Increased Expression of Myocyte HSP27 and Myometrium Contraction on New Zealand Rabbit Following Surabaya Method Uterine Compression Suture after Cesarean Delivery

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ABSTRAK

Jahitan kompresi uterus merupakan modifikasi dari prosedur BLynch sebagai salah satu teknik kompresi untuk manajemen perdarahan karena atonia uteri. Penelitian ini meneliti jumlah HSP 27 ekspresi myosit, jumlah miosit kontrak dan jumlah arteri radial terkompresi pada rahim kelinci Selandia Baru di setengah jam dan dua jam setelah kompresi Surabaya. Dua puluh kelinci Selandia Baru kelinci betina dewasa diinduksi untuk kehamilan. Semua yang melahirkan melalui operasi sesar. Populasi dibagi dua kelompok. Dari setiap kelompok sebagai kelompok paparan yang mengalami jahitan kompresi uterus menggunakan metode surabaya dan kelompok lainnya sebagai kelompok kontrol tanpa paparan jahitan kompresi uterus. Histopatologi spesimen yang diambil dari kelompok studi dan kontrol dan dihitung jumlah MSP 27, ekspresi kontraksi miosit total dikontrak arteri miosit dan total kompresi arteri yang setelah diamati pada setengah jam dan dua jam. Analisis statistik menggunakan beberapa uji T menunjukkan kenaikan 27 HSP ekspresi, total dikontrak miosit arteri, total terkompresi rahim radial pada kelompok studi dibandingkan dengan kelompok kontrol (p <0,001) pada setengah jam pengamatan. Namun pada pengamatan dua jam, terdapat perbedaan signifikan pada ekspresi HSP 27 antara dua kelompok tetapi tidak berbeda pada arteri miosit dan tekan total dikontrak. Sebagai kesimpulan, mekanik rangsangan menggunakan kompresi uterus Surabaya meningkatkan ekspresi HSP 27, total sel kontraksi sel miosit uterus radial dan penipisan arteri setelah 30 menit dan tetap sama untuk HSP 27 ekspresi setelah dua jam. ***(MOG 2011;19:73-77)***

Kata kunci: *jahitan kompresi uterus metode Surabaya, ekspresi HSP 27, kontraksi miosit, perdarahan post-partum*

ABSTRACT

Surabaya uterine compression suture is a modification from BLynch procedure as one of compression technique for management hemorrhage due to uterine atony. This study looks at on the number of HSP 27 expression on myosit, number of contracted myocyte and number of compressed radial uterine artery on New Zealand rabbit uterus at half hours and two hours after Surabaya compression. Twenty adult female New Zealand rabbit is induced for pregnancy. All are given birth by cesarian section. Population divided as two groups from each rabbit one uteri side as exposure group which undergone uterine compression suture using surabaya method and the other side as control group with no exposure to uterine compression suture. Histopathology specimen is taken from study and control group and count on the number of expression HSP 27, total contracted myocyte and total compressed radial uterine artery which after observed at half hour and two hours. Statistic analysis using couple T test shows increase on HSP 27 expression, total contracted myocyte, total compressed radial uterine artery at study group compare to control group (p<0.001) on half hour observation. However on two hours observation, there is still significant differ on HSP 27 expression between two groups but no differs on total contracted myocyte and compressed artery. In conclusion, mechanic stimuli using Surabaya uterus compression increases HSP 27 expression, total contracted myosit cells and thinning artery after 30 minutes and remained the same for HSP 27 expression after two hours. ***(MOG 2011;19:73-77)***

Keyword: *Surabaya method compression suture, HSP 27 expression, contracted myocyte, hemorrhagic post partum*

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INTRODUCTION

Maternal mortality rate in Indonesia in 2007 was about 228 per 100.000 living birth, the highest in South East Asia. Hemorrhage is mentioned as the most common cause of maternal death (28%). Massive hemorrhage mostly due to uterine atony (30%), placenta retention (18.9%) and laceration (13%).[2] The most important factor for homeostasis on postpartum is myometrium contraction.[9,5] Myometrium contraction happens due to interaction between actin and myosin.[7] Contraction myometrium was organized by receptor mechanism and mechanic activation between actin and myosin. Ca 2+ bind to calmodulin will activated myosin like chain kinase (MLCK) and initiate phosforilation of myosin before interaction with actin.[14,10,15] Meanwhile actin will

interaction with myosin only after converse from G actin to F ation.[5,6] HSP 27 work in the actin remodeling process. HSP 27 is a chaperone protein with regulate transformation of G-actin to F-actin. When mechanism stimulating is given to smooth muscle integrine as receptor on cell membrane will activated down stream signal. In this actin remedolling proses, HSP 27 is needed as stimulating for the formation of F-actin, stabilitation of focal adhesion and promoted cell migration.[6,12,11] In case of uterine atony post partum, conservative management can be in the form uterus massage, bimanual uterus 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 uterine artery,ovarii artery and hypogastic artery or uterus compression technique such as B-lynch, Hayman, Square technique suture) or even hysterectomy.[1,8,3.13]

