

Original article

Changes on aortic diameter and number of Endothelin-B receptor in aortic endothelium of diabetes mellitus rat model after exercise.

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Abstract:

Background: incidence and prevalence rate of diabetes mellitus (DM) were increased in recent years in various parts of the world. Diabetes mellitus had microvascular and macrovascular complications. Those complications of DM occurred due to vascular disorders. The complications include the decrease of endothelin B receptor (ETB) and reduce on blood vessels diameter. In vitro studies indicate shear stress can increase expression of ETB receptors on the endothelium. ETB receptors in endothelial have vasodilatation activity. Shear stress increases in physical exercise. Physical exercise known may improve ETB receptor number and also increase the diameter of blood vessels. **Objective:** This study aims to know the changes of aortic diameter and the number of ETB receptor expression in the aortic endothelium of DM rat model after exercise. **Methods:** This study is an experimental research design with a post-test only control group to measure the diameter of the aorta and ETB receptor expression in the aortic endothelial. Rat model of DM obtained by injecting streptozotocin 35mg/kgwb intra peritoneal Sprague Dawley male rats aged 11-12 weeks. Rats were divided into two groups, one group received regular physical exercise and other group without regular physical exercise. Physical exercise routine using a special treadmill for rat, low - moderate intensity, frequency 5x/week, for 9 weeks, with a week of adaptation using a speed of 5 m / min for 10 minutes, followed 8 weeks later using a gradual rise velocity of 5 m/min to 20 m/min for 60 minutes. Rat aortic diameter was measured by using a microscope optilab. Endothelin B receptor expression was calculated by the percentage of the ETB receptor in aortic endothelial which has been stained with immunohistochemical staining. **Results:** The mean results for the aortic lumen diameter in non-exercise group rats $913.73 \pm 62.08 \mu\text{m}$, and $1066.73 \pm 28.58 \mu\text{m}$ in exercise group (p value is 0.033). After immunohistochemistry staining for ETB, the results for non-exercise group is $49.37 \pm 5.64 \%$, while for the exercise group the ETB receptors is $78.42 \pm 5.84 \%$ (p value is 0.006). **Conclusion:** There was an increasing of ETB receptor number and aortic diameter of DM rat model after regular physical exercise compare with the group of DM rat model without regular physical exercise.

Key words: diabetes mellitus; exercise, aortic diameter; endothelin-B receptor

Bangladesh Journal of Medical Science Vol. 15 No. 03 July'16. Page : 381-384

Background:

Various epidemiological studies have shown a trend of increasing in the incidence and prevalence of diabetes mellitus (DM) in various parts of the world. World Health Organization (WHO) predicts an increase in the number of people with diabetes are

quite large in the years to come. WHO predictions for Indonesia, the increase in the number of patients from 8.4 million in 2000 to around 21.3 million in 2030¹. The amount of the estimated number of patients who suffer from diabetes is associated with reduced life expectancy ranges, increased micro-

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and macrovascular complications, and the decline in the quality of life².

Complications of diabetes include complications of acute and chronic complications. Acute complications such as iatrogenic hypoglycemia, diabetic ketoacidosis, hyperosmolar hyperglycemic non- ketotic coma, and lactic acidosis. Chronic complications including retinopathy, nephropathy, neuropathy, and diabetic coronary heart disease. The increase in diabetes complications result from blood vessel disorders, both macrovascular and microvaskular³. Vascular disorders preceded the increased viscosity of the blood flow, increasing the pressure received by the endothelium. Stresses imposed by the blood vessels which comes from the frictional forces due to blood flow (shear stress)

⁴ . Increased blood sugar levels will be followed by an increase in viscosity of the blood⁵proportional with shear stress⁶. Increased blood viscosity contributes to the increase in blood pressure with diabetes are thought to be contributing factors in the beginning of the failure of other organs in the DM complications ⁷.

Increase of blood pressure in diabetic patients is affected by an increase in blood viscosity, the formation of atherosclerosis in the blood vessels, reduced endothelin - B receptor continued to decline in the production of Nitric Oxide (NO) and prostacyclin, a decrease in insulin sensitivity, as well as an imbalance of vasoconstrictor and vasodilator⁷⁻¹¹ .

