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Effect of Hyperglycemia to The mRNA Level and Protein Expression of Perlecan at Rat Model of Osteoarthritis with Diabetes Mellitus Type 1

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ABSTRACT

Introduction: Previous research found that diabetes mellitus capable to aggravate osteoarthritis disease. In brief, the hyperglycemia condition in diabetes mellitus has an impact on protein glycation of all joint components, including molecule, such as perlecan. The protein expression of perlecan reflects the presence amount of perlecan in the matrix of articular cartilage. However, the impact of hyperglycemia on articular perlecan has not been explained. Moreover, the role of perlecan as a mechanotransducer for chondrocytes in type 1 Diabetes mellitus remains unclear. **Aim:** This research aims to analyze the effect of hyperglycemia in type 1 Diabetes mellitus to the mRNA level and protein expression of perlecan. **Methods:** Thirty-five adult male rats were divided into seven groups, such as three groups of rat model with anterior cruciate ligament transection (ACLT) at right knee (ACLT1, ACLT2, ACLT3); three groups of rats with ACLT at right knee which followed by Streptozotocin injection for diabetic mice model (DM1, DM2, DM3); and control group (N). Rat sacrificed at the third week, fourth week, and sixth week after two months of maintenance. The mRNA level and protein expression were analyzed by using PCR and Western blot. All of data was analyzed by ANOVA. **Results:** Protein expression of perlecan in ACLT mice with diabetes mellitus (DM1, DM2, DM3 group) was gradually decreased according to the increased hyperglycemia duration. Whilst, protein expression of perlecan within ACLT mice without diabetes mellitus (ACLT1, ACLT2, ACLT3 group) was increased. The similar result also demonstrated by the mRNA level of perlecan. Group of DM1, DM2, DM3 exhibited decreased mRNA level of perlecan over the hyperglycemia duration. While, ACLT1, ACLT2, and ACLT3 group had a gradually increased of perlecan mRNA level. **Conclusion:** Hyperglycemia on osteoarthritic condition decreased mRNA level and protein expression of perlecan which increased the severity of osteoarthritis disease.
Keywords: Diabetes mellitus, hyperglycemia, osteoarthritic, perlecan, proteomic.

1. INTRODUCTION

Proteomic and transcriptomic research on perlecan articular cartilage which influenced by hyperglycemia needs to be further explored. Hyperglycemia condition will increase the severity of osteoarthritis disease (1) since it induced the glycation process of all molecular and cellular protein building blocks (2). When articular protein was modified by glycation, it will have a functional changing. Perlecan as chondrocytes mechanotransducer is located at pericellular and extracellular matrix (3). It increased by the articular mechanical load but decreased by aging process (4, 5). Another function of perlecan to chondrocytes is to determine the differentiation phase of chondrocytes (6), link protein (7), hold the integrity of matrix cartilage (8), collaborate with growth factors to produce extracellular matrix and facilitate cell proliferation (9), and induce the osteophyte formation as synovial perlecan (5). However, impact of hyperglycemia to perlecan articular cartilage remains unclear, but the impact of hyperglycemia to perlecan endotelial aortic is demonstrated by previous study. It showed that high glucose level induced loss of one Heparan Sulphate chain (9). The high glucose condition at endothelial aortic is the basic of the present study to analyze the impact of hyperglycemia duration to perlecan articular carti-

	N group (mg/dl)	DM1 group (mg/dl)	DM2 group (mg/dl)	DM3 group (mg/dl)	ACLT1 group (mg/dl)	ACLT2 group (mg/dl)	ACLT3 group (mg/dl)
Blood sugar level	1) 77 2) 85 3) 71 4) 70 5) 88	1) 501 2) 400 3) 454 4) 454 5) 371	1) 486 2) 492 3) 422 4) 386 5) 528	1) 477 2) 367 3) 443 4) 377 5) 387	1) 121 2) 94 3) 87 4) 92 5) 87	1) 90 2) 87 3) 80 4) 83 5) 91	1) 84 2) 95 3) 98 4) 97 5) 112

Table 1. Blood sugar level of N group, three groups of ACLT (ACLT1, ACLT2, ACLT3), and three groups of ACLT with DM (DM1, DM2, DM3). The comparison of blood sample showed that STZ by intraperitoneal injection (IP) created hyperglycemia as like as Diabetes mellitus type 1 condition. Of note, the blood sample of ACLT group slightly increased due to the traumatic stress of ACLT procedure.

