

Difference of PAR-1 Expressions on New Zealand Rabbit Following Surabaya Method Uterine Compression Suture After Cesarean Delivery

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ABSTRAK

Jahitan kompresi uterus Surabaya merupakan modifikasi dari prosedur B-Lynch sebagai salah satu teknik kompresi untuk manajemen perdarahan karena atonia uteri. Penelitian ini mengamati jumlah PAR-1 ekspresi pada rahim kelinci New Zealand pada setengah jam dan dua jam setelah kompresi Surabaya. Dua puluh satu kelinci New Zealand dewasa diinduksi kehamilan. Semua melahirkan dengan operasi caesar. Populasi dibagi dua kelompok, satu kelompok kelinci sebagai kelompok paparan yang mendapatkan jahitan kompresi uterus menggunakan metode Surabaya dan lainnya sebagai kelompok kontrol tanpa paparan jahitan kompresi uterus. Histopatologi spesimen diambil dari kelompok studi dan kontrol dan dihitung jumlah PAR-1 ekspresi yang setelah diamati pada setengah jam dan dua jam. Analisis statistik menggunakan uji T menunjukkan kenaikan ekspresi PAR-1 pada kelompok studi dibandingkan dengan kelompok kontrol ($p < 0,05$) pada setengah jam dan dua jam pengamatan. Sebagai kesimpulan, mekanika rangsangan menggunakan kompresi uterus Surabaya menghasilkan peningkatan yang signifikan pada ekspresi PAR-1 setelah 30 menit dan dua jam. (MOG 2012;20:14-17)

ABSTRACT

Surabaya uterine compression suture is a modification from B-Lynch procedure as one of compression technique for the management of hemorrhage due to uterine atony. This study observed the expression numbers of PAR-1 in New Zealand rabbit uterus at half hours and two hours after Surabaya compression. Twenty-one adult female New Zealand rabbits were induced for pregnancy. All were given birth by cesarean section. Population was divided into two groups one group showed as exposure group which underwent uterine compression suture using Surabaya method and the other side as control group with no exposure to uterine compression suture. Histopathology specimen was taken from study and control group and count on the number of PAR-1 expressions which after observed at half hour and two hours. Statistical analysis using paired T test shows increase on expressions of PAR-1 at study group compare to control group ($p < 0,05$) on half hour and two hours observations. In conclusion, mechanic stimuli using Surabaya uterus compression produces significant increase in the expressions of PAR-1 after 30 minutes and two hours. (MOG 2012;20:14-17)

Keyword : Surabaya method compression suture, PAR-1 expression, post partum hemorrhagic

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INTRODUCTION

Haemorrhage post partum was tone of the cause of mortality dan morbidity in maternal and an obstetric emergency that threatening life. In United state. In United States, maternal death caused by haemorrhagic post partum for 17% from 4200 pregnancy.⁵ In development country, 25% death case in pregnancy mother because haemorrhagic post partum. Atony uterine is a condition when uterine cannot contraction effectively. After placenta release, haemorrhage control-led with myometrium muscle contraction and make spiraly artery stop the bleeding. If contraction not strong enough, so it will caused a haemorrhage.¹

The most important haemostasis mechanism is myometrial contraction, for pressing blood vessel lumen followed by the formation of clotting in placenta implantation.⁵ Smooth muscle contraction regulate by receptor and mechanical activation by actin and myosin protein. Intracellular calcium source are from extracellular potential membrane or from inside myocyte which is releasing from sarcoplasm reticulum.⁹ On certain stimulation, calcium concentration intracellular $/(Ca^{2+})_i$ increase.

