Research Article

Increased integrin and IKβ on the uterosacral ligament after childbirth

Rahajeng[1,2]*

ABSTRACT

Background: Uterine prolapse is one manifestation of pelvic organs prolapse that characterized by the decline of the uterus from its original position as a result of the weakness of the pelvic floor buffer, especially sacrouterine and cardinal ligament. **Objective:** The objective of this study was to evaluate the level of integrin and IKβ expression on uterosacral ligament in women after vaginal delivery. Design: This was a cross-sectional study. Setting: Biomedical Laboratory. **Patients and Methods:** This research was conducted by collecting samples of biopsy uterosacral ligament from primigravida who underwent cesarean section after obstructive labor at Stage 1 and Stage 2 using consecutive sampling technique. Uterosacral ligaments of primigravida a term that has not entered labor phase and underwent elective cesarean section surgery were taken as control. Examination of Integrin and IKβ expression by immunohistochemistry. Main Outcome and Measures: Level of integrin and IKβ expression. **Results:** There were significant differences; mean of integrin activity in pregnant women has not been in labor group (92.8 ± 2.4%) compared to women in labor (95.99 ± 3.98%). There were significant differences between means of IKβ activity in pregnant group of women who never in labor (77.55 ± 6.77%) and group of pregnant women in labor (4:12 ± 93.59%). **Conclusion:** There were increased levels of integrin and IKβ in the uterosacral ligament of primigravida women in labor. There are increased levels of integrin and IKβ in the uterosacral ligament of primigravida women who underwent cesarean section due to obstructive labor at Stage 1 and Stage 2. Limitation: This study was not evaluated the level of nuclear factor kappa beta.

KEY WORDS: IKβ, Integrin, MMP, Primigravida

INTRODUCTION

Pelvic organs prolapse is the most common manifestation of pelvic floor dysfunction, characterized by a condition where the pelvic organs such as uterus, vesica urinary, rectum, and vagina, down from its original position. Pelvic organ prolapse caused by weakness of the pelvic floor buffer.[1] The structure of pelvic floor is supported by fascia endopelvic (sacrouterine ligaments, cardinal ligaments, pubocervical fascia, and rectovaginal fascia) and the pelvic floor muscles (levator ani muscle). Uterine prolapse is one manifestation of pelvic organs prolapse that characterized by the decline of the uterus from its original position as a result of the weakness of the pelvic floor buffer, especially sacrouterine and cardinal ligament.[2,3] The prevalence of pelvic organ prolapse is about 7–23% and expected to rise with the increasing of women life expectancy.[4]

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

The cell receives signals from the physical environment through mechanotransduction mechanism. Mechanotransduction describes as a cellular process in translating mechanical force stimulus into biochemical signals. Mechanical strength will be responded by the cell, then converted into a biochemical signal to obtain cellular and molecular cascade.[5,6]

There are several mechanotransduction pathways that have been identified, namely integrins, ion channels, G-protein, and a growth factor. Intracellular signaling pathways activation involved in the maintenance and regulation of cell function that is interfered by mechanical forces. Mechanical load can be detected by mechanosensor membrane as well as activated ion channels, cell layer membrane, G-protein–coupled receptors, growth factor receptors, and integrins.

[1]Department of Obstetrics and Gynecology, Division of Urogynecology, Saiful Anwar Hospital of Malang, Jl. Jaksa Agung Suprapto 2A Malang, Indonesia, [2]Medical Faculty, Brawijaya University, Malang, Indonesia

***Corresponding author:** Dr. Rahajeng, Department of Obstetrics and Gynecology, Division of Urogynecology, Saiful Anwar Hospital of Malang, Indonesia, Medical Faculty, Brawijaya University, Malang, Indonesia. E-mail: jen_urogyn@yahoo.co.id

Received on: 21-02-2018; Revised on: 24-03-2018; Accepted on: 26-04-2018

The signaling pathway activated by integrin and related protein through mechanical stimulation is mitogen-activated protein kinase (MAPK) and NFkB pathway.[7] The initiation of MAPK pathway for cell adhesion mediated by integrin is important in various strained and torn cell types (heart cells, smooth muscles, and endothelial).[8,9] Another pathway activation due to mechanical stress is NFkB pathway, which is activated and translocated toward the nucleus.[10] Mechanical load is also known to trigger signaling through NFkB pathway. Members of the family MAP3K have been shown to activate the IKβ kinase (IKK), a complex that phosphorylates IKβ.[11] Therefore, this study aim is to evaluate the level of integrin and IKβ expression on uterosacral ligament in women after vaginal delivery.

