EXPLORATION OF ACTIVITIES AND GENETIC PATTERN OF ELASTASE IN PATIENTS WITH THREATENED PRETERM LABOR

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Introduction [1]

Preterm labor (PTL) is the delivery in gestational age between 20 weeks and more than or same with 37 weeks, while normal labor between 37 to 42 weeks of pregnancy.

The incidence of PTL vary between 5 to 12 % from all delivery with the low incidence in the developed country between 5 – 7 %. In Indonesia we still not have the national rate, but some researchers give the hospital rate.

Joesoef reported the incidence of PTL in several hospital in Jakarta in 1991 about 13.3 %, while in our hospital, Hasan Sadikin Hospital in Bandung in 2001 the incidence reported 9.9 %.

Most of PTL are spontaneously about 72 %, while the remain occur because the pregnancy must be terminated pretermly because of medical indications.

Neonatal death in PTL contributed 70 % of perinatal mortality in Indonesia, and neonatal death of preterm babies in the first year of their live in Hasan Sadikin Hospital range between 40–70%. The preterm infant death in Hasan Sadikin Hospital Bandung was 56,6% of all perinatal death.

Beside the failure of respiration system as the cause of preterm infant death, the morbidity raise as well as cerebral hemorrhage, nervous system disturbance, mental retarded, deafness and growth disturbance.

Primary prevention of PTL by eliminated risk factors were impossible done because risk factors known for PTL such as history of PTL, history of recurrent abortions and habitual factors. In this stage of prevention we have to perform close antenatal care, and perform secondary prevention.

Secondary prevention are include early detection of PTL. Clinically we can perform detection by evaluating of uterine contraction and the condition of the cervix. We can measure the cervical length by ultrasound, biochemical markers those are pH of vagina and fetal fibronectin from cervicovaginal secret. Unfortunately those efforts did not give the real advantages to be applied as mode of detection of PTL widely.

Morbidity and mortality rate of preterm infant will be higher if the baby born too early or the baby weight too small, so the effort to prolong the gestational period was the important thing to be done in threatened PTL.

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The effort has been done through medication, but the result unsatisfied, because of advances of the labor process.

There are many suggestion that the pathophysiology of PTL is the inflammation process in the pregnancy compartment. This process will activate cytokine network which resulting Interleukin-1 (Il-1), Interleukin-6 (Il-6) and Tumor Necrosis Faktor α (TNF α) because of activated macrophag.

Both cytokine will stimulate the process of Interleukin-8 (Il-8) formation by decidua, chorion and amnion and then together with Colony Stimulating Factor-1 (CSF-1) recruit polymorphonuclear (PMN) cell into the pregnancy compartment those include uterine cervix, and then the PMN produce the elastase.

Elastase is the proteolytic enzyme which found in pancreas, spleen and neutrophyl. In the neutrophyl elastase is found in neutrophyl lysozyme, and neutrophyl was found in the pregnant uterine cervix as the consequence of leucocyte infiltration in that area.

In the normal pregnancy, elastase detected at 20 weeks of pregnancy in the endocervix and then raise gradually as the raise of gestational age, and the concentration raise abruptly 4 – 5 weeks before initial of labor.

This enzyme cleavage the elastin, the protein of connective tissue which resistant to many proteinase, and this connective tissue are the essential component in the chorion, amnion and the cervix. The increases of activity of this enzyme in pregnancy compartment will cleavage of extracellular matrix and resulting changes in these tissue. Uterine cervix become soften, the cervix will be dilated and shorten those are the early sign of labor. In the chorion and amnion membrane this enzyme will cleavage the extracellular matrix and resulting the rupture of the membrane. The increase of elastase activity in pregnancy compartment those include in canalis cervicalis in the patient with threatened PTL, can be choose as a tool to predict the PTL.

Research method

This research conducted through analytic comparative study with cross-sectional design.

The research was done in 14 months period and the end in 2005. In that period we recruit 32 patients with criteria threatened preterm labor, and in the same time we choose 34 women with normal pregnancy as a control at random. Both of two groups of patients with PTL and control group are include in inclusion and exclusion criteria, and both of the subjects received the same investigation.

