

## Lower Serum Catalase Level is Associated with Preterm Labor among Pregnant Women at Sanglah Hospital Denpasar, Bali-Indonesia

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**Background:** Preterm labor is still become a serious health problem in Obstetric and Perinatology with no sensitive biomarker currently approved. Several studies show that decrease antioxidant activity may play significant role in preterm labor. However, only few studies had been conducted to evaluate blood catalase level in preterm labor and assess its role in preterm labor. **Objective:** The aim this study was to identify the differences of maternal serum catalase level in preterm labor compared with preterm pregnancy. **Methods:** An *observational analytic cross sectional* study was conducted from February to December 2014 using pregnant women with 28-36 weeks' gestational age. Blood catalase level was evaluated by colorimetric method and the data was analyzed by *SPPS for Windows 17.0 program*. **Results:** 12 subjects were enrolled and divided into preterm and control group. No significant differences between mean age, gestational age, and parity between preterm and control group. However, blood catalase level was significantly lower in preterm group compared with control group ( $81.82 \pm 20.38$  vs  $159.38 \pm 35.79$ ;  $p=0.001$ ). **Conclusion:** Serum maternal catalase level were significantly lower in preterm labor compared with preterm normal pregnancy.

**Keywords:** serum *catalase*, preterm pregnancy, preterm labor.

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### INTRODUCTION

Preterm labor is still a serious health problem in the field of obstetrics and perinatology because of its high mortality and morbidity associated with immaturity of their organ system. Therefore, preventive measure in term of early detection of those who at risk of preterm labor is mandatory. However, there is no current valid biomarker that can be used to determine risk of preterm labor.

The incidence of preterm labor is varied between countries with higher incidence in developing countries compared with developed ones. In 2005 the rate of preterm birth worldwide is 9.6%. Approximately 85% of these preterm births occur in Africa and Asia.<sup>1</sup>

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There's no data about the incidence of national preterm labor in Indonesia. However, the incidence of low birth weight (LBW) may roughly reflect the incidence of prematurity. The incidence of nationwide hospitalize LBW is 27.9%.<sup>2</sup> In Sanglah Hospital, the incidence of preterm delivery from January 2008 to October 2011 is 9,12%; as 802 preterm birth with gestational age 28-36 weeks of 8797 pregnant patients with 28-36 weeks of gestational age who came for antenatal care (ANC) in Obstetric Department within the same period.<sup>3</sup>

The mechanisms underlying preterm birth is not yet fully known. However, several factors are considered as causes of preterm labor: activation of the fetal maternal adrenal hypothalamic pituitary axis, infection and inflammation, decidua bleeding and excessive stretch of the uterus, causing a series of clinical symptoms and synchronization between the uterus muscle contractions (myometrium), fetal membranes rupture (chorion and amnion), and the ripening of the cervix (cervical ripening).<sup>4</sup> Myometrium smooth muscle contraction can also be triggered by an imbalance of reactive oxygen species (ROS) and antioxidants in the body that

shifted toward an increase in ROS.<sup>5</sup> It is known that ROS are continuously formed as byproducts of cellular metabolism.<sup>6</sup>

In normal conditions, ROS and antioxidants are in balance. Any alteration in antioxidant system (increase ROS production or decrease anti-oxidant level) will ended up in oxidative stress. ROS normally neutralized by enzymatic or non-enzymatic antioxidant system. Enzymatic antioxidant is particularly important because of its involvement in many disease pathogenesis. There are 3 enzymes in antioxidant system that considered being important: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx) and glutathione reductase. Among them, catalase is play particularly important role in premature rupture of membrane (PROM) and pre-term labor.

Several studies have demonstrated an association between high risk preterm labors with antioxidants, wherein the antioxidant is a protective factor against the potential toxic effects of oxidative processes. Administration of exogenous antioxidant supplementation on some small samples of women with preterm pregnancies can reduce oxidative stress at the time of delivery. In Indonesia there's no research has been done for catalase serum levels in women who experience preterm labor. In the preliminary study, we obtained the mean maternal catalase serum levels in preterm pregnancy is 137.45 nmol/min/ml and the mean maternal catalase serum levels in preterm labor is 92.06 nmol/min/ml. We assume that it is very important to investigate catalase serum levels in preterm labor, to conduct better understanding in pathophysiology of preterm labors; hereby it can be carried out preventive measures to reduce the impact of preterm labor.

