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Increased MAPK and NF- IKβ on Uterosacral Ligament after Childbirth

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Abstract

Objective: To evaluate the level of Integrin and IKβ expression on Uterosacral ligament in women after vaginal delivery.

Method: 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 by using consecutive sampling technique. Uterosacral ligaments of primigravida a term that have not entered labor phase and underwent elective Caesarean section surgery were taken as control. The method in this study was cross sectional. Examination of Integrin and IKβ expression by immuno histochemistry in this study, level of Integrin and IKβ activity between samples and control will be compared.

Result: There were increased levels of Integrin and IKβ in the Uterosacral ligament of primigravida women in labor

Conclusion: 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

Keywords: Integrin; IKβ; 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 endo pelvic (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]. Prevalence of pelvic organ prolapse is about 7-23%, and expected to rise with the increasing of women life expectancy [4]. The cell receives signals from the physical environment through mechanotransduction mechanism. Mechano transduction 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 are interfered by mechanical forces Mechanical load can be detected by Mechano sensor membrane as well as activated ion channels, cell layer membrane G-protein coupled receptors, growth factor receptors and integrins. The signaling pathway activated by Integrin and related protein through mechanical stimulation is MAPK and NF kB 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]. Pathway activation due to mechanical stress is NF kB pathway, which is activated and translocated towards the nucleus [10]. Mechanical load is also known to trigger signaling through NF kB pathway. Members of the family MAP3K has been shown to activate the I-kB Kinase (IKK), a complex that Phosphorylates I-kB [7]. Therefore, this study aim is to evaluate the level of Integrin and IKβ expression on Uterosacral ligament in women after vaginal delivery

Method

Ethical clearance

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

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 labors. Samples were collected from Uterosacral ligament of pregnant women who have not entered the phase of labor and undergoing elective Caesarean section surgery were used as control. The method in this study was cross sectional. In this study, Integrin and IKB phosphorylation examination was performed to see the activity of Integrin and IKB 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 of department Obstetrics and Gynecology of Dr. Saiful Anwar Hospital Malang while examinations were conducted at the Biomedical Laboratory, Faculty of Medicine, and University of Brawijaya.

The study population was healthy women aged 20 to 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 by 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 in order 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 20-25ºC and then prepared for immunohistochemical examination.

Protein expression analysis

Calculation procedure of Integrin and IKβ expression: Examination conducted on each slide using a light microscope at 400x magnifications. Each visual field then was shot 10 times. Imaging results in the form of files (.jpeg) uploaded to then processed through applications JPEG 2000 Immuno Ratio virtual microscope slide(online application from the Institute of Biomedical Technology Tampere Finland)This application calculates the percentage of nuclear area that were positive smeared (labeling index) by using algorithms to separate components of color deconvolution outward appearance. Results obtained from the output in the form of presentation DAB smeared area of the total area of the nucleus. To ensure the representation and reduce errors in the results analysis, observations were performed to approximately 10 field of view with 400x magnification.

Statistical Analysis

The results of the data analysis have been tested using several normality tests: Normal Probability Plot analysis, 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 laid between -2 and +2 either for both women in labor/never in labor group, it can be concluded that the data were normally distributed and has met the prerequisites of parametric test. Furthermore, the data were analyzed with statistical parametric test in order 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 test parametric test data normality by using test Normal Probability Plot, 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 [9] Calculation for all data analysis were conducted using software tools (software) GENSTAT Procedure Library Edition Release 16.1 Release PL24.1.

Results and Discussion

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).

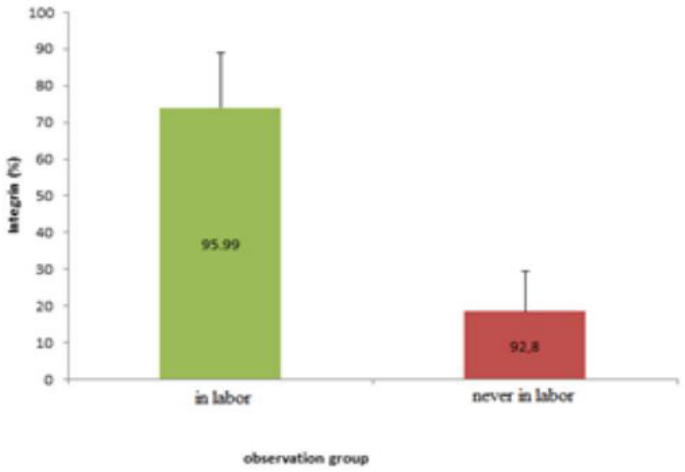

Figure 1: Compartion of MAPK expression of woman in labor compare to woman who is never in labor (significancy, p<0.001).