Threatened PTL is the pregnancy with gestational age 28 weeks or more and less than 37 weeks with the signs such as uterine contraction with interval less than 10 minutes and observe in 30 minutes minimally using cardiotocography, the dilation of cervix $\leq$ 4 cm by internal examination and the membranes still intact. As the control group are the pregnant women in the same gestational age without any signs of labor.

Both of groups received many investigation such as :

1. Taken out of 10 cc of blood from the cubiti vein for haemoglobin, leucocyte count, C-Reactive Protein and for DNA examination.
2. Taken out the swab from canalis cervicalis for elastase activity examination.

Hb, leucocyte count and CRP examination using standard examination in our hospital, elastase examination using Elisa Method and for DNA elastase examination from

isolated leucocyte from the blood and amplified elastase gen by PCR technique. This amplification was done using the pair of primer those F and R elastase primer :

5'-GTATCACGGGGCCCTGGATAA-3' (F)
5'-CGGCCCGCCCGTGCCTCCCCG-3' (R)

This pair of primer amplified the promoter area of elastase gen which range from nt 800 to nt 1, totally 800 pb.
Test of significancy was done by Chi-square test for differences of percentation for data in contingency table. Mann-Whitney test for comparing the differences of two median value from the data with no normal distribution, t-test for comparing the differences of two average data with normal distribution, and Kruskal-Walis test for comparing the differences of 2 median value with no normal distribution. Significancy is decided with p value <0.05.

RESULT

In this research we have 32 patients with threatened PTL and 34 patients normal pregnancy as a control .

Table 1. Characteristic

Characteristic	Group		Significancy
	Threatened PTL (n = 32)	Normal Pregnancy (n = 34)	
1. Age (year)			
< 20	4	1	
20-24	7	8	
25-29	6	10	
30-34	9	9	
≥ 35	5	6	
$\overline{X}$ (SB)	27,5 (6,2)	28,9 (6,3)	t = 0,93 p = 0,355
2. Parity			
0	16	18	X^2 = 0,06
1-3	15	15	p = 0,972
≥ 4	1	1	
3. Gestational Age (w)			
28-30	10	10	
31-33	8	17	
34-36	14	7	
$\overline{X}$ (SB)	32,0 (2,6)	31,6 (2,1)	t = 0,76 p = 0,448

Note : t = t-test; X^2 = chi-square test.

Table 1 shows the characteristic of the subject those are include age of the women, parity and gestational age.

Most of the women of the both groups with the age range between 20-35 year, the optimal reproductive age. The differences no significant statistically

(p>0.05). Most of the women of both groups with the parity 0, more than 50 % for both groiups. The differences no significant statistically (p>0.05).
Fifty percent of control group with the gestational age of 31–33 weeks, while most of the patient with threatened PTL with the gestational age of 34–36 weeks. The differences no significant statistically (p>0.05).
Based on this homogeneity both groups are comparable.

Table 2 Elastase concentration in canalis cervicalis in both groups

Elastase concentration (ng/ml)	Group		Significancy
	Case (n = 32)	Control (n = 34)	
$\bar{X}$ (SB)	2,68 (3,99)	1,31 (1,94)	Z_{M-W} = 3,06
Median	1,72	0,52	p = 0,002
Distance	0,09-22,07	0,11-9,98	

Note : Z_{M-W} = Mann-Whitney test

Table 2 shows the significance difference of elastase concentration in canalis cervicalis between two groups (p=0,002). The concentration of elastase in patient with threatened PTL is 1.72 ng/ml more than 1 ng/ml, while in normal pregnancy is 0.52 ng/ml, less than 1 ng/ml yaitu 0,52 ng/ml.