METHODS

Ethical Clearance

This research was equipped with a feasibility study of ethics approval from Research Ethics Committee, Faculty of Medicine, University of Brawijaya.

Sample Preparation

This research was conducted by collecting samples in consecutive sampling from biopsy uterosacral ligament of pregnant women who undergo cesarean section due to obstructed labor on Stage 1 and Stage 2 labor. Samples were collected from uterosacral ligament of pregnant women who have not entered the phase of labor and undergoing elective cesarean section surgery were used as control. The method in this study was cross-sectional. In this study, integrin and IKβ phosphorylation examination was performed to see the activity of integrin and IKβ in the samples. The examination carried out after samples of uterosacral ligament were stained by immunohistochemistry will be compared whether there are differences between the sample and the control. Sampling was carried out in the delivery room at the Department of Obstetrics and Gynecology of Dr. Saiful Anwar Hospital Malang while examinations were conducted at the Biomedical Laboratory, Faculty of Medicine, University of Brawijaya.

The study population was healthy women aged 20–40 years. The samples were taken at the uterosacral ligament from the population according to the inclusion criteria and variables that have been determined. The sample is selected using purposive sampling technique of inclusion and exclusion criteria that have been determined.

Each sample group minimum consisted of 11 people. Uterosacral ligament samples were taken at operating room during cesarean section. Uterine was stretched to expose the uterosacral ligament. Uterosacral ligament then was held with tweezers and cut using Metzenbaum scissors to get ± 3 mm–5 cm samples. Samples were inserted into formalin containing tube that was labeled in accordance with the specimen. Samples stored at 20–25°C and then prepared for immune histochemical examination.

Protein Expression Analysis

Calculation procedure of integrin and IKβ expression: Examination conducted on each slide using a light microscope at ×400 magnifications. Each visual field then was shot 10 times. Imaging results in the form of files (.jpeg) uploaded to then processed through applications JPEG2000 ImmunoRatio virtual microscope slide (online application from the Institute of Biomedical Technology, Tampere, Finland). This application calculates the percentage of nuclear area that was positive smeared (labeling index) using algorithms to separate components of colored convolution outward appearance. Results obtained from the output in the form of presentation DAB smeared area of the total area of the nucleus. To ensure there presentation and reduce errors in the results analysis, observations were performed to approximately 10 field of view with ×400 magnification.

Statistical Analysis

The results of the data analysis have been tested using several normality tests: Normal probability plot analysis and the ratio of the value of the ratio of skewness and kurtosis. The results of normal probability plot analysis showed that all the data values of the activity of integrin and IKβ are around a diagonal line (green) and delivered two red lines, except for the activity integrin. Furthermore, the skewness and kurtosis ratio are lied between −2 and +2 either for both woman in labor/never in labor group, it can be concluded that the data were normally distributed and have met the prerequisites of parametric test. Furthermore, the data were analyzed with statistical parametric test to prove the research hypothesis that has been proposed. Before the sample data were analyzed using *t*-test (one side/one-tailed) mentioned above, the data were analyzed with the prerequisite of parametric test data normality using test normal probability plot and the value of the ratio of skewness and kurtosis ratio. The decision criteria when the normal probability plot observed values around a diagonal line (green) and no observed values are out of the red boundary lines, and the value of the ratio of skewness and kurtosis ratio is between −2 and +2, then concluded the data were normally distributed. Calculation for all data analysis was conducted using software tools (software) GENSTAT Procedure Library Edition Release 16.1Release PL24.1.

