Profile of matrix metalloproteinase activity, markers of collagen and elastin degradation and remodeling during pregnancy, delivery, and puerperium in pelvic organ prolapse 3 months after childbirth

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ABSTRACT

Background: This study aims to assess the condition of pelvic organ prolapse related to changes in matrix metalloproteinase activity, elastin degradation and markers of collagen, and remodeling observed during pregnancy, childbirth, and the puerperal period 3 months postpartum. This study included 39 primigravida women who underwent vaginal delivery with a gestational age >36 weeks. This study conducted laboratory tests using blood and urine samples including procollagen type I N-propeptide (PINP), procollagen type-III N-propeptide (PPIINP), telopeptide carboxyl-terminal collagen type I (ICTP), matrix metalloproteinase-9 (MMP)-9), desmosin, and tropoelastin. The second and third blood samples will be examined 24-48 hours postpartum and 6 weeks postpartum.

Method: The Mann-Whitney test and Repeated ANOVA were used to assess the median differences between biomarkers.

Results: In the pelvic floor dysfunction and control groups, a significant relationship was found between serum MMP-9 levels 6 weeks postpartum (p=0.025). There were changes in biomarker levels of collagen and elastin during pregnancy, post-partum, and six weeks postpartum. The amount of degradation (MMP-9) seems to be correlated with the event of POP after childbirth.

Conclusion: There were changes in the biomarker levels of collagen and elastin from pregnancy, post-partum, and six weeks postpartum. In addition, the difference was significant in MMP-9 level six weeks after postpartum in POP group compare to control.

Keywords: collagen, elastin, MMP-9, pelvic organ prolapse.


INTRODUCTION

Pelvic organ prolapse (POP) is a condition that has a negative effect on the quality of life in millions of women. POP has a multifactorial etiology. The most influential factors in the process of pelvic organ prolapse include pregnancy, labor, and delivery. However, the failure of pelvic organ support caused by pregnancy and childbirth is unknown. Hormonal adaptation of pregnant women which is the cause of changes in the structure of the pelvic floor to give birth by the mechanical forces of the gravid uterus and fetus which results in downward pressure on the uterus is a hypothesis that pregnancy can cause pelvic floor dysfunction. On the other hand, childbirth also causes stretch injury to the vaginal fibroelastic tissue and pelvic floor muscles. Proteases in the vaginal wall and supporting tissue fibers are activated and cause degradation of fibroelastics such as elastin and collagen due to injury due to stretching of the pelvic floor and vaginal muscles during delivery.

The pelvic floor then undergoes a healing process after giving birth to regain its strength and the pelvic organs to function properly and keep in place. During both pregnancy, childbirth, and the puerperium, the specific processes regarding protease regulation and remodeling of elastin and collagen are not well defined especially in humans, and little information is known about these processes.

Recent results have shown that burst of elastic fiber assembly and cross-linking have occurred in the vaginal wall after delivery. After vaginal delivery, the synthesis and assembly of elastic fibers are important factors in the recovery of pelvic organ support. We suggest that degradation of the extracellular matrix during vaginal wall delivery and remodeling processes may fail to restore pelvic floor support function after delivery if reliant on an relationship between the failure synthesis of elastin and collagen fibers in pelvic organ prolapse, thereby...
causing pelvic organ prolapse. Although many studies have been conducted in experimental animals such as mice, the mechanism of pelvic organ prolapse may be unique to women.

This study aims to observe matrix metalloproteinase activity changes, collagen and elastin degradation markers and remodeling during pregnancy, delivery, and puerperium in pelvic organ prolapse 3 months after childbirth.