Table 3 *Cut off Point* of elastase concentration in predicting threatened PTL

Cut off Point Elastase concentration (ng/ml)		Group		Sens. (%)	Spec. (%)	Accuracy (%)
		Case (n = 32)	Control (n = 34)			
1.	≥ 4	5	2	15,6	94,1	56,1
	< 4	27	32			
2.	≥ 3	8	4	25	88,2	57,6
	< 3	24	30			
3.	≥ 1,7	16	6	50	82,4	66,7
	< 1,7	16	28			
4.	≥ 1,0	21	12	65,6	64,7	65,2
	< 1,0	11	22			
5.	≥ 0,75	23	14	71,9	58,8	65,2
	< 0,75	9	20			
6.	≥ 0,55	27	16	84,4	52,9	68,2
	< 0,55	5	18			
7.	≥ 0,52	28	16	87,5	52,9	69,7
	< 0,52	4	18			
8.	≥ 0,5	29	18	90,6	47,0	68,2
	< 0,5	3	16			

Table 3 shows 8 elastase concentration in canalis cervicalis are designed as a *cut off point* and are analyzed in two groups. In those concentrations are evaluated sensitivity, specificity and accuracy. From eight elastase concentrations which are evaluated as a *cut off point* in predicting PTL, the elastase concentration of 0.52 ng/ml is choosed as a *cut off point* with sensitivity of 87.5%, specificity of 52.9% and accuracy of 69.7%. If we use the elastase concentration with higher sensitivity, the specificity will be lesser. If we use the elastase concentration with higher sensitivity, the specificity will be lesser while the accuracy remain not changes. We arranged the eight elastase concentrations in the curve of *Receiver Operating Characteristic* as follows :

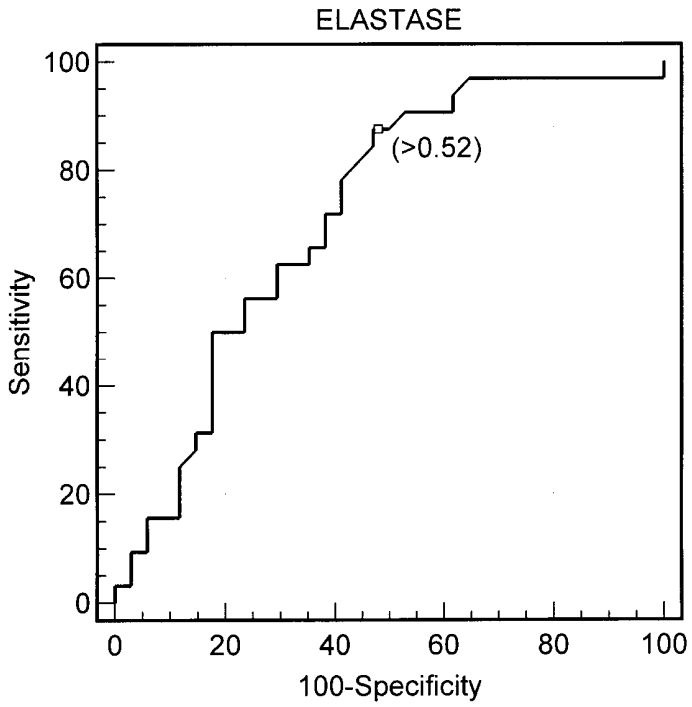


Figure 1. ROC of Elastase Concentration

After the eight concentrations are arranged in the curve of ROC (*Receiver Operating Characteristic*), apparently the 7th elastase with concentration of 0.52 ng/ml, has a longest distance from diagonal line of the curve. The selected *cut-off point* was elastase concentration of 0,52 ng/ml.

DNA Elastase Examination Analyzes

Analyzed DNA from isolated leucocyte taken out from the blood are examined by PCR technique to amplify elastase gene. This amplification was done by using the pair of F and R primer of elastase :

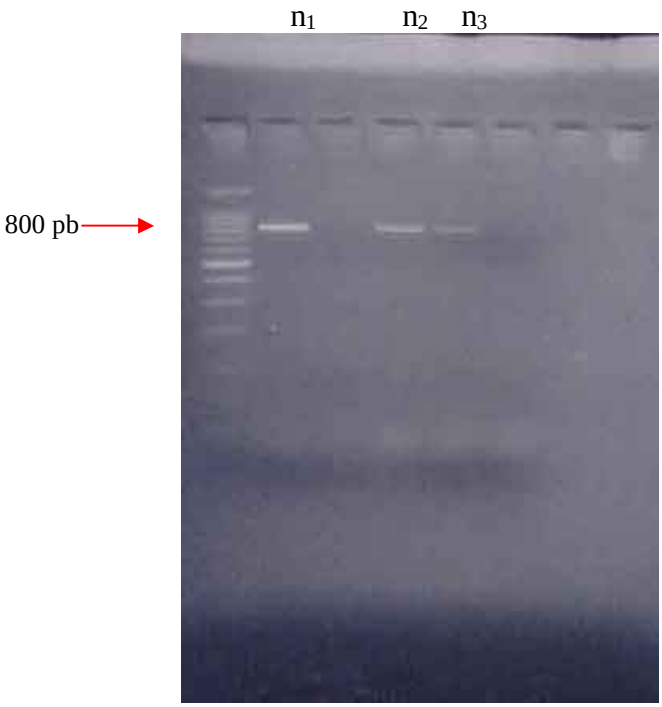
5'-GTATCACGGGGCCCTGGATAA-3' (F)
5'-CGGCCCGCCCGTGCCTCCCCG-3' (R)

