

Research Article

## Difference of Serum MMP-9 and TNF- $\alpha$ Level in Preterm and Term Premature Rupture of Membranes

### *Perbedaan Kadar MMP-9 dan TNF- $\alpha$ Serum pada Ketuban Pecah Dini Preterm dan Aterm*

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#### Abstract

**Objective:** To examine the difference between matrix metalloproteinase-9 (MMP-9) and Tumor Necrosis Factor  $\alpha$  (TNF- $\alpha$ ) serum levels in preterm and term premature rupture of membranes (PROM).

**Method:** Our study employed an observational cross sectional approach. Seventy samples were divided into two groups, 35 samples with preterm PROM (28-36 weeks gestational age) and 35 samples with PROM at term pregnancy (37-42 weeks gestational age). Both groups underwent examination for serum MMP-9 and TNF- $\alpha$  concentration using ELISA method. Statistical analysis was done using t-test.

**Result:** Serum levels of MMP-9 in the preterm PROM group was 2860.68 $\pm$ 627.32 ng/ml, which was significantly higher than in the PROM at term pregnancy group 2549.74 $\pm$ 657.15 ng/ml ( $p=0.04$ ). Likewise, the average serum level of TNF- $\alpha$  in subjects with preterm PROM was 12,086.60 $\pm$ 5384.51 ng/ml, significantly higher in comparison to PROM at term pregnancy, which was 6422.51 $\pm$ 2645.32 ng/ml ( $p=0.00$ ).

**Conclusion:** Serum levels of MMP-9 and TNF- $\alpha$  in preterm PROM is significantly higher than that in PROM at term pregnancy.

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**Keywords:** MMP-9, premature rupture of membranes, preterm, term, TNF- $\alpha$

#### Abstrak

**Tujuan:** Untuk mengetahui perbedaan kadar serum matrix metalloproteinase-9 (MMP-9) dan Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) pada ketuban pecah dini (KPD) preterm dan KPD pada hamil aterm.

**Metode:** Studi kami menggunakan metode observasional dengan pendekatan potong lintang. Tujuh puluh sampel dibagi dalam dua kelompok yaitu 35 sampel dalam kelompok KPD preterm (usia kehamilan 28-36 minggu) dan 35 sampel dalam kelompok KPD pada hamil aterm (usia kehamilan 37-42 minggu). Kedua kelompok tersebut menjalani pemeriksaan konsentrasi MMP-9 dan TNF- $\alpha$  serum dengan metode ELISA. Analisis statistik dilakukan dengan uji t.

**Hasil:** Kadar MMP-9 serum pada kelompok KPD preterm didapatkan 2860,68 $\pm$ 627,32 ng/ml, yang secara signifikan lebih tinggi dibandingkan kelompok KPD pada hamil aterm 2549,74 $\pm$ 657,15 ng/ml dengan nilai  $p=0,04$ . Kadar TNF- $\alpha$  pada subjek dengan KPD preterm 12.086,60 $\pm$ 5384,51 ng/ml, lebih tinggi dibandingkan KPD pada hamil aterm dengan kadar 6422,51 $\pm$ 2645,32 ng/ml ( $p=0,00$ ).

**Kesimpulan:** Kadar MMP-9 dan TNF- $\alpha$  serum pada KPD preterm secara signifikan lebih tinggi dibandingkan dengan pada KPD aterm.

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**Kata kunci:** aterm, ketuban pecah dini, MMP-9, preterm, TNF- $\alpha$

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## INTRODUCTION

Premature rupture of membranes (PROM) is an important matter in obstetrics in relation to complications of pregnancy in the form of prematurity, chorioamnionitis infection, and sepsis, which increases perinatal morbidity and mortality for both mother and baby. Currently, the incidence of PROM is still high, with ten percent of deliveries being preceded by PROM.<sup>1,2</sup>