**RESEARCH DESIGN AND METHODS**

This research was conducted from January 2015 to July 2019 at National Referral Hospital in Jakarta. This study design was nested case-control. The research samples were patients who meet the inclusion criteria. They were approached and examined before delivery. At the time of enrollment, they were asked regarding their pregnancy history and Questionnaire for Urinary Incontinence Diagnosis (QUID). They were also asked about the symptoms of Urinary Incontinence and if bulging was present. In addition, they underwent Pelvic Floor Ultrasonography at the time of enrollment. Women with a history of pelvic surgery, malignancy, pre-pregnancy pelvic floor dysfunction, coronary heart disease, diabetes mellitus, chronic pulmonary disease, childbirth-associated urinary tract infection, levator ani avulsion, and severe malnutrition were excluded from the study. If the patients were not able to be followed up 12 weeks after delivery, the patient completes delivery by cesarean section, and experiences severe delivery complications such as sepsis and postpartum hemorrhage, then the patient will be considered as dropping out.

Pregnant women who agreed will be asked for their consent to participate in the study. Blood and urine samples for laboratory examination included procollagen type I N-propeptide (PINP), procollagen type-III N-propeptide (PIIINP), telopeptide carboxy-terminal collagen type I (ICTP), matrix metalloproteinase-9 (MMP-9), desmosine, and tropoelastin will be collected after examination at 3 months postpartum. The data were then analyzed using the SPSS 20 program. Repeated Mann-Whitney tests and ANOVA were performed to determine differences in serum biomarker measurements in pelvic floor dysfunction and non-POP groups during pregnancy, postpartum, and six weeks postpartum.

Comparison of these variables in the cystocele group would also be carried out with this analysis. The difference between generalized dysfunction and a cystocele were put separately to determine which compartment is most affected in vaginal delivery.

The Health Research Ethics Committee of the local institution approved and gave ethical approval to this study (ethical clearance register number: 179/UN.2/F1/ETIK/2015). Patients who have agreed to participate in this study would fill out a written informed consent that was collected together with the data obtained.

**RESULTS**

Table 1 shows the patient demographic data. Meanwhile, Table 2 shows the median values for markers of degradation and synthesis of collagen, elastin, and serum MMP-9 enzymes obtained during pregnancy, after delivery, and 6 weeks postpartum. The results showed that the median difference was a decrease in MMP-9 levels at six weeks postpartum and a quite large difference in serum MMP-9 levels and ICTP levels between pregnancy and 24-48 hours after delivery.

The assessment of degradation and synthesis of collagen I, III, levels of MMP-9, and elastin during pregnancy, postpartum and 6 weeks postpartum using the repeated ANOVA test (Table 2)

MMP-9 and ICTP as degradation markers of collagen type I are increase after delivery and decrease at 6 weeks, PINP decrease after delivery, while PIIINP level relatively the same 24-48 hours after delivery compared to pregnancy level, but decrease significantly at 6 weeks. Tropoelastin level increase after delivery and decrease after 6 weeks.

On bivariate analysis of serum markers with POP and cystocele as dependent variable only MMP-9 at 6 weeks has a significant correlation.

In addition, the changes in biomarker levels between groups were identified and presented in graphical form. Figure 1 presents a trend of changes in biomarker levels during pregnancy, childbirth, and 6 weeks postpartum between the pelvic floor dysfunction group, the cystocele group, and the control group.

**DISCUSSION**

The high level of PINP and PIIINP during pregnancy could be due to the quick increase of size and weight of uterus and the accumulation of collagen in pelvic floor for preparation to accommodate stretch during delivery. From the end of the first trimester through pregnancy, in the uterus the collagen content increases 8 to 10 folds. In humans, the uterus increases approximately 11 times to accommodate fetal growth. Our result was similar to that of Shynlova et al., who assessed increased collagen type I and III levels during pregnancy. At this stage, part of the collagen fiber is degraded due to myometrial remodeling that occurred which made an increasing of extracellular matrix synthesis.

In addition, higher desmosine and lower tropoelastin level during late pregnancy in the POP compared to the non-POP group might be one factor contributing to the low capacity of the pelvic floor tissue to recoil to its original length after stretch during delivery that can cause postpartum POP.