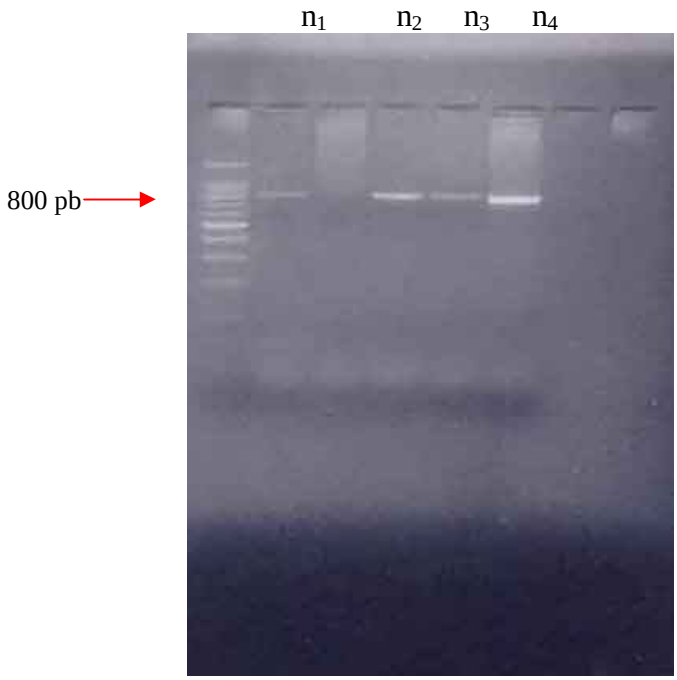
The pair of primer amplified the promoter region elastase gene which lied between 800 till 1 nt, the amount of 800 base pair.

Note : pb : Pair of bases ; c : cases of PTL

Figure 2 PCR amplification result of Elastase gene in Threatened Preterm Labor Patients

Figure 2 show 6 sample of DNA from 6 patients with threatened preterm labor with the size 800 pair of bases. patients with Threatened Preterm Labor after amplificate with the primers. The DNA tape which isolated