PROM can be categorized based on the time of occurrence. Breakage of fetal membranes before contractions begin in term pregnancy is called PROM at term, whereas rupture occurring during preterm pregnancy is called preterm PROM. The

proportion of PROM ranges from 3% to 18% of all pregnancies. Approximately 30-40% of preterm deliveries are caused by PROM. The proportion of PROM at term cases in Sanglah Hospital was 12.92% out of 2113 deliveries in 2005 whereas the proportion of preterm PROM cases was 16.77% out of 328, including those who went through delivery and conservative hospitalization.<sup>3-5</sup>

This research is based on the reasoning that increase of serum TNF- $\alpha$  in the pregnant woman can trigger the expression of MMP-9 in such a way that it can raise the degradation of collagen type IV in the fetal membranes, therefore, causing PROM. Another research claims that TNF- $\alpha$  triggers the ex-

pression of MMP-9 in human amniotic epithelial (hAE) cells. This is partially consistent with previous reports stating that in-vitro TNF- $\alpha$  triggers the secretion of MMP-9 in human amnion and trophoblast.<sup>6</sup>

## METHODS

This research was conducted in dr. Moewardi Hospital of Surakarta. The population of this research were patients with the diagnosis of PROM, either at term or preterm who gave birth in the hospital from July to August 2012 and were willing to provide informed consent as requirement for sample collection.

This research employed observational, cross-sectional design and clinical test approach on the female clients with either preterm PROM or PROM at term. All of our subjects had to fulfill the following inclusion criteria, pregnant women aged 20 up to 35 years old who had delivery in the delivery room of dr. Moewardi Hospital of Surakarta with either preterm PROM or PROM at term case, no abnormality of the baby, and willing to participate in the research by signing an informed consent form.

The minimum number of sample needed to confirm a difference between preterm PROM and PROM at term was 35. The levels of MMP-9 and TNF- $\alpha$  for both groups were assessed using ELISA method. Venous blood sample was collected from the subjects, which was then processed in Prodia Laboratory in Jakarta. The concentrations of MMP-9 and TNF- $\alpha$  in both groups were compared using t-test. All statistical analysis was conducted using SPSS version 17.0 for Windows.

## RESULTS

The subjects of this research were 70 pregnant women who were gave birth in dr. Moewardi Hospital of Surakarta with PROM. They were divided into 2 groups, 35 women with preterm PROM and 35 women with PROM at term. The characteristics of research subjects are presented in Table 1.

The normality of the samples was tested using Kolmogorov-Smirnov test. There was no significant difference in terms of age of the subjects and average gestational age between the preterm PROM and PROM at term group.

The average serum MMP-9 concentration in the preterm PROM group ( $2860.68 \pm 627.32$  ng/ml) is higher than that in the PROM at term group ( $2549.74 \pm 657.15$  ng/ml) as shown in Table 2 below.

**Table 1.** Characteristics of Research Subject

Variable	Category	Preterm PROM	PROM at Term
Age	$\geq 30$ years	10 (28.6%)	17 (48.6%)
	$\leq 30$ years	25 (71.4%)	18 (51.4%)
Parity	Primipara	24 (68.6%)	10 (28.6%)
	Multipara	11 (31.4%)	25 (71.4%)
Gestational age	28 - 32	19 (54.3%)	0 (0%)
	33 - 36	16 (45.7%)	0 (0%)
	37 - 39	0 (0%)	23 (65.7%)
	40 - 42	0 (0%)	12 (34.3%)
Education level	Primary School	8 (22.9%)	11 (31.4%)
	Middle School	19 (54.3%)	18 (51.4%)
	High School	8 (22.9%)	6 (17.1%)
Occupation status	Housewife	13 (37.1%)	23 (65.7%)
	Working	22 (62.9%)	12 (34.3%)

The difference in the average serum concentration of MMP-9 between the preterm PROM group and the PROM at term group is found to be statistically significant ( $p=0.04$ ).

The MMP-9 level of both sample groups is homogenous, as indicated by the Kolmogorov-Smirnov normality test, the  $p$ -value generated for each respective group was 0.994 for the preterm PROM group and 0.495 for the PROM at term group.