Number of endothelin - B receptors in the blood

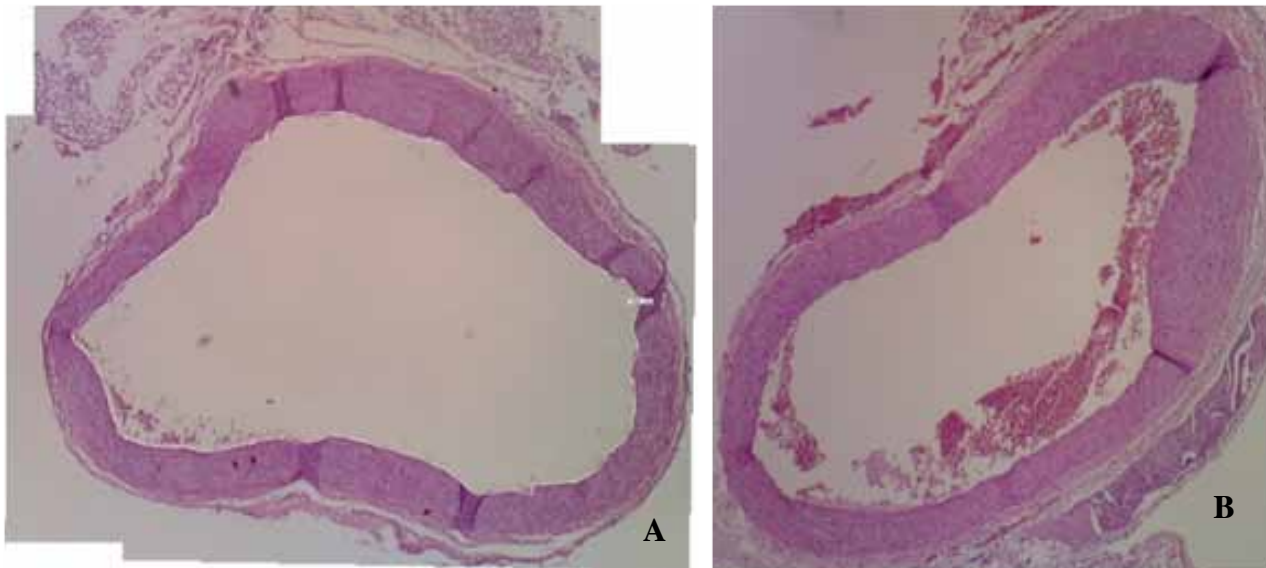


Figure 1.aortic lumen circumference measurement results using optilab
a. DM group with exercise b. DM group without exercise

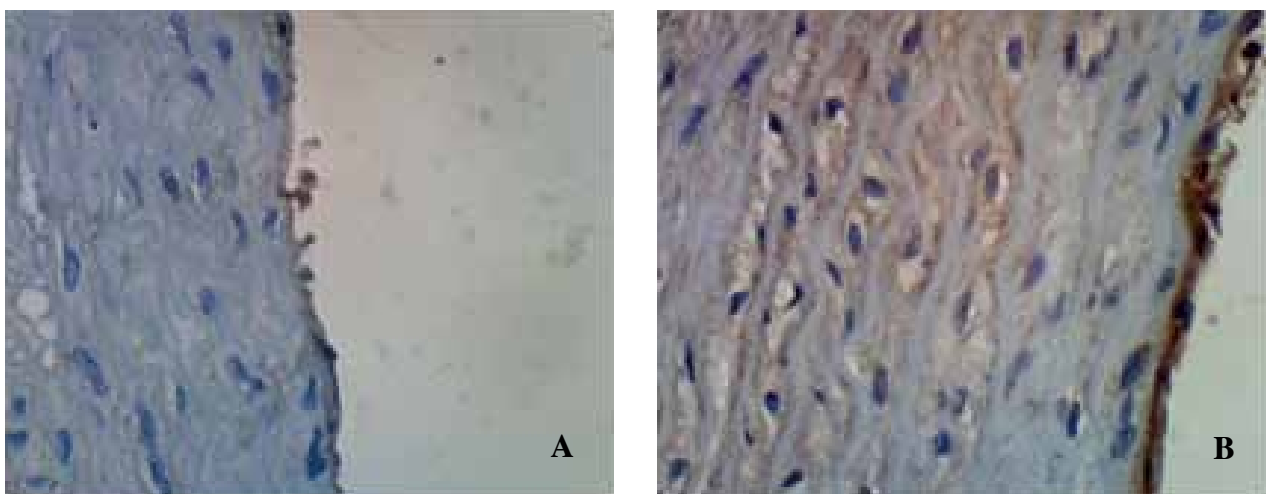


Figure 2.picture a ETB receptors in non-exercise group.
Picturesb ETB receptors in exercise group

Table 1. Results of statistical analysis of the aortic lumen diameter, endothelin-B receptor, and the difference in blood pressure between groups