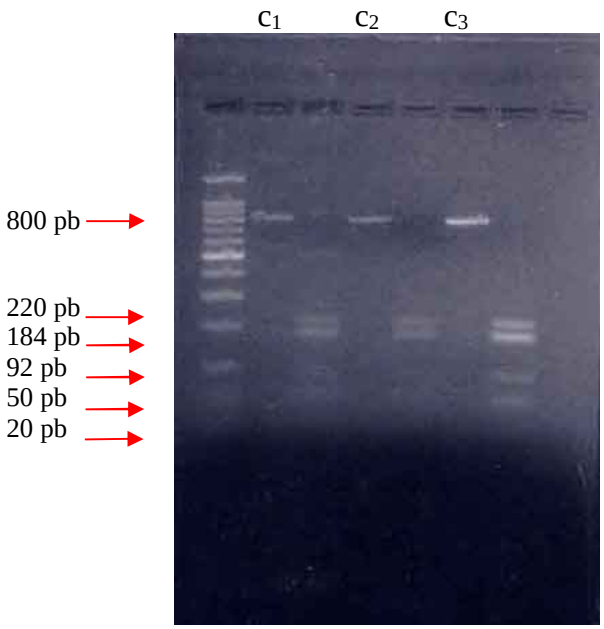


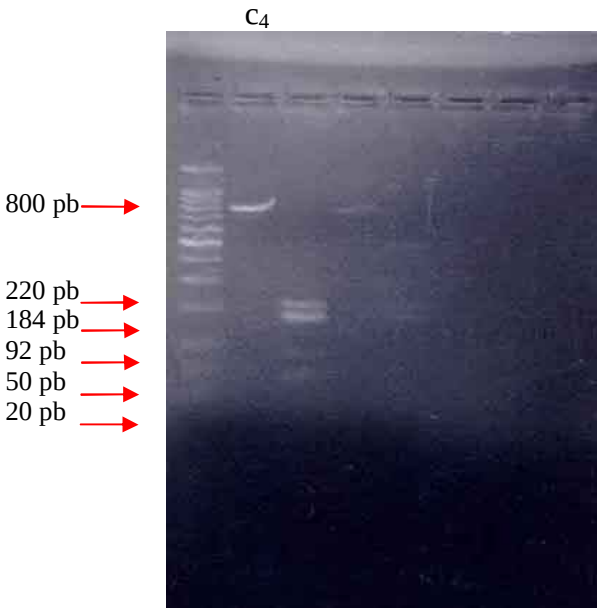


Note : pb : Pair of bases ; n : normal cases

Figure 3 PCR amplification result of Elastase Gene in normal pregnancy

Figure 3 shows 7 sample of DNA patients with normal pregnancy after amplification with these primers. The DNA tape which isolated from 7 patients with normal pregnancy has size of 800 pair of bases.



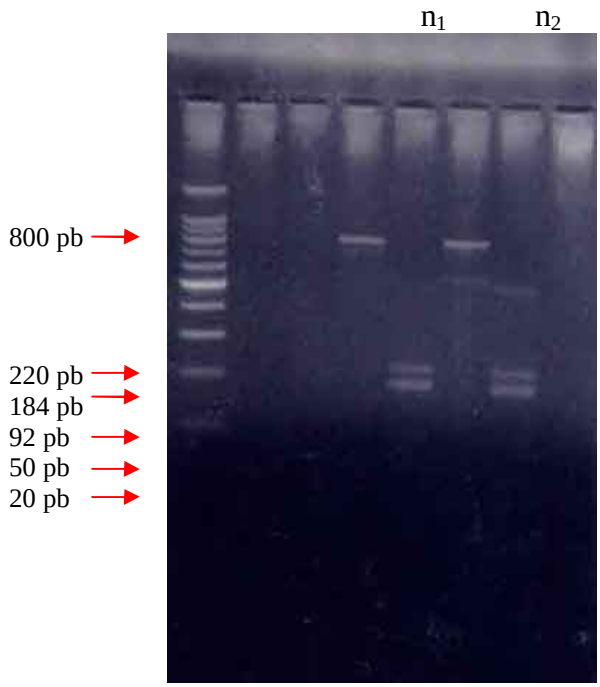


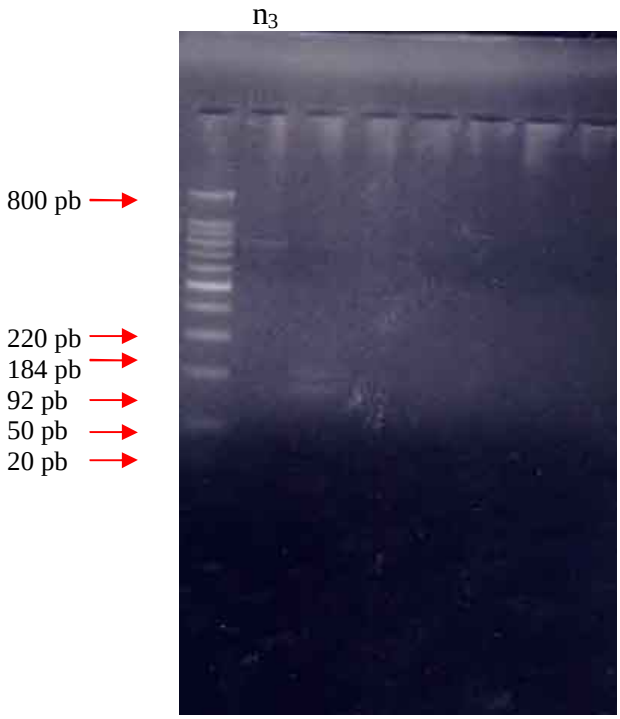
Note : pb : Pair of bases ; c : PTL cases

