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The Differences Of Matrix Metalloproteinase 9 and Tissue Inhibitor Matrix Metalloproteinase 1 Expression Between Nullipara and Postpartum on Vaginal Wall Of Rattus Norvegicus

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ABSTRACT

The pathophysiology of how the labor process changes the molecular and biochemical changes of the pelvic floor, especially the vaginal wall, causing the female pelvic organ prolapse is not clear. This change is presumed through the mediation of changes in Matrix Metalloproteinase 9 (MMP-9) and Tissue Inhibitor Matrix Metalloproteinase 1 (TIMP-1). Therefore, this study aims to determine changes in MMP-9 and TIMP-1 in postpartum. This research is an observational cohort study on female rats Rattus norvegicus Wistar strain. Replication used was 5 individuals for each nulliparous group, postpartum day-1, day-3 and day-7. Selection of mice by using random allocation. The vaginal wall tissue slides were stained with immunohistochemistry. MMP-9 and TIMP-1 expressions were calculated and analyzed using Analysis of Variance (ANOVA). The results of this study indicate that there is a significant difference (p = 0.007) mean expression of MMP-9 per visual field on the vaginal wall among nullipara (11.2 ± 1.304), postpartum day-1 (13.2 ± 2,387), day- 3 (12.6 ± 2.191), and day-7 (8.0 ± 2,550). There was an increasing trend of MMP-9 expression on the 1st day postpartum, then MMP-9 gradually decreased until postpartum on the 7th day. The results in this study also showed that there was a significant difference (p = 0.028) in the mean of TIMP-1 expression on the vaginal wall between nulliparous rats (11.6 ± 1.342), postpartum day -1 (15.4 ± 3.130), day -3 (13.2 ± 2.683), and day-7 (10.8 ± 1.483). There was increased expression of TIMP-1 day-1 postpartum than nullipara, then decreased gradually until the 7th day postpartum.

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1. Introduction

Pelvic organ prolapse is a health problem in nearly half of women over 50 years of age (Barber and Maher, 2013). Of course in the future this number will grow rapidly and need the attention of gynecologists (Herschorn, 2004). Studies show that prolonged stretching of the vaginal wall can produce a contributing effect on the progressive deterioration of pelvic organ support (Drewes et al., 2007)

The synthesis and degradation of elastin and collagen in the vaginal wall plays an important role in the development of pelvic organ prolapse. The process of collagen and elastin degradation mainly depends on the activity of the Metaloproteinase Matrix (MMP) and its regulation (Ruiz-Zapata et al., 2016). Childbirth is a form of stress that activates MMP. Matrix metalloproteinase 9 (MMP-9) is the largest contributor in tissue remodeling. The work of the matrix metalloproteinase is limited by both exogenous and endogenous MMP inhibitors such as the tissue matrix metalloproteinase inhibitor (TIMP). TIMP-1 is an inhibitor that binds stoichiometry to almost all MMPs so that it irreversibly inhibits the action of MMP. Changes in TIMP-1 levels will of course greatly affect the expression of MMP-9 (Ruiz-Zapata et al., 2016).

The pathophysiology of how labor changes the molecular and biochemical changes of the pelvic floor, especially the vaginal wall, causing prolapse is not clear. There are no studies on the ratio of MMP and TIMP after delivery. Whether these changes in collagen and elastin are mediated by changes in MMP-9 and TIMP-1 which play an active role in the degradation of collagen and elastin is also unclear. It is hypothesized that there are changes in MMP-9 and TIMP-1 in postpartum. These changes underlie changes in collagen, elastin, and the occurrence of pelvic organ prolapse.

2. Method

The sample of this study was a female white rat, Rattus norvegicus, wistar strain. The rats were 8-10 weeks old, the rats weigh 120-150 gr. The sample used was 5 for each group. This study used an observational cohort study design with one control group and three observation groups. This study is a study to assess levels of MMP-9 and TIMP-1 in nulliparous, postpartum day 1, day 3 and day 7. The experiment site is in the Pharmacology Laboratory and Physiology Laboratory, Faculty of Medicine, Brawijaya University Malang. After delivery, the mice were sedated with ketalar ip and underwent surgery to remove the vaginal wall tissue. The vaginal wall is then subjected to embedding, cutting, and immunohistochemical staining to observe the number of MMP-9 and TIMP-1 expressions.

Embedding. The vaginal wall was immersed in a fixative solution in the form of formalin or PFA (1-7 days), then immersed in 70% ethanol for at least 24 hours and followed by 80% ethanol for 2 hours. Then immersed in 90% and 95% ethanol respectively for 30 minutes each. Then soaked in xylol 2 times for 30 minutes each. Then proceed with dipping the vaginal wall in liquid paraffin. The preparation on the paraffin block is inserted in the microtome block holder. The slices are cut into 5 micrometers.

Permeabilization of Cells with PBS. Incubate cells at 1% BSA (Bovine Serum Albumin) for 1 hour at room temperature.

