endocervix is more significant compared to its concentration in the vagina. In his study, it was also shown that MMP-8 is the physiologic constituent of the lower genital tract, that involves with the host response towards inflammation[18]. The increasing level of MMP-8 in the endocervix will be more than 90% in the first and mid-second trimester, correlates with the process of spontaneous preterm labor[16]. Other than become as the proteinolytic enzyme, MMP-8 also involves in preventing the infection/inflammation transmission from the vagina towards uterine cavity. Initially, MMP-8 can be found in the distal of cervical mucus plug (CMP). The existence of MMP-8 in all parts of CMP of preterm labor, shows that the infection has already transmitted from the vagina towards uterine cavity. The mean value of MMP-8 CMP in the preterm labor is 2-5 fold higher compared to the term labor[19]. If the MMP-8 reaches amniotic fluid by the level of 23 ng/mL, then the MMP-8 can be as the strong predictor towards preterm labor that occur before 32 weeks gestational age[20]. As the result, endocervical inflammation will cause the formation and release of endocervical MMP-8, increase the degradation of cervical collagen, and finally causing the cervix to soft and funnel.

The next question will be, what is causing the inflammation in the uterine endocervix? More study still need to be conducted to prove that the inflammation is probably caused by the transmission of microorganism from the vagina to the endocervix.

CONCLUSION

In the spontaneous preterm labor, the shorter uterine cervix will have higher endocervical MMP-8 level.

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