In post-partum period, MMP-9 levels increased in all groups at 24-48 hours after delivery. Similar results were also obtained in a study by Cecilia et al, which showed...
**Table 1. The Patients’ Demographic Data.**

<table>
<thead>
<tr>
<th>Demographic Data</th>
<th>POP (N = 22)</th>
<th>Non-POP (N = 17)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>24</td>
<td>24</td>
<td>0.819</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.25</td>
<td>60</td>
<td>0.989</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.565</td>
<td>1.55</td>
<td>0.820</td>
</tr>
<tr>
<td>BMR (kg/m²)</td>
<td>25.64</td>
<td>25</td>
<td>0.921</td>
</tr>
<tr>
<td>Newborn weight (g)</td>
<td>2900</td>
<td>3100</td>
<td>0.274</td>
</tr>
</tbody>
</table>

*Mann Whitney rank test; p < 0.05 is statistically significant

**Table 2. Differences in median serum markers levels of synthesis and degradation of elastin, collagen, and MMP-9 during pregnancy, after delivery, and 6 weeks postpartum.**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Pregnancy</th>
<th>After Delivery</th>
<th>Six Weeks Post-Partum</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICTP</td>
<td>62.94</td>
<td>123.75</td>
<td>105.82</td>
<td>0.000</td>
</tr>
<tr>
<td>PINP</td>
<td>128.64</td>
<td>107.35</td>
<td>104.44</td>
<td>0.002</td>
</tr>
<tr>
<td>PIIINP</td>
<td>262.12</td>
<td>267.96</td>
<td>175.89</td>
<td>0.007</td>
</tr>
<tr>
<td>Desmosin</td>
<td>0.98</td>
<td>1.12</td>
<td>1.14</td>
<td>0.421</td>
</tr>
<tr>
<td>MMP9</td>
<td>580.3</td>
<td>1056.8</td>
<td>418.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Tropoelastin</td>
<td>0.83</td>
<td>0.97</td>
<td>0.71</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Repeated ANOVA test; p < 0.05 is statistically significant.

**Figure 1.** Biomarker levels between pelvic floor dysfunction, cystocele, and control group in pregnancy, after delivery, and 6 weeks postpartum. (A) ICTP, (B) PINP, (C) PIIINP, (D) desmosine, (E) MMP-9, (F) tropoelastin.

that 24-48 hours after delivery, there was a 10-fold increase in MMP-9 mRNA activity. MMP-9 is a protease enzyme that has function to degrade elastin and collagen. Degradation of collagen and elastin tissue is triggered by stretching of the vaginal walls during labor. The activity of protease enzymes mediates this condition. It increases the degradation
Table 3. Differences in median serum markers levels of synthesis and degradation of elastin, collagen, and MMP-9 in POP and Non-POP Group during pregnancy, after delivery, and 6 weeks postpartum.

| Biomarker | Pregnancy |  | After Delivery |  |  | Six Weeks Post-Partum |  |
|-----------|-----------|----------------|----------------|----------------|---------------------|----------------|
|           | POP (n=22) | Non-POP (n=17) | POP (n=22) | Non-POP (n=17) | POP (n=22) | Non-POP (n=17) | POP (n=22) | Non-POP (n=17) |
| ICTP      | 61.015 | 226.33 | 62.94 | 5.82, 293.09 | 0.671 | 113.31 | 12.99, 350.43 | 0.269 | 96.11 | 1.59, 231.45 |
| PINP      | 274.71 | 711.24 | 104.44 | 15.83, 71.21 | 0.269 | 283.465 | 7.5, 625.1 | 0.183 | 223.88 | 5.02, 663.75 |
| PIIINP    | 307.05 | 706.78 | 217.1 | 80.13, 531.13 | 0.336 | 261.38 | 50.19, 685.9 | 0.865 | 201.41 | 34.61, 557.71 |
| Desmosin  | 1.3 | 0.41, 22.04 | 0.97 | 0.37, 8.5 | 0.197 | 1.615 | 0.51, 1.08 | 0.116 | 1.51 | 0.31, 27.93 |
| MMP9      | 553.95 | 147.9, 1898.1 | 591.6 | 268.4, 889.5 | 0.843 | 1089.65 | 231.6, 1964 | 0.365 | 352.5 | 57.9, 1124.1 |
| Tropoelastin | 8.965 | 0.12, 18.15 | 0.72 | 0.1, 18.22 | 0.165 | 4.8 | 0.15, 17.35 | 0.097 | 0.685 | 0.11, 5.95 |