lage. Perlecan at present study was analyzed based on the protein expression at extracellular and pericellular matrix of cartilage. Impact of hyperglycemia to microcellular homeostasis will change the respond of chondrocytes to their enviroment (10). The previous study of morphological chondrocytes changing was concluded that hyperglycemia had an impact on intracellular matrix strain of chondrocytes at the superficial layer of articular cartilage (11). When perlecan protein expression was decreased, the mRNA level of perlecan as chondrocytes respond at articular cartilage matrix need to be explored. The proteomic and transcriptomic change of perlecan which produced by chondrocytes under the osteoarthritic condition and under diabetic needs to be measured. A present study showed that hyperglycemia had an impaction to the severity level of osteoarthritis disease based on the protein expression and mRNA level of perlecan.

2. AIM

Therefore, this research aims to analyze the impact of hyperglycemia duration on the severity of osteoarthritis based on the mRNA level and protein expression of perlecan. The result of this research is expected to be fundamental to prevent degeneration of cartilage and reduce severity osteoarthritis. Hence, the risk of hyperglycemia at osteoarthritis patient can be controlled.

3. METHODS

The present study had approved by the Research Ethics committee of Brawijaya University, Malang, Indonesia. Thirty-five of male rats (*Rattus norvegicus* strain Wistar) with 5-6 months old and 300-400 g body weight was addressed as a subject in this research. Rats divided into seven groups, such as three groups of rat with anterior cruciate ligament transection (ACLT) at right knee (ACLT1, ACLT2, ACLT3); three groups of rat with ACLT at right knee which followed by Streptozotocin injection for diabetic mice model (DM1, DM2, DM3), and control group (N group). All groups were fed twice a day in the morning and afternoon by given 30 g of the standard diet. Furthermore, ACLT was performed to all ACLT group and all DM group, then wait for one week for healing process.

Three groups of DM group were administered by streptozotocin 25 mg/kg body weight with intraperitoneal injection after maintenance within a month. Then, it was maintenance again after injection. After two months of maintenance, the group of ACLT 1 and DM 1 was sacrificed at the third week; the group of ACLT 2 and DM 2 was sacrificed at the fourth week; then control group, ACLT 3 and DM 3 was sacrificed at the sixth weeks. The blood sample was collected before the rats were sacrificed. It was addressed to measure the blood glucose level and body weight. Right knee articular of cartilage was gathered for further analysis. Both condylar femur bone processed by using Western Blot to observe the protein expression of perlecan. Moreover, the articular cartilage of tibial bone was also processed by using real-time Polymerase Chain Reaction (PCR) to measure the mRNA level of perlecan. Furthermore, all of the data was analyzed with statistical analysis by ANOVA.

4. RESULTS

The blood sugar level was measured before rats were sacrificed. All the data is presented in Table 1.

Based on the result in Table 1, the blood sugar level at DM groups was significantly increased. It indicates that STZ injection induced hyperglycemia as diabetes mellitus condition. It demonstrated by the level of blood glucose which higher than 200 mg/dl (12) and decreased body weight. Furthermore, the protein expression and mRNA level of perlecan within each group were addressed for further analysis.

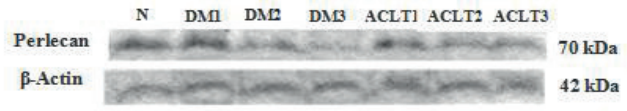

Figure 1. Protein expression of perlecan on the control group (N group), ACLT with streptozotocin injection (DM1, DM2, DM3 group), and ACLT model (ACLT1, ACLT2, ACLT3 group) by Western Blot. The band of DM groups looked gradually infrequent: DM1>DM2>DM3. It demonstrated that protein expression of perlecan decreased over time which increased the duration of hyperglicemia.

Protein expression of perlecan by Western Blotting

Results showed that protein expression of perlecan at DM groups was gradually decreased from 146.301 int/mm2 (DM1 group) to 124.743 int/mm2 (DM2 group), then more decreased into 103.344 int/mm2 (DM3 group). This decreased protein expression is in line with the hyperglycemia duration. The duration of hyperglycemia was 7 weeks and it decreased the protein expression of perlecan about 35.239 int/mm2. In detail, the protein expression of perlecan decreased from 181.540 int/mm2