Sulistyono introduce modification of B-Lynch namely Surabaya compression technique by three suture on uterus with chromic number 2 on 3 cm from uterine lower segment, 4 cm from left lateral go through uterine lower segmen. After Surabaya compression,bleeding is expected to stop due to obliteration of blood vessels. Myometrium contraction will close blood vessels than open on placental site. Study by Shynlova and friends (2002), found that uterus myosin have direct respond on mechanic activation to increase C-Fos mRNA. C-fos mRNA expression have positive correlation with contraction associated protein (CAPs). Minimal duration needed for mechanic stimuli to activated gen C-Fos mRNA, between 30 minutes and peak at 2 hours. This study want to evaluate the increasing of HSP 27 expression myocyte, number of contracted myocyte and number of compressed radial uterine artery on 30 minutes and two hours evaluation.

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, where used as the subject for this study. Rabbit is chosen as subject at this study due to rabbit uterus is bicorn. Any sick rabbit and rabbit less than 25 grams are excluded from study.Sample that cannot be read were also excluded from study. Adult female rabbit were given Pregnant Mare Serum human chorionic gonadotropin (HCG 30 IU). Than those rabbit was mating with male rabbit. Gonadotropin (PMSG) 30 IU. 40 hours later injected by If vaginal was closed 12 hours latter than the female rabbit was diagnose 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 sagital incision. One site of rabbit uterus was undergone Surabaya B Lynch modification compression with three vertical suture at lower uterus segment uterus through the other site of SBR of the uterus to produce compression effect and stop the bleeding. On the 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 immunohistokimia staining (to measure HSP 27 expression) and HE staining to measure number of myocyte there it contractions and number of compressed artery.

RESULTS AND DISCUSSION

This study recruited 21 pregnant rabbits that meet inclusion and exclusion criteria. Three of them dropped out, 2 fetus had birth weight less than 25 grams and 1 rabbit had abdominal pregnancy. 18 pregnant rabbits was 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 fetal between left and right uterus. As shown from histogram (figure 1) average HSP 27 expression at control group at 30 minutes and 2 hours are 3.49 ± 1.25 and 4.83±1.58 which significantly differ from control groups. Percentage of contracted myocyte between control and study groups on 30 minutes and 2 hours are 35.63±9.61 and 72.61±10.73. while on control group are 82.40±9.83 and 82.98±12.65. There is significant difference between two groups on 30 minutes evaluation but not on two hours (figure 2).

As can be seen from the figure 3, percentage of compressed radial artery on control group at 30 minutes and 2 hours were 42.95±16.45 and 79.11±12.71. While on treatment group were 83.71±7.72 and 84.04±7.92. There were significant difference at 30 minutes evaluation between treatment group and control group but not at 2 hours.

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.[3] Further was found this compression was mechanic stimulus which can initiate interaction between actin and myosin. This mechanic stimulus will activate P38

mitogen which activated protein kinase (p38-MAPK) as mediator for phosforilation HSP27 at actin.[11] Phosforilated HSP27 will capping barbed end of actin filament. This mechanic stimuli will cause depolarization and open L-Type Channel which lead Ca intracelluler and initiation contraction (actin and myosin interaction).This study found that HSP27 at uterine myocyte with Surabaya compression technique compare to control group with no manipulation found significant HSP27 expression at 30 minutes and 2 hours. This finding is relevant with study by Hirano and friend at 2004. Mechanic stimuli on fibroblast cell embryo mice culture lead to an increase HSP27 expression which has association with F actin formation. Beside mechanic stimulation HSP27 phosforilasion also induced by heat shock and other stimuli such as cytokine, growth factor and peptide. As in Bitar 2002, HSP27 expression increases with agonist stimuli revealed that mechanic stimuli on smooth muscle will lead integrine on myosit membrane to active Focal Adesion Cynase (FAK)

Tabel 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	35.52 ± 8.96	34.33 ± 8.71	0.62
Number of fetus	2.93 ± 0.88	2.80 ± 1.08	0.50

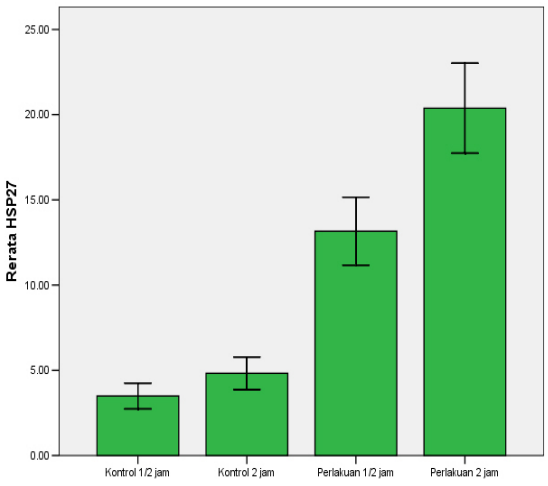

Figure 1. Distribution average HSP27 between control and study group.