*p-Mann Whitney test; p < 0.05 is statistically significant

Table 4. Differences in median serum markers levels of synthesis and degradation of elastin, collagen, and MMP-9 in Cystocele and Control Group during pregnancy, after delivery, and 6 weeks postpartum.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cystocele (n=12)</th>
<th>Non-Cystocele (n=17)</th>
<th></th>
<th>After Delivery</th>
<th></th>
<th></th>
<th>Six Weeks Post-Partum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POP (n=22)</td>
<td>Non-POP (n=17)</td>
<td>POP (n=22)</td>
<td>Non-POP (n=17)</td>
<td>POP (n=22)</td>
<td>Non-POP (n=17)</td>
<td>POP (n=22)</td>
<td>Non-POP (n=17)</td>
</tr>
<tr>
<td>ICTP</td>
<td>40.03</td>
<td>158.32</td>
<td>62.94</td>
<td>5.82, 293.09</td>
<td>0.232</td>
<td>73.61</td>
<td>12.99, 236.2</td>
<td>0.042</td>
</tr>
<tr>
<td>PINP</td>
<td>309.16</td>
<td>674.92</td>
<td>104.44</td>
<td>15.83, 712.1</td>
<td>0.132</td>
<td>283.465</td>
<td>7.5, 625.1</td>
<td>0.057</td>
</tr>
<tr>
<td>PIIINP</td>
<td>328.89</td>
<td>706.78</td>
<td>217.1</td>
<td>80.13, 531.13</td>
<td>0.101</td>
<td>301.27</td>
<td>68.4, 685.9</td>
<td>0.376</td>
</tr>
<tr>
<td>Desmosin</td>
<td>1.395</td>
<td>22.04</td>
<td>0.97</td>
<td>0.37, 8.5</td>
<td>0.121</td>
<td>1.38</td>
<td>0.62, 1.08</td>
<td>0.241</td>
</tr>
<tr>
<td>MMP9</td>
<td>560.7</td>
<td>1898.1</td>
<td>591.6</td>
<td>268.4, 889.5</td>
<td>0.929</td>
<td>1084.7</td>
<td>468.3, 1907.1</td>
<td>0.425</td>
</tr>
<tr>
<td>Tropoelastin</td>
<td>12.27</td>
<td>0.12, 18.15</td>
<td>0.72</td>
<td>0.1, 18.22</td>
<td>0.116</td>
<td>9.41</td>
<td>0.18, 17.35</td>
<td>0.177</td>
</tr>
</tbody>
</table>

*p-Mann Whitney test; p < 0.05 is statistically significant
of collagen, the main component of the vaginal wall, which can weaken the pelvic organ support systems. This statement is following the study of Drewes et al., which showed that 48 hours to 7 days after delivery, there will be increased levels of desmosin (a marker for the degradation of mature elastin fibers) in the vaginal tissues.

The ability and distensibility of the tissue to gain to its initial length before stretching is regulated by elastin. In this study we found tropoelastin during pregnancy is higher than 6 weeks postpartum and increases 24-48 hours after delivery. This result is the same with study by Soderberg et al. They found an increase in elastin synthesis in the last trimester of pregnancy which may facilitate labor by causing increased distensibility of the pelvic floor. Increased synthesis of tropoelastin and fibulin-5 after delivery is necessary to repair damaged elastin fibers due to regeneration of elastic fibers in vaginal tissues and vaginal delivery. The occurrence of POP after delivery can be caused by disruption of elastin homeostasis in vaginal tissue after delivery as one of the factors.

