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RESEARCH ARTICLE

Effect Of *Low* Doses of Acetylsalicylic Acid (ASA) On the Histopathological Features of Fibroblasts in The Amniotic Membrane

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Abstract: *Acetylsalicylic acid (ASA) is a drug with several indications such as analgesic, antipyretic, and antithrombotic/antiplatelet. ASA is efficacious as antithrombotic at low doses and used for thrombosis therapy in stroke, antiphospholipid syndrome (APS), pre-eclampsia and eclampsia. However, the administration of ASA made pharmacokinetic interactions that cause a vitamin C deficiency condition. This study aims to determine the effect of low doses of Acetylsalicylic acid (ASA) on the histopathological appearance of fibroblasts on fetal membranes. This study used a cross-sectional design using 54 paraffin membrane blocks as samples processed into histopathological preparations. The case group received low-dose ASA and the control group did not receive low-dose ASA. Histopathological preparations were observed using a microscope to see images / count the number of fibroblast cells. The results showed that there were significant differences between the number of fibroblasts in the case group and the control group with p = 0.001 or p <0.05*

Keywords: *low-dose ASA, histopathological fibroblast*

INTRODUCTION

Acetylsalicylic acid (ASA), also known by other names, aspirin is a drug with various properties, namely analgesic, anti-inflammatory, and antithrombotic/antiplatelet, which is a drug that works by inhibiting platelet attachment/aggregation.[1–3] ASA works as an antithrombotic/antiplatelet in the low dose range (80-325mg).[4,5,6] The benefits of using ASA to prevent thrombosis in cardiovascular disease and other vascular diseases (stroke), antiphospholipid syndrome (APS), pre-eclampsia and eclampsia have been widely

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proven so that they are used as secondary prevention to avoid the risk that occurs.[7–11]

APS is an autoimmune disease that results in thrombosis of blood vessels, and recurrent abortion in pregnant women due to thrombosis in the placental blood vessels.[12–14] Morbidity of pregnancy with APS is associated with *decidual vasculopathy* and placental infarction, so low-dose combined aspirin/*ASA* treatment with heparin is the therapy of choice.[15–19]

The formation of amniotic membranes during pregnancy is an important process.[20,21] One of the important components to make it up is collagen.[22,23] Collagen is a component of the membrane that functions to maintain the strength of tissues in the amnion and chorion.[20,24]

Collagen is synthesized by fibroblast cells.[25,26] Fibroblasts are stem cells that play a role in forming and laying fibres in the matrix, especially collagen fibres.[27,28] These cells secrete small tropocollagen molecules that join in the basic substance to form collagen fibres.[29,30] Vitamin C is needed for the formation of ECMS components such as collagen, laminin and fibronectin.[31–34]

However, the administration of ASA causes pharmacokinetic interactions both at the absorption, distribution and excretion stages, in the form of transport barriers, *uptake barriers* and increased excretion of vitamin C.[35,36] This condition can affect the work of fibroblasts in synthesizing collagen and other matrix components.[37,38]

This study aims to see the effect of low-dose *Acetylsalicylic acid* (ASA) administration on the histopathological picture of fibroblasts in amniotic membranes.

METHOD

This study is descriptive-analytic with a *cross-sectional design.* The study population was women with normal (did not get low-dose ASA) and abnormal (got low-dose ASA) pregnancies. The study samples were stored biological material (SBM) from paraffin blocks of amniotic membrane tissue of women who received low-dose ASA during pregnancy (case group) and who did not get low-dose ASA during pregnancy (control group) totalling 54; comes from previous research.

The research was conducted after obtaining approval from the Research Ethics Commission Fakultas Kedokteran Universitas Muhammadiyah Sumatera Utara.

The research began with tissue *processing* in the laboratory, in the form of tissue slicing, attachment to preparations/preparations and colouring preparations using *Hematoxylin* and *Eosin* (HE) staining.

Furthermore, observation of histopathological preparations with binocular light microscopy was observed with magnification of 10x and 40x to clarify the shape of fibroblasts. Then the number of fibroblasts is read by counting the number in three fields of view. This calculation is carried out with a coefficient of variation < 10%.

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RESULT

Calculation of the number of fibroblasts from amniotic membrane histopathology preparations in 2 observation groups, namely: the case group (received low-dose ASA) and the control group (did not get low-dose ASA).

Table 1. Number of fibroblasts in the case group and control group

Group	Average	*SD*	Minimum (sel)	Maximum (sel)
Case ASA (+)	30	4,45	28	32
Control ASA (-)	51	11,68	47	56

Information: *SD (Standard deviation)*

The table above shows that the number of fibroblasts in the case group (30±4.45) was less than in the control group (51±11.68). In the case group, the least number of fibroblasts was 28 and the most was 32; While in the control group, the least number of fibroblasts was 47 and the most was 56.

From the results of the analysis with independent or unpaired T-tests in *Bootstrap,* it can be concluded that there is a real difference between the number of fibroblasts in the case group and the control group with a value of $p = 0.001$ or $p < 0.05$ (there is an effect of giving low doses of ASA on changes in the histopathological picture (number of fibroblasts) of amniotic membranes.

DISCUSSION

From the results of this study, it was found that there was an effect of long-term low-dose ASA administration on the number of fibroblasts in the amniotic membrane. This was seen in the number of fibroblasts in the sample group that received low-dose ASA (case group) less than the sample group that did not get low-dose ASA.

The use of ASA can inhibit absorption and increase the excretion of vitamin C which plays an important role in collagen synthesis so that the amount of collagen which is the main component of the extracellular matrix (ECM) is reduced.[39] Vitamin C deficiency that causes collagen synthesis disorders will affect the structure, stability and function of collagen fibrils in the metabolism of ECMs.[40,41]

Collagen plays a very important role in the stability and defence of the structural integrity of tissues and organs so disruptions in collagen synthesis will disrupt the stability and integrity and changes in the structure of amniotic membrane tissue.[42,] These biochemical changes are involved in the pathogenesis *of premature rupture of membrane (PROM).*[43] In the group with *PROM*, collagen degradation was found in the amniotic and chorion layers.[44,45]

Collagen is produced by fibroblasts and is the main structural building element of connective tissue in ECMs.[46] Vitamin C deficiency will cause fibroblast growth to also decrease.[47] A study found that fibroblast culture media that received ascorbic acid (vitamin C) 50µg showed normal growth and more amounts than culture media that received ascorbic acid (vitamin C) 1µg.[48,49] It

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is concluded that vitamin C deficiency will affect the amount of synthesis of fibroblasts.

CONCLUSION

There is an effect of low-dose ASA administration on changes in the histopathological picture of amniotic membrane fibroblasts, namely the number of fibroblasts in the case group (getting ASA) and the control group (not getting ASA) is different from real, p-value = 0.001 or p <0.05.

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