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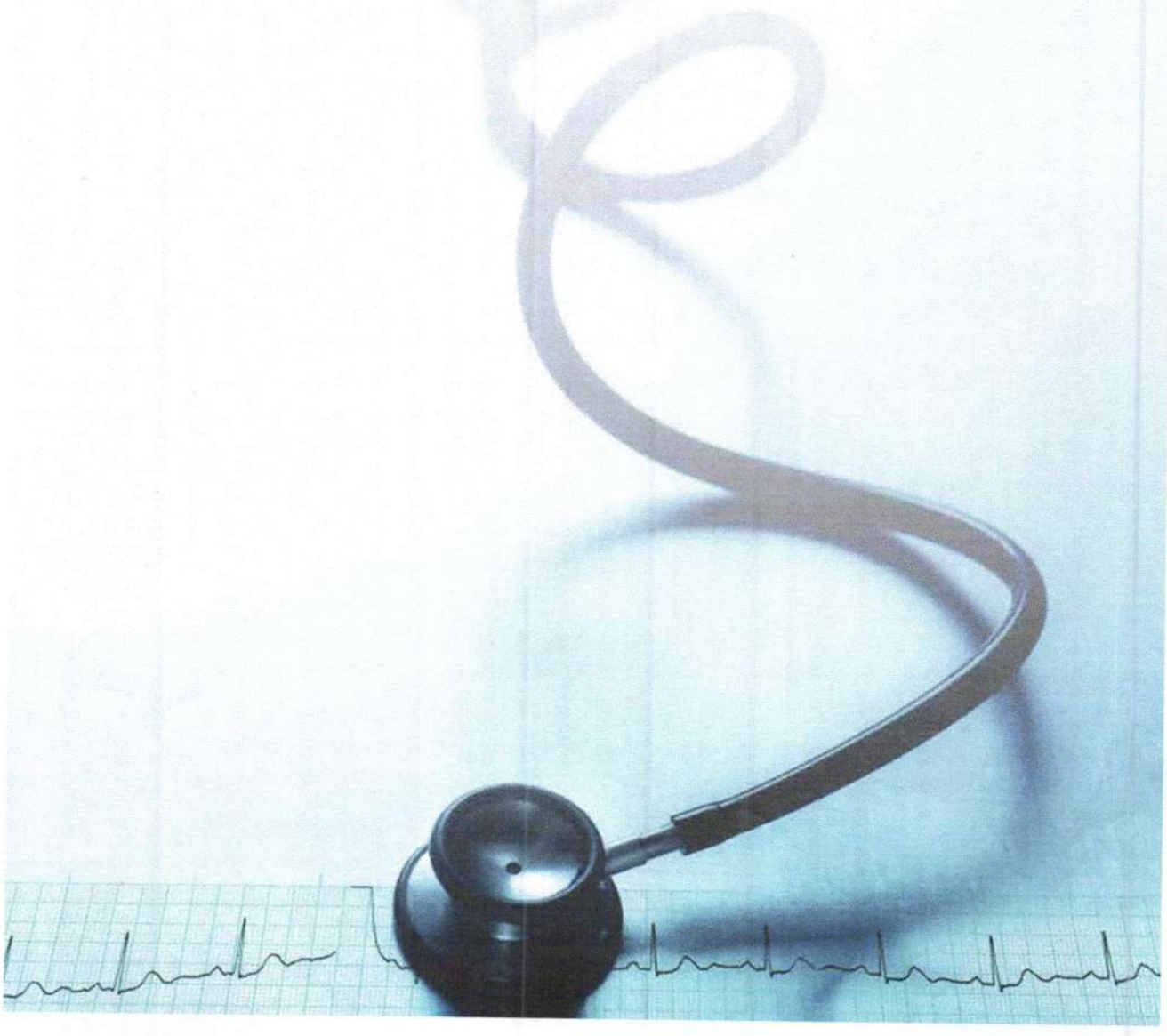

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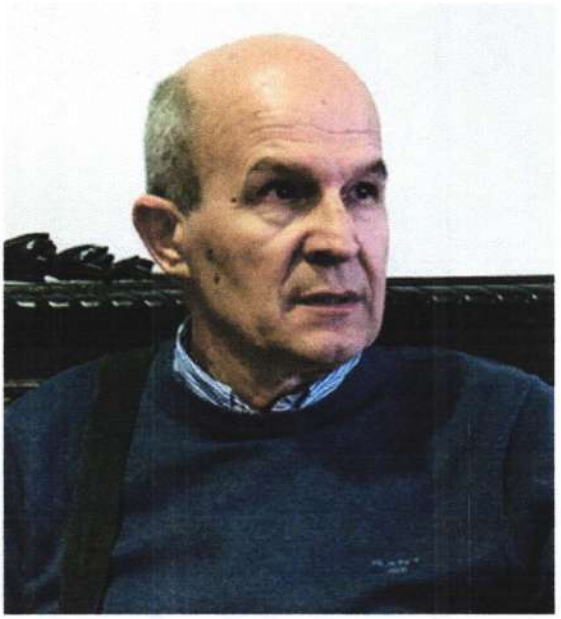