through phosforilation of HSP27.Phosforilated HSP27 will stimuli F actin, stabilized focal adhesion, stabilization and induced cell migration.[6] HSP27 will cap barbed end of actin filament which will make it more stable and ready for interaction with myosin too produce contraction.[4]

Table 2. Comparison of contracted myocyte between control and study group at 30 minutes and 2 hours evaluation

Time	Group		Delta	P value
	Control (X ± SD)	Study (X±SD)		
30 minutes	35.63 ± 9.61	82.40 ± 9.83	46.76± 4.71	< 0.001
2 hours	72.61 ± 10.73	82.98 ± 12.65	10.36± 4.60	0.03

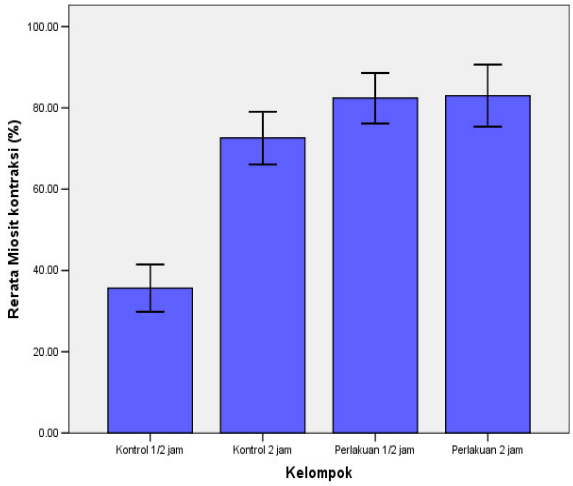

Figure 2. Distribution of contracted myocyte between control and study group

Myometrium contraction was produced through interaction between myosin and actin. Ca+ influx will in initiated stimulation of most smooth muscle contraction. This Ca influx can be induced by neurogenic, endocrine, mechanic or chemical stimuli.[7] Mechanic stimuli beside activates integrine at cell membrane as mechanic transducer in preparing actin to interac with myosin, also induced electricity activity on uterus myosit. Study done by Sidharta 2011, mechanic stimuli in the form of Surabaya compression on rabbit myocyte after CS will increase L type channel expression and increase intracellular Ca2+ which mediated by myosin Calmodulin (CAM). Ca2+ -CAM complex stimuly MLCK which will reduce auto inhibitor region flow from that kinase. MLCK will phosforilated myosin 20 kD like chain on specific sirine residu near N terminal.

Myosin phosforilation related with increase activity of actomyosin ATPase and facilitated interaction actin myosin with increase myosin head flexibility.

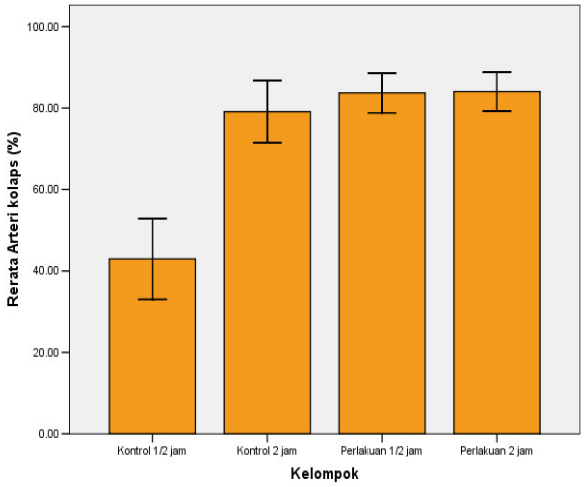

Figure 3. Distribution of compressed radial artery between control and study group

Table 3. Comparison of HSP27 ekspresion between control and study group at 30 minutes and 2 hours evaluation.