Parameter	Rats DM without physical exercise	Rats DM with physical exercise
Aortic diameter (μm)	913,73 \pm 62,08 ^{a, b}	1066,73 \pm 28,58 ^a
Receptor endotelin-B	49,37 \pm 5,64 ^b	78,43 \pm 5,83 ^b

a p=0,033, b p=0,006

vessels is related to blood pressure. Endothelin - B receptor in endothelial becomes active when bound to endothelin - 1, and then activate the NO production pathway that causes vasodilation, thereby affecting blood pressure. Endothelin - 1 acts on the endothelin A and B receptors in vascular smooth muscle and endothelin - B receptor in endothelial. Activation of these receptors in both vascular smooth muscle to produce vasoconstriction, but endothelin - B receptor activation in vascular endothelium also mediate vasodilation and clearance of endothelin - 1. Endothelin - B receptor may indicate anti - atherosclerotic effect by increasing clearance of circulating endothelin - 1^{4,10,12}.

Shear stress has been known to be a modulator in the expression of endothelin - B receptor. Regular physical exercise can increase the shear stress on the blood vessels . Episodic change of shear stress on physical exercise are factors that are thought to contribute to vascular changes when physical exercise. Based on this background, further research needs to be done to determine the role of regular physical exercise on blood pressure reduction in a rat model of diabetes mellitus by changes in endothelin - B receptor expression and aortic diameter^{12, 13} .

Objectives

This study aims to know the changes of aortic diameter and the number of ETB receptor expression in the aortic endothelium of DM rat model after exercise.

Method

This study is an experimental research design with a post-test only control group to measure the diameter of the aorta and ETB receptor expression in the aortic endothelial. Rat model of DM obtained by injecting streptozotocin 35mg/kgbw intra peritoneal Sprague Dawley male rats aged 11-12 weeks. Rats were divided into two groups, one group received

regular physical exercise and other group without regular physical exercise. Each group consist of 8 rats in the beginning.

Physical exercise routines that are used in this study is the use of physical exercise treadmill Gama special treat rat with low - moderate intensity, slope 0°, 5x/minggu frequency, the total time for 9 weeks, starting one week adaptation 5m/menit treadmill at a speed of over 10 minutes, followed by 8 weeks of the start -storey speed of 5 m / min to 20 m / min for 1

hour¹⁵ .

Rat aortic diameter is the diameter of the lumen of rat aortic preparations were stained with hematoxylin eosin staining which measured three times then taken using a microscope optilab. Expression of endothelin - B receptor is a percentage of the endothelin - B receptor in aortic endothelial which had been stained with immunohistochemicalstaining , which is calculated using formula¹⁶

$$\frac{\text{The number of positively stained endothelial cells}}{\text{Total number of endothelial cells}} \times 100 \%$$

Total number of endothelial cells

DM rat model is the male age of Sprague Dawley rats range 11-12 weeks induced with streptozotocin 35 mg / kg intraperitoneally a single dose, with a fasting blood glucose level > 140 mg / dl or blood sugar levels when > 200 mg / dl^{17,18} .

Ethical approval was taken Prior study.

Results and discussion:

This study using Sprague-Dawley male rats age range of 11-12 weeks, weighing between 250-300 g were divided into two groups. Rats divided into two groups. Exercise group and non-exercise group. Each group consisted of 8 rats. Rats were able to complete routine physical exercise protocol until the end of the study were 7 rats of exercise group, and 5 rats of non-exercise group.

The results obtained during the study are as contained in Table 1.

The mean results for the aortic lumen diameter DM group rat without regular physical exercise is 913.73 \pm 62.08 μm and DM groups of rat with regular physical exercise 1066.73 \pm 28.58 μm . Results of independent t-test trials for groups of diabetic rats with exercise and no exercise groups diabetic rats resulted in a p-value = 0.033, which means there is a significant difference between the two groups (p < 0.05).

After staining, the results obtained for the group of diabetic rats without regular physical exercise is the average percentage of $49.37 \pm 5.64 \%$, while for the group of diabetic rats group with regular physical exercise mean obtained is $78.42 \pm 5.84 \%$. Different test results using independent t - test showed no training for the DM group and DM group with the exercise value of $p = 0.006$, this indicates a significant difference between the two groups. Pearson correlation test results between the variables showed that the diameter is not correlated with the percentage of endothelin - B receptor in aortic endothelial. Significance values obtained in the correlation test was $p > 0.05$. These results indicate

that the correlation between the two variables was not statistically significant. R value for Pearson correlation test between the variable diameter and endothelin receptor number is 0,407, with a value of $p = 0.189$ which means there is no significance to the value of the correlation of two variables.

Acknowledgements:

This article was presented in CVD-IA 2016.

Conclusion:

There was an increasing of ETB receptor number and aortic diameter of DM rat model after regular physical exercise compare with the group of DM rat model without regular physical exercise.

Conflict of interest: None

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