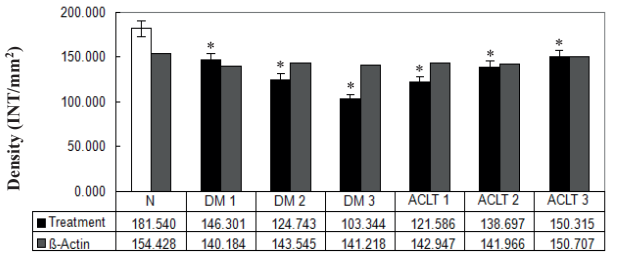

Figure 2. Comparison protein expression of perlecan among each group which exhibited by density (Int/mm2). The protein expression of perlecan decreased by increasing of hyperglycemia duration at DM groups. Whilst, protein expression of perlecan increased at ACLT groups. * = significant different level (p<0,05)

at the control group to 146.301 int/mm2 (DM1 group). Moreover, in the DM2 group, the protein level of perlecan was decreased 56.797 int/mm2 for 8 weeks of maintenance. Whilst, the protein expression of perlecan was decreased 78.196 int/mm2 for 10 weeks of maintenance in the DM3 group (Figure 2). All the data obtained from DM groups was gradually decreased simultaneously by the higher length of hyperglycemia duration.

Result data of ACLT groups was opposed to the result of DM groups. The protein expression of perlecan within ACLT groups was increased gradually during the hyperglycemia duration, but it still lower than the control group (181.540 int/mm2). The protein level of perlecan at ACLT1 group was 121.586 int/mm2, then increased into 138.697 int/mm2 at ACLT2 group. Moreover, it increased into 150.315 int/mm2 at ATCLT3 group. It showed that protein expression of perlecan gradually increased over time from eleventh weeks to fourteenth weeks of ACLT maintenance (from 121.586 int/mm2 to 150.315 int/mm2).

The mRNA level of perlecan by real time PCR

The mRNA level of DM groups and ACLT groups demonstrated the opposite result. In detail, the mRNA level of perlecan was gradually decreased from 0.080908 fold change (DM1 group) to -0.28845 fold change (DM2 group), then -0.53711 fold change (DM3 group). Whilst, the results data of ACLT groups demonstrated that the mRNA level was -0.54702 at ACLT1 group, then gradually increased to -0.19522 fold change at ACLT2 group, and 0.075356 fold change at ACLT3 group (Figure 3).

The results data of protein expression and mRNA level

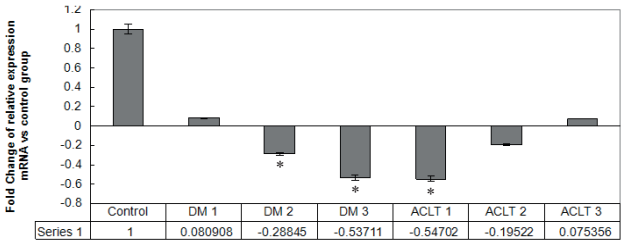

Figure 3. Comparison of mRNA level of perlecan among each group. It exhibited fold change mRNA level of perlecan on DM groups significantly decreased due to increase of hyperglycemia period. However, the mRNA level of perlecan on ACLT groups was increased. * = significant different level (p<0,05)

of perlecan within DM groups and ACLT groups exhibited that there is something happened at the extracellular matrix and inside the chondrocytes. It is demonstrated based on the comparison result of osteoarthritis rats models with hyperglycemia to the osteoarthritis rats model without hyperglycemia.

5. DISCUSSION

Perlecan is known as heparan sulfate proteoglycan 2 which has a function as chondrocytes mechanotransducer (13). It is located at extracellular matrix (ECM) (14) especially at pericellular matrix (PCM) (17). The higher accumulation of perlecan at pericellular matrix than extracellular matrix demonstrated that perlecan has an important function to chondrocytes. The previous study by Willusz et al (2012) showed that perlecan function was related to the elasticity module of PCM and contributed to the microenvironment of chondrocytes (14). The study about impact hyperglycemia to the perlecan articular cartilage remains unclear. Therefore, this present research is addressed to fulfill the research gap of what happens at osteoarthritis patient with diabetes mellitus to the severity of osteoarthritis disease.

Hyperglycemia induces the glycation of protein, including perlecan (15). The present research analyzed the protein expression of perlecan by Western blot to demonstrate the representative quantity of perlecan at extracellular cartilage matrix. It is because this part is affected by hyperglycemia when approaching to the articular cartilage layer by layer from matrix to cell. Furthermore, it is also addressed for analyzing the chondrocytes respond to produce perlecan.