RESULTS

The Level of Integrin

The result shows that there were significant differences; mean of integrin activity in pregnant women has not been in labor group (92.8 ± 2.4%) compared to women in labor (95.99 ± 3.98%). When based on the mean value ± SD integrin activity seen in woman in labor groups larger when compared with a mean value ± SD integrin activity on the group of woman who never in labor. This means that there is significant increased integrin activity in the woman in labor [Figure 1].

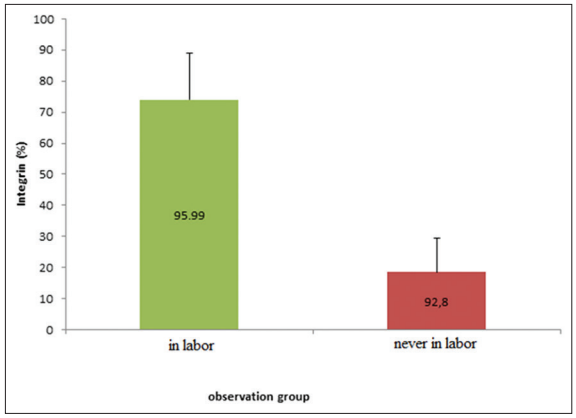

Figure 1: Comparison of integrin activity in pregnant women has not been in labor compared to women in labor (significant, $P < 0.001$)

The Level of IKβ

There were significant differences between means of IKβ activity in pregnant group of women who never in labor (77.55 ± 6.77%) and group of pregnant women in labor (4:12 ± 93.59%). It can be seen from mean ± standard deviation value of IKβ activity in the group of pregnant women in labor has greater compared to the group of pregnant women who never in labor. It means that there is an increase in activity IKβ on uterosacral ligament in labor [Figure 2].

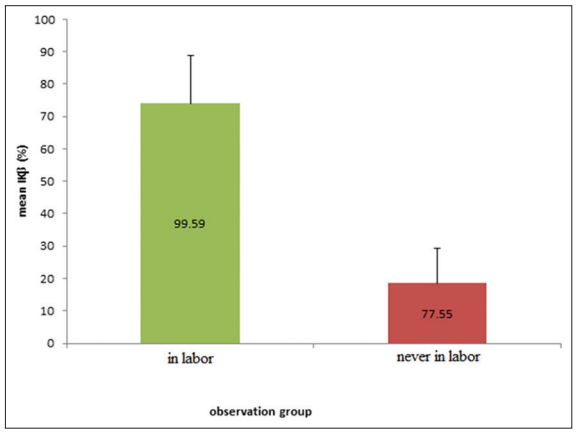

Figure 2: Comparison of IKβ activity of woman in labor compared to woman who never in labor (significant, $P < 0.001$)

DISCUSSION

The Level of Integrin

Integrins are one of the plasma membrane proteins that play an important role in cell adhesion and extracellular matrix. Integrins as transmembrane proteins on the cell surface bind to cytoskeleton participate in intercellular adhesion, hand in adhesion to the extracellular matrix. Ligand binding to integrin led to the formation of focal adhesion complex that has a role as a receptor activated for signals conduction.[11] Besides, as molecular glue that binds the extracellular matrix, integrins are also involved in conveying extracellular signals into the cell and regulate the cytoskeleton assembly. Integrin bounds to the intracellular domain of focal adhesion kinase (FAK) - protein signaling and actin filaments of the cytoskeleton. Integrins are transmembrane proteins that can penetrate the plasma membrane and bind to the extracellular matrix using its extracellular domain. Extracellular signaling mechanisms that are transferred into cells through the use of integrin cell adhesion. After extracellular matrix components are bound to integrin, signal then transduced into the intracellular domain of integrin, thereby activating FAK which is attached to the intracellular domain. As a result, the target protein in the intracellular signaling system is phosphorylated FAK, thus bypassing the extracellular signal to the intracellular signaling system. Then, the signal is transmitted into the nucleus to alter gene expression.[12]