Figure 4. Fragmen DNA fragment of Elastase Gene after cut by *Hpa*II enzyme in Threatened PTL group

Figure 4 shows the DNA tape result after PCR which come from 4 pregnant women with threatened PTL cutting by *Hpa*II restriction enzyme The segment of tape resulting after its cut are measured the length of pair of base. The restriction by this enzyme resulting of 7 segments of DNA those are 50 pb, 184 pb, 184 pb, 92 pb, 220 pb, 50 pb and 20 pb respectively of those DNA samples.

H*pa*II enzyme has ability to cut at the sequence CCGG, and from DNA tape with length of 800 pb there are 7 sequence CCGG so the cutting resulted 7 slices DNA tape.

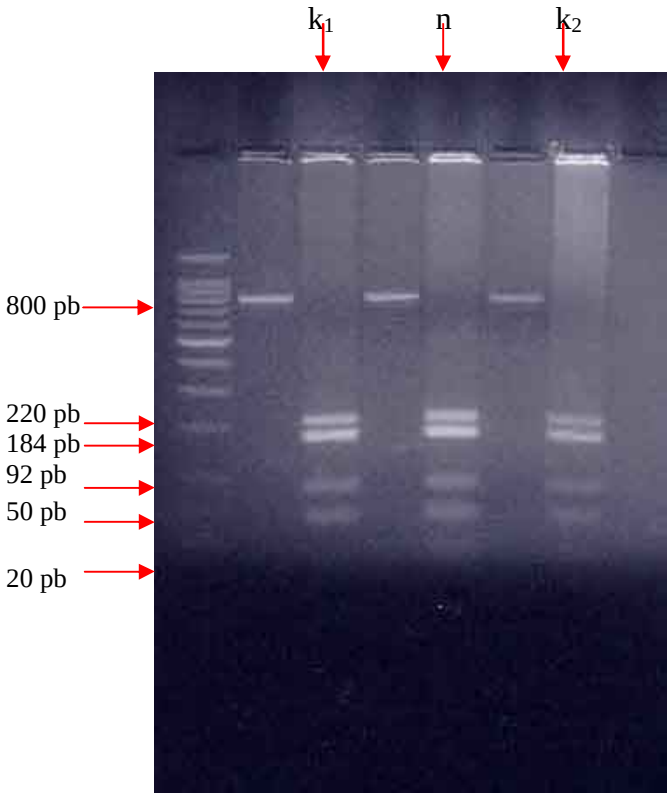




Note : pb : Pair base ; n : normal cases

Figure 5. DNA fragment of Elastase Gene after cut by *Hpa*II enzyme in Normal Pregnancy

Figure 5 show the DNA tape after PCR from three normal pregnant women which cut by *Hpa*II enzyme. The segment cutting is measured of its length of base. The result of cutting by restriction enzyme were 7 same DNA slices of both groups which are 50 pb, 184 pb, 184 pb, 92 pb, 220 pb, 50 pb dan 20 pb consecutively.



Note : k : Threat.PTL; n : Normal cases; pb : Pair base

Figure 6. DNA fragment of Elastase Gene after cut by *Hpa*II Enzyme from the patients with threatened PTL and normal pregnancy

Figure 6 show the DNA tape after PCR from two pregnant women with threatened PTL (c_1 and c_2) and one normal pregnant woman (n) which cut by *Hpa*II enzyme. The segment cutting is measured of its length of base. The result of cutting by restriction enzyme were 7 same DNA slices of both groups which are 50 pb, 184 pb, 184 pb, 92 pb, 220 pb, 50 pb dan 20 pb consecutively.

The result of segments of DNA after cutting by this restriction enzyme shows the same result between normal pregnancy and threatened PTL, and we conclude that there is no difference in gene expression in both groups.

Conclusion

1. We found the significance difference of elastase concentration in canalis cervicalis between normal pregnancy and threatened preterm labor.
2. We did not find the difference of elastase gene expression between normal pregnancy and threatened preterm labor.

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