## MATERIAL AND METHODS

Observational analytic cross sectional study was conducted to investigate the differences in catalase serum levels in preterm labor at 28 - 36 weeks' gestation from February to December 2014. Subjects of this study were taken from the accessible population by consecutive sampling, sequentially one at a time in order to obtain cases of preterm labor and 28 - 36 weeks' gestation. Subjects were selected among those with normal, 28-36 weeks' pregnant women who came to visit the ER Delivery Room and Obstetrics and Gynecology Clinic of Sanglah General Hospital. Those who had vaginal bleeding, early rupture of membrane, systemic disease (cardiovascular disorder, diabetes mellitus, chronic pulmonary disease, anemia, hypertension), uterine myoma, had history of premature labor, polyhydramnios, cervix incompetence and congenital disease were excluded. To evaluate serum catalase level, 6cc of blood was drawn from every subject. The blood will be processed and catalase level was determined by calorimetric method by using spectrophotometer.

The data was analyzed by using SPSS for windows 17.0. Normality evaluation was analyzed by Kolmogorov-Smirnov for normality test whereas homogeneity was evaluated by Levene's Test. Comparative analysis of difference in serum catalase level among pre-term group and control group was evaluated by independent t-test. This research has obtained ethical clearance from the Ethics Commission Medical Faculty Udayana University/Sanglah General Hospital.

## RESULTS

12 pregnant women were enrolled in this study. 6 women were included in preterm group and 6 were in control group. Subject's baseline characteristics were obtained from medical records and analyze for normality of data distribution. No significant difference was detected in comparative analysis of subject's age, gestational age, and parity. The result normality analysis is presented in **Table 1**.

**Table 1.** Normality Test for Age, Gestational Age and Parity of Both Groups

Risk Factor	Preterm Labor Group (n=6)		Preterm Pregnancy Group (n=6)		P
	(nmol/min ml)		(nmol/min ml)		
	Mean	SD	Mean	SD	
Age (year)	29,17	6,795	28,67	6,314	0,898
Gestational Age	33,33	3,011	34,83	1,472	0,299
Parity	0,67	0,816	1,00	1,265	0,599

Then, we analyzed the homogeneity of baseline data and serum catalase level using Levene's homogeneity test in preparation for comparative analysis. All variables were considered homogeny. The result of homogeneity test is described in **Table 2**.

**Table 2.** Homogeneity Data Test for Age, Gestational Age, Parity, and Catalase Level of Preterm Labor and Preterm Pregnancy

Variable	F	P	Interpretation
Age	0,001	0,981	Homogen
Gestational Age	2,312	0,159	Homogen
Parity	1,250	0,290	Homogen
Catalase Level	0,182	0,678	Homogen

Finally, we analyzed association between catalase level with pre-term labor. Independent T-test analysis showed significant differences between catalase level in preterm labor compared with control group (preterm pregnancy). It also appeared that catalase level was significantly lower in pre-term labor group. The result of independent T-test analysis is presented in **Table 3**.

## DISCUSSION

Oxidative stress that caused by ROS accumulation have long been taught to play crucial role in preterm labor pathogenesis. Several researches had proved that ROS associated with inflammation, senescence, and apoptosis of amniotic membrane. ROS is also involved in uterine smooth muscle contraction regulation albeit mostly occur in pathological condition.

**Table 3.** Difference of Catalase Level between Preterm Labor and Preterm Pregnancy

Subject Group	N	Mean Catalase Level (nMol/min/ml)	SB	t	P
Preterm Labor	6	81,82	20,38	4,61	0,001
Preterm Pregnancy	6	159,38	35,79		

ROS contribute to spontaneous uterine contraction mainly by facilitating intracellular calcium ( $Ca^{2+}$ ) accumulation. Intracellular  $Ca^{2+}$  initiate contraction process by binding to and activate calmodulin forming calcium-calmodulin ( $Ca^{2+}$  CALM) which triggers the activity of myosin light chain kinase (MLCK). Activated MLCK modulate the actin-myosin interaction and, hence, causes uterine contractions.<sup>8</sup> On the other hand there is a unique mechanism that modulates a calcium pump, which is activated by potassium pump ( $BK_{ca}$ ) that functions in regulating the contractility of the myometrium. Furthermore, activation of the  $BK_{ca}$  pump would trigger "negative feedback" mechanism to reduce depolarization and contraction of the myometrium. The resulting repolarization increase  $Ca^{2+}$  ion pump activity and lower cytoplasmic  $Ca^{2+}$  level that induce uterine relaxation. However, cellular ROS is proposed as one of risk factor of preterm labor mainly because of its property to induce uterine contraction. In addition, ROS is also associated with inflammation and cellular senescence of amniotic membrane which decreases its tensile strength and, hence, makes it prone to premature rupture.<sup>9</sup>