Cells attached to the extracellular matrix through specific cell receptors. Integrins are transmembrane receptors that mediate adhesion between cells and the surrounding tissue as well as extracellular matrix. In signal transduction, integrin conveys information about the chemical composition and mechanical status of the cell into the extracellular matrix. In addition to transmitting mechanical strength across the membrane, integrin also is involved in cell signaling and regulation of cell cycle, cell shape, and motility of cells.[13]

Integrins are composed of alpha and beta chain subunit of heterodimers. Integrins that are not bound to the extracellular matrix ligands will be distributed free in the plasma membrane, but after the binding of extracellular matrix to integrin on the cell surface, it will induce the formation of plaque proteins in the cytoplasmic surface. Actin microfilaments plaque protein is connected to the cell cytoskeleton. Integrins and the cytoskeleton complex build the focal adhesions. Integrin is a mediator mechanical cytoskeleton in its association with the extracellular matrix. Integration between the cytoskeleton and extracellular matrix is important for both cells, where integrin used as sensors to compose the extracellular

matrix and align internal cytoskeleton. The resulting tension from communicated cell cytoskeleton and extracellular matrix through integrin are able to regulate and lay new fiber matrix on the damaged area. In this way, the network architecture can adapt to trauma extracellular matrix.[14]

Integrin as major transmembrane protein has strategic location that directly contact the extracellular matrix that makes integrins able to detect changes in pressure due to stretching on the cell surface and converts the mechanical signal into a chemical signal.[8] Previous study has been reported that mechanical stimulation of the extracellular matrix - integrins would trigger a signal that would cause cellular adaptive responses, such as there modeling of the extracellular matrix that regulates mechanical specificity for change as expected. Integrin acts as mechanotransducer when it triggers a signal after ligand binding to response the changes in the strength of its interactions with the extracellular matrix.[7]

Role of integrins in cell signaling has been known. Initial adhesion of integrin ligands leads to activation of extracellular matrix, grouping, and assembly of focal adhesion complex. It also serves as assembly signaling pathways for: Protein kinase (FAK, ILK, Src, and Fyn), adapter proteins (She, Grb-2, and Crk), and GTPase (Rho and Ras) and will trigger the protein MAPK directly and synergy with growth factor receptor.[7,15]

The Level of IKβ

Nuclear factor kappa beta (NFkβ) is important in regulating the cellular response because it has a fast response in the primary transcription. Activation of NFkβ is initiated by signals that degrade IKβ protein. This occurs primarily through the activation of the IKK. When activated by extracellular signals, IKβ kinase phosphorylates two serine residues located in the IKβ domain. When phosphorylated on serine, IKβ inhibitor molecule is modified by a process that makes IKβ ubiquitination followed by proteasome degradation. With the degradation of IKβ, NFkβ complex then freed to enter the nucleus where it can "switch on" the expression of a particular gene which has a DNA binding site NFkβ nearby. NFkβ activation of this gene will cause cell response.[2] NFkβ activation in response to mechanical stretching associated with the phosphorylation and degradation of IKβ and IKβ kinase activation. Mechanical stretching results in increased activation of ERK ½ and p38 MAPK.

The signaling pathway activated by integrin and related protein through mechanical stimulation is MAPK and NFkβ pathway.[7] Initiation of MAPK pathway for cell adhesion mediated by integrin is important in various strained and torn cell types (heart cells, smooth muscles, and endothelial).[8,9] Another pathway activation due to mechanical stress is NFkB pathway, which is activated and translocated toward the nucleus.[10] It has been proved occur in tear endothelial cells. NFkβ activation is also required for integrin ability of fibroblasts to contract the collagen gel. IKK complex seems to be activated by NIK and MEKK1, two enzymes of the MAP kinase kinase kinase (MAPKKK).[10] This shows that there is crosstalk between MAPK and NFkβ pathway in mechanotransduction signaling on connective tissue. In addition, obtained lane indirect among which due to strain release autocrine of growth factor (angiotensin II and or PDGF) through the smooth muscle cells resulting in the activation of several protein kinase isoenzyme that can affect MAPK or NFkβ pathways either directly or indirectly.[9]