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 leds to 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 regulates the cytoskeleton assembly. Integrin bound to the intracellular domain of Focal Adhesion Kinase (FAK) - protein signaling and act in 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 is bound to Integrin, signal then transducer into the intracellular domain of Integrin, thereby activating focal adhesion kinase which is attached to the intracellular domain. As a result, the target protein in the intracellular signaling system is Phosphorylates 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 convey 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 ligand 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. Acting microfilaments plaque protein is connected to the cell cytoskeleton. Integrins and the cytoskeleton complex build the focal adhesions. Integrin role as mediator mechanical cytoskeleton due to 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 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].

Integrins as major transmembrane protein have strategic location that directly contacts the extracellular matrix. That makes integrins able to detect changes in pressure due to stretching on the cell surface and convert 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 the remodeling of the extracellular matrix that regulates mechanical specificity for change as expected. Integrin acts as mechanotranducer 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 lead 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, Fyn), adapter proteins (She, Grb-2, Crk) and GTPase (Rho, Ras) and will trigger the protein Mitogen-Activated Protein Kinase (MAPK) directly and synergy with growth factor receptor [7,15].

The level of IKβ

There were significant differences between mean 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 ± SD value of IKB activity in the group of pregnant women in labor has greater compared to the group of pregnant women who never in labor. It means there is an increase in activity IKB on Uterosacral ligament in labor (Figure 2).

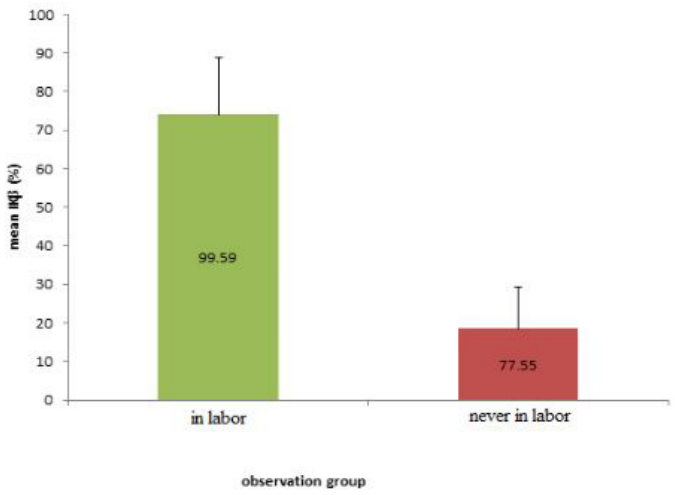

Figure 2: Comparison of IKβ activity of woman in labor compare to woman who never in labor (significancy, p<0.001).

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

The signaling pathway activated by Integrin and related protein through mechanical stimulation is MAPK and NF kβ 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 [8,16]. Pathway activation due to mechanical stress is NF kβ pathway, which is activated and translocated towards the nucleus [10]. It has been proved occur in tear endothelial cells. NF kβ activation is also required for Integrin ability of fibroblasts to contract the collagen gel. IKB Kinase Complex (IKK) seems to be activated by NIK and MEKK1, two enzymes of the MAP kinase (MAPKKK) [10]. This show there is crosstalk between MAPK and NF kβ pathway in mechanotransduction signaling on connective tissue. Additionally, 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 NF kβ 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 NF kβ pathway. Members of the family MAPKKK has been reported to activate the I-kβ Kinase (IKK), a complex that Phosphorylates I-kβ. Furthermore, NF kβ 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 (PKC) 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 is that menacing C, a component of the extracellular matrix, directly regulated by mechanical strain. In that study, Integrin activation through MAPK / NF kβ pathway is involved in the trajectory of these changes [7].

Conclusion

It can be concluded that there is increased of Integrin and IKβ levels in the Uterosacral ligaments in labor.

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