Time	Group		Delta	p value
	control (X±SD)	study (X± SD)		
30 minutes	42.95 ± 16.45	83.71 ± 7.72	40.76± 5.18	< 0.001
2 hours	79.11 ± 12.71	84.04 ± 7.92	492± 5.06	0.336

After RLC fosforilation as respond from myosin kinase will have ability to bind with actin filament. MLC phosforilation will stimuli bridge cycling along thin actin filament and induced contraction.[14,7, 15]

Statistical analysis that compare between treatment and control group on the percentage of contracted miosit myometrium and percentage of compressed radial artery on 30 minutes was found significant ($p<0.05$). However on the 2 hours evaluation no differ between control and treatment group. Shylova and friend study 2002 revealed that uterus miosit has direct respond on mechanical activity through C-Fos mRNA. C-Fos mRNA acumulation was detected 15 minutes after miosit contraction which maximum on 30 minutes (invitro study). C-Fos mRNA expression.This study found that smooth muscle will maintenance signal from mechanic stimulus when C-Fos mRNA has been stimulated eventhough the stimulus was stopped. Depolarization gap junction has important. Gap junction (connexin 43) is an intracellular canal which facilitation electric communicator and metabolic between myometrium cell. At term gap junction number increase and facilitate electric connection which needed for myometrium cell coordination for producing effective contraction. Gap junction consist of conection protein which has low electric resistance for an efficient potential interaction between cell. Smooth cell membrane connect through gap junction so than ion can flow free between myosin. Low electric resitance induce low potential action allow myocyte for contract simultaneously. This the reason why surgical compression will allow depolarization potential actin among cell are more effective and faster.

This study also revealed that Surabaya compression beside increase number of contracted myocyte and number of compressed artery up to 80% on 30 minutes evaluation. This was similar with Chen study found that mechanic activity on uterus cause increase expression on contraction associated proteins (APS) namely oxytocin receptor messenger ribonukleic acid (OTR mRNA) during pregnancy. This reseptor makes uterine more prone through mechanic stimuli. On other study revealed that mechanic stimuly on uterine will increase gap junction, activated signal transduksi consist of tyrosine kinase, nitrogen activated protein kinase (MAPK), protein kinase C (PKC), phospolipase C, phospolipase D and inositol 1,4,5-triphospate which cause phosforilation on light myosin chain so that contraction is easier and faster.

CONCLUSION

This study found increased expression of HSP27 in control group after 30 minutes and 2 hours following Surabaya method compression suture. Thirty minute after the procedure was done there is increased number of myometrial cell contraction and compressed radial artery between study group than the control group, but after 2 hours there was not difference on total contracted myocyte and compressed artery.

REFERENCES

1. Anderson JM and Etches D. Prevention and management of postpartum hemorrhage. American academy of family physicians 2007; 75: 875 – 882.
2. Al-Zirqi I, Vangen S, Forsen L and Pedersen, S. Prevalence and risk factors of severe obstetric haemorrhage. BJOG.2008; 115:1265.

3. B-Lynch C. Conservative Surgical management. In A Text Book of Postpartum Hemorrhage 1st ed. UK: Sapiens, 2006; pp. 287-298.
4. Cooper G.F. and Hausman, R.E.. The Cytoskeleton and Cell Movement. In The Cell: A Molecular Approach. 4nd Ed. The American Society for Microbiology; 2007. 12: 473-526.
5. Cunningham F.G. et al. ed, 2010. Maternal Anatomy. In William Obstetrics.. 23nd Ed.The McGraw-Hill Companies Inc, 2: 14-35.
6. Gerthoffer WT and Gunst SJ. Focal adhesion and small heat shock proteins in the regulation of actin remodelling and contractility in smooth muscle. Signal Transduction in Smooth Muscle. J Appl Physiol; 2001.vol 91: 963-972.
7. Guyton AC and Hall JE eds, Contraction and excitation of smooth muscle. In Textbook of medical physiology. 11th Ed. Elsevier Saunders. 2006; Inc, 8: 92 – 100
8. Hamamy EE and Lynch CB.. A worldwide review of the uses of the uterine compression suture techniques as alternative to hysterectomy in the management of severe post-partum haemorrhage. Journal of obstetrics and gynaecology. 2005; 25(2): 143 – 149.
9. Jacobs AJ, et al. Overview of postpartum hemorrhage. 2010. Available from: ecapp0504p. utd.com-222.124.156.242-127D622DFC-5.
10. Maul H, et al. The physiology of uterine contractions. Clinics in perinatology. 2003; 30: 665 – 676.
11. MacIntyre DA, et al. Contraction in human myo-metrium is associated with changes in small heat shock proteins. Endocrinology. The Endocrine Society. 2007; 149(1): 245-252 676.
12. Oldenhof AD, et al. Mitogen-activation protein kinases mediate stretch-induced c-fos mRNA expression in myometrial smooth muscle cells; Am J Physiol. 2002; 283: C1530-C1539.
13. Sulistyono A. Conservative Surgical Management of Postpartum Hemorrhage (PPH) using 'Surabaya Methode' (Modified B-Lynch Compression Suture). Indonesian Journal of Obstetrics and Gynecology. 2010; Vol 34 No. 3 page: 108-113.
14. Webb RC. Smooth muscle contraction and relaxation. The American Physiological Society. 2003; 27(4): 201 – 206
15. Wray S. Insight into the uterus. Experimental Physiology. 2007; 92(4): 621 – 631