Effect of *Escherichia Coli* on Decorin and Type I Collagen Levels in Fetal Membranes of Premature Balb/c Mice

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**ABSTRACT**

Introduction: Premature birth is a global problem in various countries worldwide. The pathogenesis leading to preterm labor may occur in early pregnancy and is associated with inflammatory pathways and changes in the angiogenic process. Decorin and biglycan are hypothesized to play a role in pregnancy maintenance, wherein preterm delivery, these levels are thought to be reduced. This study was examined effect of *Escherichia coli* on Decorin and Type I Collagen levels in fetal membranes of Premature Balb/c Mice.

Methods: There were 28 pregnant Balb/c mice divided into 4 groups, with a post-test-only control group design in true experimental design research. The first control group (N1) was a group of pregnant mice dissected on day-15, while the second control group (N2) were mice pregnant at term followed up to parturition. Group P1 were given *Escherichia coli* 1x10^8 CFU/ml dose on the cervix on day-15, while the second treatment group (P2) were given *Escherichia coli* in the cervical canal on day-15 followed by delivery. We examined the Decorin and Type I Collagen levels between group. One-way ANOVA was used to analyze the collected data.

Results: There was a significant difference in the mean decorin levels between the control group N1 vs P2 (12.35±2.24 ng/mL vs 6.62±1.50 ng/mL; p=0.000). In addition, a significant difference was also found in the mean level of type 1 collagen between the control group N1 vs P2 group (283.5±31.3 ng/mL vs 170.6±38.8 ng/mL; p=0.000).

Conclusion: A decrease in decorin levels affects the reduction in collagen type 1 levels in fetal membranes of Balb/C mice in premature models.

**INTRODUCTION**

Premature birth is defined by the World Health Organization (WHO) as a birth occurring before 37 weeks of pregnancy or within 259 days of a woman's last menstrual period. Preterm birth affects 5 to 18 percent of all deliveries worldwide, with the prevalence rising during the previous three decades in affluent countries. Preterm deliveries are expected to occur 15 million times per year, with 1.1 million newborns dying due to complications. The research results in Indonesia, Riskesdas, 2013 showed that from 48,336 births during the period from January 2010-June 2013 there were 17,576 premature deliveries (36.4%). The pathophysiology of preterm labor is grouped into four mechanisms. First is activating the maternal-fetal hypothalamus-pituitary-adrenal (HPA) triggered by stress. Second, inflammation and infection are associated with the pathogenesis of preterm labor and pregnancy complications. The third is the occurrence of placental bleeding that can cause myometrial contractions, and the last is excessive uterine stretching which can be caused by multiple pregnancies and polyhydramnios [1–3].

The pathogenesis leading to preterm labor may occur in early pregnancy and is associated with inflammatory pathways and changes in the angiogenic process. Angiogenesis has been linked to collagen types...
I, III, IV, and VI, as well as glycoproteins like fibronectin, vitronectin, laminin, and cellular matrix proteins like thrombospondin and SPARC (Sequestered Protein Acidic and Rich in Cysteine). Heparan sulfate, PGs perlecan and syndecan, decorin and biglycan, chondroitin sulfate, fibromodulin PGs keratan sulfate, lumican, and lumican hyaluronan all have a role in angiogenesis [4]. Araujo et al. (2015) showed that decorin and biglycan function in maintaining pregnancy. The most frequently expressed proteoglycans in human fetal membranes are biglycan and decorin, which are both members of the short leucine-rich proteoglycan (SLRP). Biglycan and decorin are also engaged in collagen fibrillogenesis and contribute to connective tissue mechanical characteristics. The tensile strength of connective tissue is reduced when these proteoglycans are missing [5].

Atalay et al. said that biochemical changes, including changes in collagen and cervical connective tissue components, occur towards the end of pregnancy. The concentration of collagen fibrils decreases while reorganization of the collagen fibrillar network occurs in the cervical tissues before delivery. Therefore, changes in cervical decorin levels can be found in women with preterm labor because decorin has a role in the reformation of type 1 collagen [6]. This study is expected to explain the mechanism of Decorin and type 1 collagen in relation to the occurrence of preterm labor.

**MATERIAL AND METHODS**

**Study Design**

This study was conducted with a post-test-only control group in an experimental laboratory held in the Research Laboratory and Experimental Animals (LPHC) Faculty of Medicine Universitas Brawijaya Malang from July to September 2021. The pregnant mice Balb/c utilized in this study were obtained from the Laboratory of Research and Experimental Animals (LPHC) Faculty of Medicine, Universitas Brawijaya Malang. The preparation of fetal membranes or mice and the measurement of decorin and type 1 collagen level were carried out in the Biomedical Laboratory of the Faculty of Medicine, Universitas Brawijaya Malang.