So there is bound with calmodulin. Ca^{2+} - complex calmodulin caused myosin light chain kinase phosphorylated (MLCK), form bound with actin and caused contraction. This is showed that Ca^{2+} - calmodulin-MLCK pathway is important in mechanical

activity in uterine.¹³ Any vascular lesion because of releasing placenta in uterine post partum, consequent thrombin activity in specific and temporally in the site. Thrombin through its receptor Protease Activated Receptor-1 (PAR-1) caused increase calcium through extrinsic pathway, which is increasing in calcium influx and through intrinsic pathway which from sarcoplasmic reticulum. Besides that, PAR-1 increasing myocyte oxytocin receptor.⁴ PAR-1 is the plentiest receptor which activated by thrombin and in bigger concentration in smooth muscle cell compared to PAR-3 and PAR-4. Though PAR-2 is a receptor which activated by trypsin.

In case of uterine atony post partum, conservative management can be in the form of uterine massage, bimanual uterine compression and medication with uterotonic drug. If this attempt has not been able to control the hemorrhage, more invasive action such as intra uterine tamponade or operative procedure such as ligation of uterine artery, ovarian artery and hypogastric artery or uterus compression technique such as B-Lynch, Hayman, Square technique suture) or even hysterectomy.^{1,8,12}

Sulistyono introduced modification of B-Lynch namely Surabaya compression technique by three sutures on uterus with chromic number 2 on 3 cm from uterine lower segment, 4 cm from left lateral go through uterine lower segment. After Surabaya compression, bleeding is expected to stop due to obliteration of blood vessels. The mechanical stimulation would induce expressions of PAR-1 by myometrial cells. This study wants to evaluate the roles of PAR-1 in uterine contraction.

MATERIALS AND METHODS

This study is true experimental with post test only control group design. Adult New Zealand rabbit ages more than 6 months with at term pregnancy and weight about 2000-3500 grams, were used as the subject for this study. Rabbit is chosen as subject at this study, because the study cannot be performed on human. Any pregnant rabbit with disorders or intrauterine fetal death are excluded from study. Adult female rabbit were given Pregnant Mare Serum Gonadotropin (PMSG) 30 IU.

40 hours later injected by human chorionic gonadotropin (HCG 30 IU). Then those rabbit was mating with male rabbit. If vaginal was closed 17 hours later than the female rabbit was diagnosed as pregnant day I. When the female rabbit pregnancy is at term (gestation week 28-31 days) cesarean section was conducted under anesthetic ketamin 1 mg per kilograms

weight with sagittal incision. One site of rabbit uterus was undergone Surabaya B Lynch modification compression with three vertical sutures at lower uterus segment uterus through the other site of SBR of the uterus to produce compression effect and stop the bleeding.

Control group was left without any manipulation. Histopathology specimen was taken from uterus by 30 minutes after Surabaya compression procedure and at two hours later both from control or study groups. Samples were sent to laboratory for immunohistochemistry staining (to measure expressions of PAR-1).

RESULTS AND DISCUSSION

This study recruited 21 pregnant rabbits that meet inclusion and exclusion criteria. Three of them dropped out, 2 fetuses had birth weight less than 25 grams and 1 rabbit had abdominal pregnancy. 11 pregnant rabbits were finally recruited as subject of this study. Average weight was about 2300-3100 grams with average fetal rabbit weight 25-52 grams (Table 1). There is no significant difference between average fetal weight between left and right uterus. There is no significant difference between number of fetuses between left and right uterus.

Table 1. Comparison between weight and number of rabbits fetus on right and left uterus

Variable	Uterus		P value
	left (X ± SD)	right (X ± SD)	
Rabbit fetal weight	34.05 ± 8.78	32.64 ± 7.33	0.224
Number of fetus	2.91 ± 0.83	2.55 ± 0.52	0.104

As shown from histogram (Figure 1) and Table 1, PAR-1 expression mean at control group at 30 minutes and 2 hours are 15.36 ± 1.20 and 23.46 ± 1.04 , which is significantly different from the control group.