Cell incubation on MMP-9 antibody and TIMP-1 antibody was followed by cell incubation on secondary antibodies (gout-anti rabbit IgG biotin labeled). Primary antibodies MMP-9 and TIMP-1 Mouse Monoclonal IgG 200 µg / ml (Santa Cruz

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Biotechnology). Counterstain with Mayer's hematoxilen for 10 minutes. The expression of MMP-9 and TIMP-1 that can be observed on the preparation is the presence of a brown color notation. To ensure representation and reduce error in results, it is necessary to observe a total of 20 fields of view with a magnification of 400 times, each containing approximately 1500 cells.

Oneway ANOVA analysis. The level of significance chosen was = 0.05 or it also means that the 95% confidence interval was chosen.

3. Results and Analysis

3.1 Vaginal Expression of MMP-9

The following is a Fig of MMP-9 expression in the vagina:

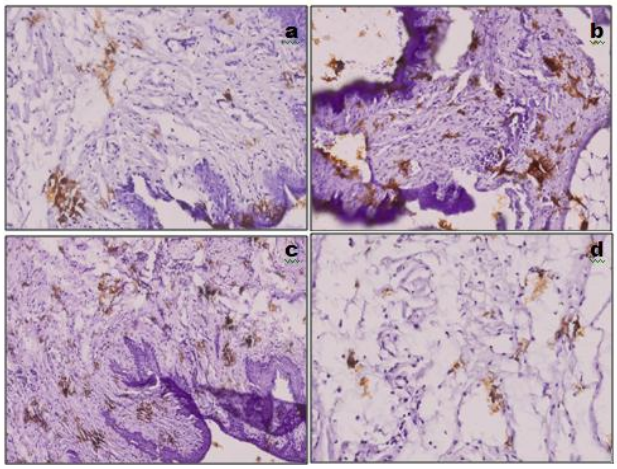

Fig. 1. Fig. Expression of MMP-9 in the Vagina

Note: MMP-9 expression is marked with dots with thick indents. There appears to be a trend of increasing mean vaginal MMP-9 expression at postpartum day 1 followed by a significant decrease in MMP-9 expression on day 7 postpartum. a) Control, b) post delivery on day 1, c) post delivery on day 3, d) post delivery on day 7.

Data analysis was performed with the one-way Anova test to compare vaginal MMP-9 levels between the control group, the 1st, 3rd and 7th day postpartum groups. The results of the analysis are shown in table 1 below:

TABLE 1

ANOVA TEST RESULTS OF METALLOPROTEINASE 9 MATRIX LEVELS IN THE VAGINA

Variable	MMP-9 Mean ± SD (Per Field of View)	*p-value*
Control	11.2 ± 1.304	
Day 1 Postpartum	13.2 ± 2.387	0.007
Day 3 Postpartum	12.6 ± 2.191	
7th day after delivery	8.0 ± 2,550	

Information: p-value> 0.05, there is no significant difference

Table. 1. indicates that there is a significant difference (p = 0.00 $<^{\propto}$ vaginal MMP-9 expression between the control group (11.2 ± 1.304), the 1 day postpartum group (13.2 ± 2.387), the 3 day postpartum group (12.6 ± 2.191) and the day 1 group -7 postpartum (8.0 ± 2,550). This means that labor greatly increased vaginal MMP-9 expression in female rats until it peaked on the first postpartum day. However, the increase in MMP-9 gradually decreased until it was lower than when it was not pregnant on the 7th postpartum day.

The expression of MMP-9 in the vaginal tissues is very important. This is because MMP-9 regulates vaginal regulation of collagen types I and III and elastin. Type I collagen is stronger and increases the tensile strength for pelvic floor support. Collagen type III in the vagina is very important for fetal accommodation during delivery. The connective tissue of the vagina is composed of type I and type III collagen in equal proportions. When this proportion changes and the amount of type III collagen increases compared to type I, it will cause pelvic organ prolapse(Van Doren, 2015). Therefore, the strategic role of MMP-9 indicates the need to develop MMP-9 expression engineering technology in the future for the regulation of pelvic floor support.

The increase in MMP-9 in postpartum is due to the fact that the delivery process stimulates stretching of the birth canal. In vaginal delivery, when the baby's head enters the pelvis, the largest part of the baby's head will stretch the muscles of the pelvic floor, ligaments, vagina and existing connective tissue so that it is known as crowning of the baby head.(Dietz and Wilson, 2005). This process of stretching the baby's head allows stress to stretch the pelvic floor tissue. These strain stimuli are then received by the extracellular matrix (MES) and affect TGFβ. These stimuli will be delivered to fibroblast cells through the role of integrins and fibronectins. This mechanical stimulation will signal the fibroblasts to differentiate and signal the transduction pathway for upregulation of MMP-9 and TIMP-1.(Jin et al., 2000).

Various studies describe MMP-9 transduction pathways (multisignal pathways) due to stretching(Wang et al., 2017). These pathways include intracell signal transduction, signal transduction involving the MAPK pathway, the JNk / SAPK pathway, as well as through cytokine and interleukin signals such as: EGF (Epidermal growth factor), NGF (Nerve growth factor), VEGF (Vascular endothelial growth factor), PDGF (Platelet derived growth factor), TNF-α (Tumor necrotizing factor-

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α), TGF-β (Transforming growth factor-β), ROS (Reactive oxygen species)(Ahmad et al., 2014).