The average serum TNF- $\alpha$  concentration in preterm PROM ( $12,086.60 \pm 5,384.51$  pg/ml) is higher than that in PROM at term ( $6,422.51 \pm 2,645.32$  pg/ml) as shown in Table 3 below.

Serum TNF- $\alpha$  concentration is homogeneously distributed in both groups. This is verified by the  $p$ -value obtained by Kolmogorov-Smirnov test, with  $p=0.495$  for the preterm PROM group and  $p=0.497$  for the PROM at term group. The average serum TNF- $\alpha$  concentration of the preterm PROM group is significantly different from the PROM at term group, as verified by independent samples  $t$ -test analysis ( $p=0.00$ ).

### DISCUSSION

Inflammatory cytokines (IL-1, IL-6, and TNF- $\alpha$ ) have been found in higher concentrations in the amniotic fluid of pregnant women with PROM. However, TNF- $\alpha$  is the cytokine with the most distinct characteristic out of the cytokines, which seems to influence the family of MMP during preg-

nancy. This member of the pro-inflammatory cytokines have been proven to trigger the expression of MMP in various tissues in such a way that it causes the active release of MMP-9 from the fetal membrane and triggers apoptosis in the fetal membrane. The activation of MMP-9 will eventually lead to PROM.<sup>7,8</sup>

Demographic characteristics of the preterm PROM group and the PROM at term group are presented in Table 1 and it was discovered that there was no significant difference between the groups for the variables of education level, parity, occupation, age, and gestational age. Thus, we can conclude that the two groups are comparable in terms of demographics.

Kolmogorov-Smirnov Z tests for MMP-9 and TNF- $\alpha$  in both groups provided  $p$ -value more than 0.05. Therefore, we can conclude that the distribution of values in all of the groups are normally distributed.

From results of independent  $t$ -test, the level of MMP-9 is significantly higher in preterm PROM than PROM at term ( $p$ -value=0.04). This result is in line with findings by Romero et al conducted in 2002 in which the concentration of MMP-9 is significantly higher in preterm PROM than that in premature delivery ( $p=0.035$ ). However, the research did not compare two groups with PROM although we can see similar findings that there is an increase in the level of MMP-9 in PROM cases.<sup>9-11</sup>

**Table 2.** Average Serum MMP-9 Concentration in Preterm PROM and PROM at Term

Group	Number of Sample (n)	Average Serum MMP-9 Concentration (ng/ml)	$p$
Preterm	35	$2860.68 \pm 627.32$	0.04
At Term	35	$2549.74 \pm 657.15$	

**Table 3.** Average Serum TNF- $\alpha$  Concentration in Preterm PROM and PROM at Term

Group	Number of Sample (n)	Average Serum MMP-9 Concentration (ng/ml)	$p$
Preterm	35	$12,086.60 \pm 5,384.51$	0.00
At Term	35	$6,422.51 \pm 2,645.32$	

The average concentration of TNF- $\alpha$  in preterm PROM is almost twice the average concentration in PROM at term. The difference in the level of TNF- $\alpha$  between that in preterm PROM and that in PROM at term was found to be significant ( $p=0.00$ ). A similar research was conducted by Sabarudin in dr. Hasan Sadikin Hospital of Bandung and network hospitals in Bandung from May up to November 2009. It is generally claimed that gestational age from 28 up to 34 weeks bear greater risk to undergo PROM. As has been stated by Noriko et al, TNF- $\alpha$  in human chorionic cells has been verified to promote the production of MMP-9 and Prostaglandin E2 and suppress the tissue inhibitor of metalloproteinase (TIMP). Thus, TNF- $\alpha$  has the tendency to cause weakening and rupture of membranes through degrading extracellular collagen matrix.<sup>12-14</sup>

### CONCLUSION

There is a significant difference in the levels of MMP-9 and TNF- $\alpha$  between preterm PROM and PROM at term, in which the levels of MMP-9 and TNF- $\alpha$  in preterm PROM are greater than PROM at term. This verifies that the role of MMP-9 and TNF- $\alpha$  in the occurrence of preterm PROM is higher than that in PROM at term.

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