Postpartum PINP was higher in POP than non-POP group, although the differences was not statistically significant. This result is similar to some study shows that postpartum POP vagina had the lower type 3 collagen and higher ratio of collagen I/III and stiffer than control. In our study, desmosin levels did not differ significantly in the POP group compared to the non-POP group at 24 – 48 hours postpartum. This is similar to the study by Jameson et al which was conducted in a postnatal rat model. They demonstrated that altered elastin structure and low elastin degradation are associated with POP after delivery due to the accumulation of damaged elastin fibers. Jameson concluded that an inability to process the molecular mechanisms necessary to destroy damaged elastin fibers in the pelvic floor tissue resulting from childbirth causes POP after delivery.

The PIIINP and tropoelastin level decreased compared with pregnancy levels, however, the level was still higher in POP than in the non-POP group although the difference was not statistically significant. PINP level was lower in the non POP compare to POP group, it shows that after delivery there was an increase synthesis of collagen type 1 as a remodeling effort after injury.

The biomarker profile in the form of a predominance of collagen and elastin degradation markers is shown at 24-48 hours postpartum. This explains the effect of labor on the degradation of elastin and collagen. The increase in MMP-9 in the POP group was significantly higher than in the non-POP group. The condition may be happened because the process of degradation of elastin and collagen in the POP group is higher.

PINP levels in the POP group were higher than those in the non-POP group six weeks postpartum, although there was no statistically significant difference. During this time there was a decrease in PINP, PIIINP, and tropoelastin levels compared to 24-48 hours postpartum. When six weeks postpartum, the healing process due to injuries during childbirth can occur. Compared to collagen type III, collagen type I is less elastic but stronger. This study is in line with Zhou L et al’s research regarding the relationship between POP and PINP. They analyzed vaginal wall biopsies and vaginal stiffness to assess collagen I and III densities in patients with both premenopausal and menopausal who experienced POP. The analysis showed that the vagina of patients with POP was stiffer and had lower collagen III density than the non-POP group. Study by Zhou et al. also in line with several other studies showing an increased ratio of collagen I/III and a stiffer vagina found in patients with POP than controls. Collagen fibers in patients with POP are also wider and thicker with a more irregular pattern when seen from histopathological examination.

The non-POP group had higher levels of MMP-9 and ICTP than the POP group, which might indicate failed in the elimination process of damage collagen I from the pelvic floor that persist at 6 weeks postpartum. The result is accordance with study by Ruiz zapatta et al, they found that vaginal tissue of POP patient is stiffer than non POP apparently due to failure of protease enzyme to degrade collagen fiber during remodeling process of wound healing.

The role of collagen and elastin in the occurrence of pelvic floor dysfunction after delivery can be study using serum markers, serum metabolite products of collagen and elastin were used to represent collagen and elastin levels in the supporting tissues of the pelvic floor.

This study aims to look at all markers of elastin and collagen degradation and remodeling using a prospective cohort design followed by a nested case control study. This study has several limitations despite having strict inclusion and exclusion criteria. First, the sample’s size of this study is small. Second, the best time for sampling to get a better description of the processes that occur is not yet known. Third, because of ethical considerations, vaginal tissue biopsies were not performed to determine local tissue conditions and were not compared with systemic markers. Fourth, this study did not have a control group, non-pregnant women and no prenatal POP-Q measurements. Therefore, we suggest conducting further research with a larger sample size. In addition, to assess the remodeling process, blood can be taken 3 days to 2 weeks after delivery, while the second blood draw is done 24-48 hours only to see the magnitude of the degradation process.

CONCLUSION

There were changes in the biomarker levels of collagen and elastin from pregnancy, post-partum, and six weeks postpartum. In addition, the difference of MMP-9 level six weeks after postpartum was significant in POP group compare to control.

DISCLOSURE

Author Contribution
All authors have contributed similarly to this research process, including conception and design, data collection, data analysis and interpretation of the data, critical revision of the article for important intellectual content, and final approval.

Conflict of Interest
The author reports no conflicts of interest in this work.
Ethical Approval
This study was approved and given ethical acceptance by the Health Research Ethics Committee of the local institution (ethical clearance register number: 179/UN.2F1/ETIK/2015).

Consent for Publication
The patient’s informed consent was obtained directly. Written consent to participate in the study as well as presentation of data obtained from patients who had agreed to participate in this study were collected and received.

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REFERENCES