The role of the vagina is very important in producing longitudinal support and central pelvic floor support(Wah and Hi, 2017). In addition, disruption of support in the Levator Ani muscle will cause the vagina to replace the support so that pelvic organ prolapse does not occur(DeLancey, 2016).

This study also showed that the increase in postpartum MMP-9 expression peaked at postpartum day 1, then gradually decreased until postpartum day 7. This is due to the role of a tissue inhibitor (TIMP-1) which increases after 24 hours postpartum. This increase in TIMP-1 after 24 hours postpartum aims to prevent excessive degradation by protease enzymes, including MMP-9.(Nissi, Santala and Talvensaari-Mattila, 2021). The presence of TIMP control causes the degradation of the extra cellular matrix by MMP-9 after 24 hours postpartum to not be massive and causes a pelvic floor support defect.

3.2 Vaginal Expression of TIMP-1

The following is a Fig of TIMP-1 expression in the vagina:

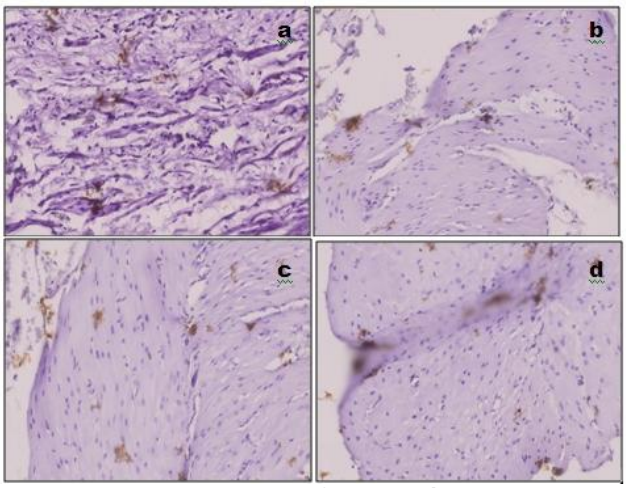

Fig. 2. Fig. Expression of TIMP-1 in the Vagina

Note: TIMP-1 expression is marked with a thick indented dot. There was an increasing trend in the mean TIMP-1 expression in the vagina after delivery on the 1st day, which was then followed by a decrease on the 3rd and 7th day after delivery. a) Control, b) post delivery on day 1, c) post delivery on day 3, d) post delivery on day 7.

Comparative test of vaginal TIMP-1 levels between the control group, the 1 postpartum day group, the 3rd postpartum day and 7th day postpartum day with the one way Anova test in detail and completely is shown in table 2 below:

TABLE 2

ANOVA TEST RESULTS OF TISSUE INHIBITOR LEVELS IN THE VAGINAL METALLOPROTEINASE 1 MATRIX

Variable	TIMP-1 Vagina Mean ± SD	*p-value*
Control		
Day 1 Postpartum	11.6 ± 1,342	
Day 3 Postpartum	15.4 ± 3,130	0.028
7th day after delivery	13.2 ± 2.683	
	10.8 ± 1.483	

Information: p-value> 0.05 there is no significant difference, p-value <0.05 there is a significant difference.

Table. 2. indicates that there is a significant difference (p = 0.00 <αTIMP-1 vaginal expression between the control group (11.6 ± 1.342), the 1st postpartum group (15.4 ± 3.130), the 3rd postpartum group (13.2 ± 2.683) and the day-1 group -7 postpartum (10.8 ± 1.483). This means that there was a significant increase in TIMP-1 on the 1st postpartum day, but then decreased significantly on the 3rd to 7th day postpartum.

This study shows that the expression of TIMP-1 in the vagina increases and reaches a peak at postpartum day 1. This is a perfectly natural control mechanism, as increasing TIMP-1 after 24 hours postpartum will prevent excessive degradation by MMP-9. This also explains that at postpartum there will not necessarily be a pelvic floor support defect if the MMP-9 and TIMP-1 controls are balanced. The increase in TIMP-1 which has reached its peak at postpartum day 1 aims to prevent excessive degradation of the vaginal wall so that it is hoped that the homeostasis of elastin tissue in the vaginal wall can be maintained.(Nissi, Santala and Talvensaari-Mattila, 2021). The increase in MMP-9 in the vagina is not massive and must be immediately controlled by an increase in TIMP-1 because the increase in MMP-9 in the vagina only functions as a remodeling of the vaginal connective tissue to turn into a more relaxed and accommodating connective tissue for the next labor(Geng, Huang and Jiang, 2016).

4. Conclusion

There were differences in vaginal expression of MMP-9 and TIMP-1 between nulliparous and postpartum rats. This study showed that labor alters the expression of the protease enzyme MMP-9 and its inhibitor TIMP-1 in pelvic floor tissues such as the vaginal wall and that these changes are time-dependent.

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