Surgical uterine compression post partum management to control post partum bleeding was firstly introduced by Christopher B-Lynch at 1998 with special aim to produce vascular continue pressure on placenta site.² Further was found this compression was mechanic stimulus which can initiate interaction between actin and myosin. This mechanic stimulus would raise expression of CAPs on smooth muscle. This was the way the mechanic stimulus modulate the activation of myometrium importantly.¹⁰

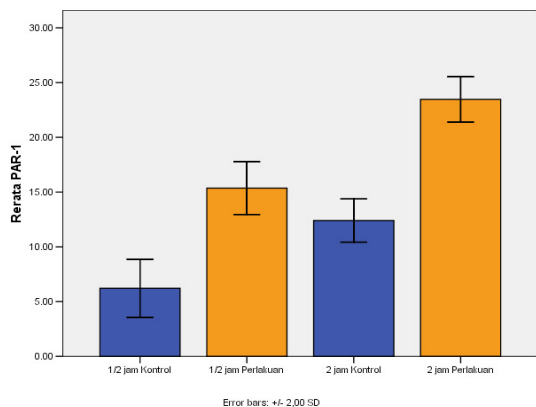


Figure 1. Distribution of PAR-1 expression mean between control and study groups

Table 2. Comparison of PAR-1 expression between control and study group at 30 minutes and 2 hours evaluation

Time	Group		Delta	P value
	control (X ± SD)	study (X ± SD)		
30 m	6.22 ± 1.33	15.36 ± 1.20	9.14 ± 2.06	<0.0001
2 h	12.40 ± 0.99	23.46 ± 1.04	11.06 ± 1.60	< 0.0001

As shown from histogram (Figure 1) and Table 1, PAR-1 expression mean at control group at 30 minutes and 2 hours are 6.22 ± 1.33 and 12.40 ± 0.99 , which is significantly increased.

The mechanic stimulus of Surabaya method would increase the expression of PAR-1 significantly ($p < 0.05$). This protease-activated receptor 1 (PAR 1), stimulate the activation of transduction signal pathways, and resulted to oscillation of cytosolic calcium. This would produce influx of Ca^{2+} .^{6,11}

While the bond of thrombin and PAR 1, would bind with G_{aq} which would activate the PLC. This PLC would hydrolyze the IP_2 which would release the IP_3 and DAG. This IP_3 would release ion of SR calcium, so intracellular Ca^{2+} would raise and formed Ca^{2+} -calmodulin. This would activate the MLC-kinase, produce P-myosin which would bind with F-actin, and this bond would create contractions.

At this research, we found that expretion on PAR-1 is increasing at rabbit uterine, which it conducted with B-Lynch suture uterine compress technic Surabaya modification methode, it is supported the latest research before by another researcher. Increasing in expression

PAR-1 are happen in uterine with treatment and at control uterine in all time interval. Differences at PAR-1 expression was seen at uterine in treatment group compared to control group. This increasing at PAR-1, caused increasing at calcium influx through increasing of L type calcium channel. Increasing PAR-1 is happen to through G protein which are $G_{a12/13}$ and G_{aq} where PAR-1 can coupled with $G_{a12/13}$ to bound Guanine nucleotide exchange factors (RhoGEF) and then change Rho A guanidine diphosphat protein (Rho A GDP) become Rho A guanidine triphosphat (Rho A GTP) which increasing phospholipase C (PLC) and separating phosphatidilinositol 4,5 phosphat (PIP2) to be inositol 1,4,5 phosphat (IP_3) and diacylglycerol (DAG) and then causing fosforilation at myosin light chain (MLC).(6,4). Beside that, for fosforilated MLC through activating protein kinase C (PKC), DAG is activating mitogen activated protein kinase (MAPK) then fosforilated heat shock protein-27 (HSP-27). Activated HSP-27 can change aktin-G into aktin-F. Aktin-F will be bound with MLC which has been phosphorylated and cause contraction.³ At ligature uterin compression with B-Lynch Surabaya modification methode, as the result of continous compression so it will give chance to produce thrombin at the implantation placenta, because thrombin is only produced at vascular lesion, inflammation or, tissue damage.^{7,9}

CONCLUSION

Expressions of PAR-1 increased after 30 minutes and 2 hours following Surabaya method compression suture.

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