Since ROS play a significant role in preterm labor, it's logical that the level of oxidative stress could be determined by measuring enzymatic antioxidant such as catalase, SOD, and glutathione peroxidase (Gpx). Because of its presence in serum, catalase is the best candidate as biomarker of oxidative stress. Study conducted by Juniaux in 2006 states that an imbalance between ROS production and antioxidant defense mechanisms mainly depletion in catalase level, potentially trigger intracellular calcium homeostasis. The oxidative stress increase  $Ca^{2+}$  efflux from reticulum endoplasmic by activating inositol-1,4,5, triphosphate ( $IP_3R$ ) receptor and ryanodine receptors pathway that result in increased

cytoplasmic  $Ca^{2+}$  level. As stated in previous section,  $Ca^{2+}$  is strong inducer of uterine contraction that potentially lead to preterm labor. Thus, suppression of oxidative stress by antioxidant system, primarily by catalase, play very important role in preventing preterm labor.<sup>10</sup>

Catalase is homotetramer protein that use Fe as a cofactor and encoded by gene in 11<sup>th</sup> chromosome. Inactivating mutations in this gene cause an condition mark by low catalase level and high oxidative stress state known as acatalesemia. Catalase is a hemoprotein containing four heme groups, a compound that commonly found in animals and plants. Catalase is evolutionary preserved protein and present in almost every organism. However, most organisms have more than one type of catalase. A study has revealed that there is a relationship between the differentiation of cells with catalase because it metabolize hydrogen peroxide or organic peroxides.<sup>11</sup> In human, this enzyme can be found in the blood, bone marrow, mucous membranes, kidneys and liver. In addition to support SOD activity, catalase hidrolize a wide variety of peroxides and free radicals into oxygen and water. This enzyme suppress or inhibit the formation of free radicals by breaking the oxidative chain reaction and turn it into more stable products which is known as chain-breaking-antioxidant. Catalase and glutathione peroxidase have the same nature in catalyzing  $H_2O_2$ . However GPx has higher activity against  $H_2O_2$  than catalase. This is due to differences in their kinetics. Catalase catalyzes  $H_2O_2$  linearly in accordance with the concentration of  $H_2O_2$ , while GPx become saturated if  $H_2O_2$  concentration below 10-5 mol/L. When  $H_2O_2$  concentration is very low or at normal conditions, GPx have more dominant role than catalase to catalyze  $H_2O_2$ .<sup>12</sup>

In this study, we found significantly lower level of catalase in preterm labor group compared with normal preterm pregnancy. The result confirmed several previous studies and the theoretical association between oxidative stress and preterm labor. Several studies have demonstrated an association between risks of preterm labor with antioxidants, wherein the antioxidant is a protective factor against the potential toxic effects of oxidative processes. Exogenous administration of antioxidant supplementation to women with preterm pregnancies can reduce oxidative stress at the time of delivery. However, under conditions of increased ROS production without adequate antioxidant defense mechanism, it will trigger oxidative stress. ROS production particularly increases at late secretion phase just before menstruation and continued to decrease in early pregnancy, especially during decidua. Catalase is one of enzymatic antioxidants in the cells that found in the peroxisomes in almost every organ of the body, but mainly concentrated in the liver. Catalase serves to

catalyze  $H_2O_2$  into  $H_2O$  and  $O_2$ .  $H_2O_2$  has effect to induce contractions of the myometrium through increasing calcium influx into the cell and mediate the release of prostaglandins, which trigger preterm labor. This occurs because of an imbalance of  $H_2O_2$  oxidants and catalase antioxidants. In addition, catalase also plays a role in reducing the negative effects of  $H_2O_2$  in a manner that is preventing a sustainable  $H_2O_2$  chain reaction. Thus the effect of  $H_2O_2$  that can induce myometrium contractions in pregnancies over 20 weeks to 36 weeks can be eliminated so it preterm labor do not happen.<sup>13</sup>

## CONCLUSION

This research concludes that catalase level is lower in preterm labor compare with preterm pregnancy. However, further research is needed to determine adverse cut-off point in catalase level to enhance its clinical application as biomarker for preterm pregnancy risk. In addition, administration of antioxidants can be considered as efforts to prevent preterm labor in patients with low levels of catalase.

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