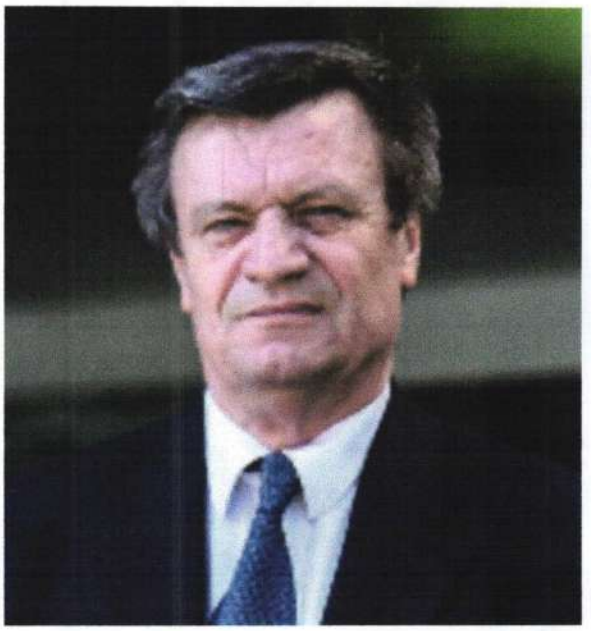

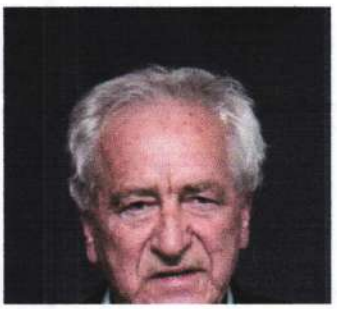

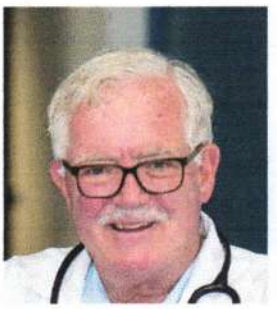

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Chondrocyte Intracellular Matrix Strain Fields of Articular Cartilage Surface in Hyperglycemia Model of Rat: Cellular Morphological Study

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ABSTRACT

Introduction: Chondrocyte is one cell in articular cartilage was products many proteins, molecules, and other factors. The external influence of cartilage, such as: hyperglycemia was entering joint capsule and impact to the chondrocytes and the cartilage. Hyperglycemia caused modification of heparan sulfate proteoglycan 2 (perlecan) proteins through glycation process. **Aim:** The aim of this study was to analyze morphological changing of chondrocytes pericellular matrix by the influence of hyperglycemia. **Material and Methods:** Eighteen adult male rats were divided into six groups: control, rat treated with sugar intake was 0.5 mg/kg, 0.75 mg/kg, 1mg/kg, 1.5 g/kg and 2 mg/kg of body weight. The animal model was dislocated and left knee was taken to observe changing of chondrocytes pericellular matrix strain fields by Scanning Electron Microscope (SEM) from perpendicular to femoral condylus cartilage. **Results:** Changing of chondrocytes intracellular matrix strain fields as changing of cell diameters and cell distances at group control and group I to group V, which cell diameters was lower level and cell distances was the highest level at over diet 2. This changing of intracellular matrix strain fields was corresponding to changing chondrocytes morphology in hyperglycemia condition, due to hypertrophic stage as adaptive responses. This research as based on next research for accomplish of hyperglycemia influence to morphology articular cartilage changing to prevent degeneration of cartilage towards osteoarthritis. **Conclusions:** Present study concludes that hyperglycemia influence to chondrocyte intracellular matrix strain fields changing.

Keywords: cartilages, chondrocytes, heparan sulfate proteoglycan 2 (perlecan) proteins, hyperglycemia,

1. INTRODUCTION

Research on the effect of excessive carbohydrate intake on joint health, is still not explained yet in detail about changes in the morphology of chondrocytes in the articular cartilage. Previous studies have suggested that enlargement of chondrocyte size due to the influence of hyperglycemia in diabetes patients (1). The chondrocytes in the hypertrophy phase is not beneficial for cartilage health, because according to previous studies, it was found that hypertrophic chondrocytes secrete catabolic factors over anabolic factors, thus risking the integrity of the articular cartilage matrix (2,3). In addition to other previous studies, it was found that type 2 diabetes mellitus was a common predictor of severity progression of osteoarthritis disease (4).