According to data in this research, it showed that the duration of hyperglycemia had an effect on the protein expression of perlecan. It exhibited that protein expression of perlecan gradually decreased within a certain period of hyperglycemia.

This data supports the previous result by Catherine et al which stated that hyperglycemia had an effect on the perlecan of endothelial aorta. It demonstrated by decreased perlecan of endothelial aorta was about 30% as equal to lose one of heparan sulfate chain of total four heparan sulfate chain of perlecan. Moreover, the interesting result exhibited that protein expression of perlecan was gradually decreased over time within DM groups. It means the osteoarthritis patient who suffering the hyperglycemia by diabetes mellitus will have a higher possibility of articular cartilage damage as higher severity of the osteoarthritis disease. Whilst, the data from ACLT groups without diabetes mellitus type 1 induction showed that protein expression of perlecan was lower at ACLT1 group as the result of cellular shock during ACLT treatment. Then, the protein expression of perlecan was gradually increased at ACLT2 group and ACLT3 group. It exhibited that the perlecan quantity at articular cartilage is increased as a response of chondrocytes to produce perlecan in the transcription process. Therefore, it can be analyzed at the mRNA level.

Another data showed that hyperglycemia reaches into the intracellular part after it attained the perlecan of articular cartilage matrix. When hyperglycemia reduc-

es the perlecan of articular cartilage matrix, the function of perlecan as chondrocytes mechanotransducer is also decreased. Therefore, the chondrocytes respond to this condition is an interesting issue to be analyzed. The hyperglycemia had the ability to induce a different response of chondrocytes to produce perlecan. It could be analyzed by mRNA level measurement. The mRNA level of perlecan within DM groups was gradually decreased: 0.080908 fold change (DM1 group) to -0.28845 fold change (DM2 group) to -0.53711 fold change (DM3 group). This result showed that hyperglycemia had an effect on osteoarthritis disease which demonstrated by protein expression and mRNA level of perlecan as chondrocytes response. Moreover, the mRNA level within ACLT groups was increased from -0.54702 fold change to -0.19522 fold change whereas from 11 weeks to 12 weeks of maintenance. It means that without the hyperglycemia at articular cartilage, the chondrocytes respond to produce perlecan could be gradually increased over time. This condition was supported by the other data at 14 weeks maintenance which showed that mRNA level of perlecan increased into 0.075356 fold change at ACLT3 group.

Hyperglycemia impacted to perlecan aorta endothelial by increasing the atherosclerotic process (9), as demonstrated by present research. Result showed that duration of hyperglycemia had an effect to perlecan articular cartilage by decreasing the protein expression of perlecan. Moreover, the duration of hyperglycemia also had an impact on mRNA level of perlecan by decreasing them. When this condition was continued over time, the perlecan level will be lower. The decreased of perlecan articular cartilage will reduce the articular cartilage matrix integrity (16) and change the differential phase of chondrocytes to hypertrophy (17). The chondrocytes hypertrophy will increase the catabolic factors which promote the degradation of articular matrix (18). All of this process is badly impacted to articular cartilage. So that, osteoarthritis disease which followed by diabetes mellitus disease will decrease the perlecan of articular matrix over time. Moreover, it will be followed by decreased response of chondrocytes to produce perlecan articular matrix. It seems that hyperglycemia had ability to reduce the molecular function and cellular response. This result is opposite to the ACLT groups which showed that protein expression of perlecan was increased gradually. It supported by chondrocytes response which increased the perlecan production through transcription process resulted increased mRNA level of perlecan over time. Therefore, chondrocytes will produce perlecan articular cartilage without the influence of hyperglycemia condition. The present research needs to be further explored to identify whether hyperglycemia had ability to induce another molecular functional and other cellular responses. So that, molecular and cellular pathology of osteoarthritis and diabetes mellitus will be exhibited.

6. CONCLUSION

Duration of hyperglycemia increases the severity of articular cartilage damage by decreasing the protein expression of perlecan. Moreover, it also reduced the chondrocytes to respond as repairing system of the microcellular environment of articular cartilage by decreasing the mRNA level of perlecan over time. The decreased protein expression of perlecan was depicted the amount of perlecan quantity at articular cartilage tissue. It is a fundamental part of the next research of proteomics. Moreover, decreased mRNA level of perlecan represents the cellular response to produce a new perlecan which can be used to the next genomic research of perlecan articular cartilage under hyperglycemia condition at rat model. This research can be used as a guide for reducing the severity of osteoarthritis disease by controlling carbohydrate intake to avoid the hyperglycemia condition.

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