Previous study illustrated that the strain that happened to bond the extracellular matrix and integrin is responsible to trigger the MAP kinase pathway (MAPKKK, MAPKK, and MAPK) through GTPase. MAPK translocate to the nucleus to activate transcription factors such as AP-1 or SRF. Mechanical load is also known to trigger signaling through NFkβ pathway. Members of the family MAPKKK have been reported to activate the IKK, a complex that phosphorylates Ikβ. Furthermore, NFkβ is released to the nucleus and binds to the promoter sequence of the target. In addition, there is indirect pathway in the regulation of gene expression through the release of growth factors that activate protein kinase C and MAPK pathway.[7]

Previous research has been done to prove that the mechanical strain can cause a rapid induction of the extracellular matrix components in fibroblasts. The composition of the extracellular matrix specifically to adapt to changes in mechanical load is given. Evidence has been found that Tenac Inc., a component of the extracellular matrix, is directly regulated by mechanical strain. In that study, integrin activation through MAPK/NFkβ pathway is involved in the trajectory of these changes.[7]

REFERENCES

1. Hughes, D. Pelvic Organ Prolaps. In: Shcorge JO, editor. Williams Gynecology. New York: Mc-Graw Hill Medica; 2010. p. 1066-70.
2. Goepel C. Differential elastin and tenascin imunolabelling in the uterosacral ligaments in postmenopausal women with and without pelvic organ prolaps. Acta Histochem 2007;110:204-9.
3. Jelovsek J, Maher C, BarberMD. Pelvic organ prolapse. Lancet 2007;369:1027-38.
4. Hunskaar S, Burgio K, Clark A, Lapitan MC, Nelson R, Sillen U, *et al*. Epidemiology of Urinary and Faecal Incontinence and

Pelvic Organ Prolaps. In: Abrams P, Cardozo L, Khoury S, Wein A, editors. Incontinence. Paris: Health Publication; 2005. p. 255-331.
5. Ingber DE. Mechanobiology and diseases of mechanotransduction. Ann Med 2003;35:564-77.
6. WuM P. Regulation of extracellular matrix remodeling associated with pelvic organ prolapse. J Exp Clin Med 2010;2:11-6.
7. Chiquet M, Flu¨ck M. Early Responses to Mechanical Stress: From Signals at the cell Surface to Altered Gene Expression. In: Storey KB, Storey JM, editors. Cellular and Molecular Responses to Stress. Vol. 2. Amsterdam: Elsevier Science; 2001. p. 97-110.
8. Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. Ann Rev Cell Dev Biol 1996;12:463-519.
9. Schmidt C, Pommerenke H, Durr F, Nebe B, Rychly J. Mechanical stressing of integrin receptors induces enhanced tyrosine phosphorylation of cytoskeletally anchored proteins. J Biol Chem 1998;273:5081-5.
10. Mercurio M, Manning AM. Multiple signals converging on NFkB. Curr Opin Cell Biol 1999;11:226-32.
11. Hynes RO. Integrins: Bidirectional allosteric signaling machines. Cell 2002;110:673-87.
12. Benjamin L. Cells. London: Jones & Bartlett Learning; 2007. p. 671.
13. Humphries MJ, Campbell LD. Integrin structure, activation, and interactions. Cold Spring Harb Perspect Biol 2011;3:a004994.
14. Davies PF, Barbee KA, Volin MV, Robotewskyi A, Chen J, Joseph L, *et al*. Spatial relationships in early signaling events of flow-mediated endothelial mechanotransduction. Ann Rev Physiol 1997;59:527-49.
15. Jaalouk DE, Lammerding J. Mechanotransduction gone awry. Nat Rev Mol Cell Biol 2009;10:63-74.

Source of support: Nil; Conflict of interest: None Declared