This study is expected to fulfill the research gap before diabetes emerged, it starts hyperglycemia condition, there has been a change in chondrocytes which at the risk of causing articular cartilage degeneration. Other literature states that osteoarthritis also increases the risk of type 2 diabetes mellitus (5). Various results from previous studies have not been explained about changes at the cellular level, when hypertrophy of chondrocytes happens, it will have an impact on the increase in secretion of catabolic factors over catabolic factors. The matrix degradation was starting at the superficial layer of articular cartilage due to mechanical compression during joint movement (6). This condition lasts will have an impact on articular cartilage erosion. The entire process has not been carried out research on the length of the chondrocyte diameter and the distance between chondrocytes in the

	C	SI 0,5	SI 0,75	SI 1	SI 1,5	SI 2
Blood Sugar level (mg/dl)	86.33±0,58	143,33±1,53	160.67±0,58	127.83±1,04	201,77±1,97*	202±2,64*

Table 1. Blood sugar level of control group and sugar intake treatment group. C = control; SI: Sugar intake (0,5mg/kg BW; 0,75mg/kg BW; 1mg/kg BW; 1,5mg/kg BW; 2mg/kg BW). * = significant different level (p<0,05)

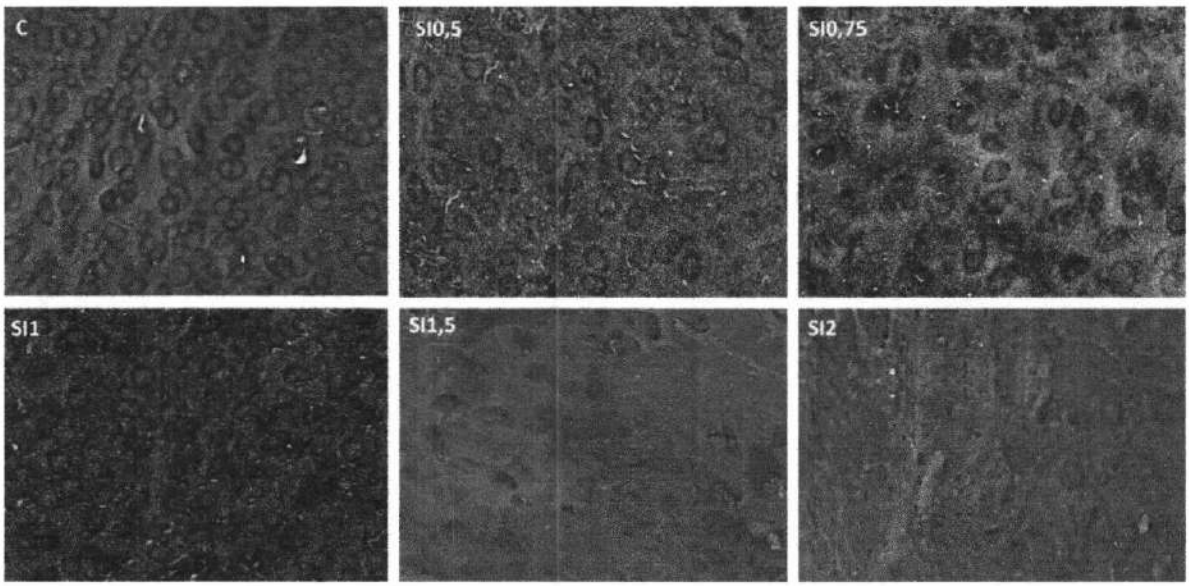


Figure 1. Morphology of chondrocyte and pericellular matrix (1000x) by Scanning Electron Microscope (SEM). C = control; SI: Sugar intake (0,5mg/kg BW; 0,75mg/kg BW; 1mg/kg BW; 1,5mg/kg BW; 2mg/kg BW).

surface layer of chondrocytes in hyperglycemia condition.

The parameters are expected to represent the chondrocytes intracellular matrix strain fields, which are needed to reflect the integrity of the articular cartilage matrix. The results of morphological changes in chondrocytes include superficial cell diameters and cell distance at the superficial layer of articular cartilage giving answers to the morphological changes in chondrocytes when cartilage degeneration begins, which is the starting point for osteoarthritis.