**Sample**

This study divided 28 pregnant Balb/c mice into two large groups with 14 animals. A control group and a treatment group made up this huge cohort. The control group was divided into two small groups of 7 mice. The first control group (N1) was a group of pregnant mice dissected on day 15, while the second control group (N2) were mice pregnant at term followed up to parturition; this was not given *Escherichia coli*. The second large group is the treatment group, also divided into 2, namely the first treatment (P1); this group of pregnant mice, on day 15, were given *Escherichia coli* on the cervix and waited 38 hours, and then we did the surgery. While the second treatment group (P2) mice pregnant on day 15 were given *Escherichia coli* in the cervical canal followed by delivery. This study included pregnant female Balb/C mice at two months of age, with an average weight of 20-25 grams and a health condition characterized by active movements and bright eyes. These mice experienced their first pregnancy.

**Interventions**

**a. Acclimatization of Balb/C Mice**

Twenty-eight female Balb/c mice and 28 male Balb/c mice were needed for fertilization. The percentage of success in one fertilization cycle is 70-80%. The acclimatization of Balb/c mice was carried out for 14 days to condition the animals to a laboratory atmosphere, relieve stress due to transportation, and adapt to their environment. Male Balb/c mice and female Balb/c mice were placed in separate cages, and Balb/c female mice were given pheromone hormones. Female Balb/c mice in the estrus phase were placed in a cage with male Balb/c mice for 1x24 hours. The next day, we examined the vagina of the female Balb/c mice. If a vaginal plug was found, it was considered the -0 gestational day. On the ninth day of gestation, the abdomen of the female Balb/c mice was palpable, and if there were signs of a fetus, it confirmed that the female Balb/c mice were pregnant on the ninth gestational day.

**b. Escherichia coli Breeding**

Eosin Methylene Blue agar and Mac Conkey agar medium were used to grow *Escherichia coli* colonies, incubated at 37°C for 18-20 hours. Observe the growing colonies, and identify them using a simple biochemical test using Triple Sugar Iron media and the indole reaction.

**c. Escherichia coli Exposure Process**

The treatment group was female Balb/c mice with a gestational age of 15 days given treatment in the cervical canal area by exposure to *Escherichia coli* 1x10⁶ CFU/ml for one time. Mice were placed in a supine back position and held with paper tape, and the perineal area was washed with 70% isopropanol. The syringe is bent at an angle of 300, directed through the vagina, and visually inserted 3 mm into the vaginal canal. 100L of saline containing *Escherichia coli* (10⁶ CFU) was introduced. After injection, animals were placed in individual microisolators and observed after parturition, namely 4 hours and 24 hours [7].

**d. Termination of Trial Animals**

According to McCarthy et al., 2018, the mice model was premature after 15 days of coitus. The estrus phase
experienced by mice is characterized by the external genitalia, namely the swollen and reddish vulva. After 24 hours from the time of mating, the vaginal plug was observed, which was a mixture of female seminal vesicle secretions and hardened male ejaculate. The appearance of a vaginal plug is counted as day zero of pregnancy.

e. Fetal Membrane Specimen Collection
Balb/c mice were pregnant on the 15th-18th day of gestation and underwent surgical removal of fetal membranes. The sample was put into a tube and labeled. Tissue samples were stored at -4°C and sent to the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya Malang.

f. Decorin and type 1 Collagen
Decorin is located on the collagen fibrils. Decorin has been shown to regulate fibrillogenesis, cell organization and stabilization with type I collagen in vivo. The effect of Transforming Growth Factor Beta 1 (TGF-α 1) reduces the synthesis and chain length of decorin. Decorin is located on the collagen fibrils. Decorin has been shown to regulate fibrillogenesis, cell organization and stabilization with type I collagen in vivo. The effect of Transforming Growth Factor Beta 1 (TGF-α 1) reduces the synthesis and chain length of decorin.

Then the Elisa test was carried out on decorin and type I collagen with the first step being to determine the number of wells used in the microtitr. For standard curve preparation, 100 l of assay buffer was pipetted into the well (as a blank well), 100 l of decorin and type I collagen were pipetted. Standard 1-7 into a predetermined well. For treatment samples, the thing to do is pipette 100 l of each treatment sample and put it into the well. The microtitr was incubated at 37°C for 2 hours. Each well was washed with 3x 400 l of wash buffer for 5 minutes each. Each well was filled with 100 l of antibody, except for the blank. The microtitr was incubated at 37°C for 1 hour. Each well was washed with 3x 400 l wash buffer. Each well was filled with 100 l of conjugate, except for the blank. The microtitr was incubated at 37°C for 30 minutes. Each well was washed with 3x400 l wash buffer. 100 l of TMB substrate was pipetted and added to each well, then incubated for 30 minutes at room temperature. Stop solution (HCl) was pipetted as much as 100 l and added to each well for 5 minutes. Absorbance value readings were carried out at OD 450 nm.

Ethics
The Health Research Ethics Committee, Faculty of Medicine, Brawijaya University, Malang, Indonesia, authorized all methodologies used in this investigation.

Statistical analysis
The data analysis technique used in this study consisted of three stages of calculation:
1. Normality testing of sample data with the Shapiro-Wilk test
2. Comparative test using independent sample t-test if the data were normally distributed, but the Mann-Whitney test if the data were not normally distributed
3. The One-Way Anova test (F test) (if the data were normally distributed) or the Kruskal Wallis test (if the data are not normally distributed) (if the data was not normally distributed).

SPSS for Windows 19.0 was used to complete all calculations.

Fig. 1. Histogram of Mean Decorin Levels in Fetal Membranes
RESULTS

Table 1 shows that decorin and type 1 collagen levels in fetal membranes have p-values greater than 0.05. From Table 2, it can be interpreted that the administration of Escherichia coli in preterm pregnant mice has a significant effect on decreasing decorin levels. Where there was a substantial distinction in the mean decorin level between control group 1 (N1) (12.35 ± 2.24 ng/mL) and group P2 (6.62 ± 1.50 ng/mL), the mean value of decorin levels in the control group P2 was much smaller than the mean levels of decorin in control group 1. Likewise, the mean value of decorin levels between control group 2 (N2) (12.91±2.65 ng/mL) and group P1 (9.47±0.71 ng/mL) showed a significant difference. This means that the treatment with Escherichia coli can reduce decorin levels in mice pregnant prematurely to parturition compared to normal pregnant mice without Escherichia coli. Furthermore, the average decorin levels in the four sample groups are presented in full, as shown in Fig. 1.

Table 2. Comparison of Decorin levels (ng/mL) in Fetal Membranes

<table>
<thead>
<tr>
<th>Observation Group</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>12.35±2.24</td>
<td>a</td>
</tr>
<tr>
<td>N2</td>
<td>12.91±2.65</td>
<td>b</td>
</tr>
<tr>
<td>P1</td>
<td>9.47±0.71</td>
<td>c</td>
</tr>
<tr>
<td>P2</td>
<td>6.62±1.50</td>
<td></td>
</tr>
</tbody>
</table>

Information:
The rank of letters represents the Tukey test findings on the means; if it contains different letters, there is a significant difference (p-value<0.05), whereas if it contains the same letters, there is no significant difference (p-value>0.05).

Table 3. Comparison of Type I collagen levels (ng/mL) in Fetal Membranes

<table>
<thead>
<tr>
<th>Observation Group</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>283.5±31.3</td>
<td>a</td>
</tr>
<tr>
<td>N2</td>
<td>376.7±51.9</td>
<td>b</td>
</tr>
<tr>
<td>P1</td>
<td>196.5±25.7</td>
<td>c</td>
</tr>
<tr>
<td>P2</td>
<td>170.6±38.8</td>
<td>c</td>
</tr>
</tbody>
</table>

Information:
The rank of letters represents the Tukey test findings on the means; if it contains different letters, there is a significant difference (p-value<0.05), whereas if it contains the same letters, there is no significant difference (p-value>0.05).
*Escherichia coli* can reduce collagen type 1 in prematurely pregnant mice compared to normal pregnant mice without *Escherichia coli*. Furthermore, the average levels of type 1 collagen in the four sample groups are presented in full, as shown in Fig. 2.

**DISCUSSION**

Premature labor can occur between 25%-40% due to infection. Infection can develop through 2 main routes—an intrauterine infection that can arise systemically or bacteria that can develop through the genital tract [11]. The bacteria most often used to induce infection are *Escherichia coli* or toxic to the surface of gram-negative bacteria, lipopolysaccharide (LPS) [10].

Decorin is a short-chain proteoglycan, one of the small leucine-rich proteoglycans (SERPs) found in the extracellular matrix. Biglycan and decorin function to maintain a pregnancy to term [9]. In this study, the lowest decorin levels were found in the treatment group (P2), namely the 15th-day pregnant mice were given *Escherichia coli* 10⁶ CFU/ml followed by delivery, followed by the treatment group (P1), namely 15th-day pregnant mice were given *Escherichia coli* 10⁷ CFU/ml were sacrificed after 38 hours, followed by control groups N1 and N2. However, no statistically significant difference was found. These results explain decreased decorin levels in preterm labor caused by *Escherichia coli* infection.

Decorin can bind TGFβ and modulate extracellular matrix assembly, collagen, and skeletal muscle differentiation [12]. Decorin also directly interacts with collagen to modulate collagen fibril assembly. Collagen fibrils form various types of collagen, namely types I, II, III, V, and XI, which have their respective functions and strengthen tissues and the integrity of all connective tissues in the body [13]. Biglycan and decorin, according to Araujo et al. (2015), have a function in supporting pregnancy by controlling inflammation and the response of fetal membranes to inflammation [8]. Cytokines produced during infection can also activate levels of matrix metalloproteinases in the cervix and decidua, which play a role in extracellular matrix degradation, rupture of fetal membranes, and cervical changes in the uterus. If inflammation occurs, TGFβ decreases, thereby reducing decorin chain synthesis, causing a decrease in decorin levels which affect the fetal membranes [14].

Inflammation can reduce TGFβ levels [10]. The choriodecidual membrane is a liaison between maternal and fetal tissues that has an essential role in maintaining the uterus and initiating labor through immunological and hormonal regulation [15]. The molecular mechanisms of labor must be systematically identified in the inflammatory process that increases COX2 levels in the fetal membranes [16]. Increased levels of COX2 regulate prostaglandins that can induce uterine contractions and the choriodecidual membrane, which is the origin of the dynamic changes that initiate preterm labor [17].

The amnion (the innermost layer of the intra-amniotic cavity) and the chorion (fetal tissue connected to the maternal decidua) form the fetal membrane, which is connected to the maternal decidua collagen-rich extracellular matrix proteins and forms the innermost layer of the intra-amniotic cavity [18]. Type I collagen is fibrillar collagen, which makes up a large part of the interstitial membrane [19]. Collagen type I is the most common and primary structural component of numerous tissues [20].

In our study, the level of type 1 collagen was the lowest in the treatment group (P2). In this group, 15th-day pregnant mice were given *Escherichia coli* 10⁸ CFU/ml followed by delivery, followed by the treatment group (P1), and 15th-day pregnant mice were given *Escherichia coli* 10⁷ CFU/ml and sacrificed after 38 hours. Then followed by the control group N1, namely normal pregnant mice on the 15th day, then sanitized, followed by the control group N2, namely normal pregnant mice at term followed by parturition. These results explain decreased collagen type 1 levels in preterm labor caused by *Escherichia coli* infection.

Decreasing decorin levels are also associated with decreased levels of type 1 collagen because decorin can bind TGF-β [21]. TGF-β regulates the stability of fetal membrane proteins such as MMPs, TIMPs, and collagen because TGF-β expression is partly regulated by biglycan and decorin; TGF-β expression in fetal membranes in mice represents early and late developmental stages, respectively [22]. Overall, the findings suggest that TGF-β expression in fetal membranes is dynamic and differs during pregnancy [23].

Decorations can also modulate the extracellular matrix and collagen arrangement and directly interacts with collagen to modulate collagen fibril assembly. Collagen fibrils form various types of collagen, namely types I, II, III, V, and XI, which have their respective functions and strengthen tissues and the integrity of all connective tissues in the body [13]. Decreased levels of type 1 collagen can cause cervical ripening resulting in preterm labor [24]. Changes in the structure of the cervix during labor are characterized by a decrease in the concentration of collagen, a decrease in the extracellular matrix, and an increase in water content which indicates that the cervical tissue provides low resistance [25]. As the uterus contracts, the cervical tissue undergoes a process of thinning and dilatation. During cervical ripening, there is a process of dissociation and degradation of collagen, resulting in changes in collagen structure during this period [9].
CONCLUSION

There was a decrease in decorin levels and type I collagen levels in mice in Balb/c fetal membranes in a premature model. Decreasing levels of decorin affect decreasing levels of collagen type 1 in fetal membranes of Balb/c mice in premature models.

ACKNOWLEDGMENT

We want to express our gratitude to everyone who contributed to this research and assisted with data collection.

CONFLICT OF INTEREST

The authors declare no competing interests.

REFERENCES