2. AIM

The aim of this study to analyze occurrence of hyperglycemia will increase the risk of osteoarthritis due to degeneration of the superficial layer of the articular cartilage caused by chondrocytes during the hypertrophy phase secreting catabolic factors over anabolic secretion.

3. MATERIAL AND METHODS

The experimental animal model was eighteen of male rats (*Rattus norvegicus* strain Wistar), 1.5 to 2 months old and 100 to 150 g body weight, divided into six groups of control group and rat was oral administration with sugar intake 0.5 mg/ kg BW (SI0,5); 0.75 mg/ kg BW (SI0,75), 1mg/kg BW (SI1), 1.5 g/kg BW (SI1,5) and 2 mg/kg BW (SI2). Sugar intake treatment for three times daily (in the morning, afternoon and evening). All groups were fed once a day in the afternoon was given 30 g of the standard diet.

All treatment was maintained for two months, after that all animal models were taking a blood sample to check the blood glucose level before being sacrificed. All treatment above was doing at standard above and sugar intake treatment until hyperglycemia condition. The sample was right condylar of femur bone, and processing to observe at the highest point of condylar with the perpendicular laser shooting of Scanning Electron Microscope device. Each condylar was shot for three times. All data of morphological chondrocytes changing was processed by Olympus TM 1000. Then the data continue to be analyzed with statistical analysis by one-way ANOVA. This study had approved by the research Ethics committee of Brawijaya University, Malang, East Java, Indonesia

4. RESULTS

The blood sugar level of SI2 group highest level significantly among of all treatment was 202±2,64* (Table 1). Whereas, SI 1,5 group was higher blood glucose level compared with control group, low sugar intake and standard. Control group and lower sugar intake (S10,5; SI0,75 and SI1) have still range 86 mg/kg BW until 160.67 mg/kg BW.

The morphological changing of chondrocytes at the superficial layer of articular cartilage by Scanning Electron Microscope for measure cell diameters and distance can show in Figure 1 and 2.

The group of SI1, 5 and SI2 shows that the cell is not compact and rarely (Figure 1 and 2). Vice versa, Control group and the lower sugar and standard sugar intake still show compact cell. This condition also supported with the cell diameter of chondrocyte show that SI1 and SI2 was shortest diameter significantly among of all groups (Figure 3A). Whereas, the cell distances of chondrocyte

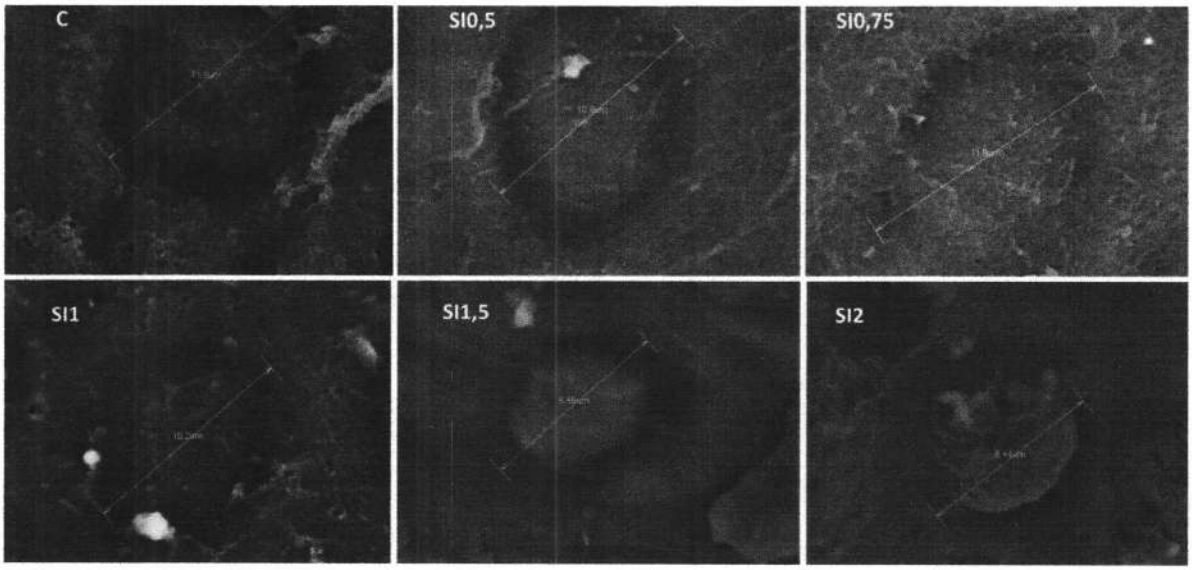


Figure 2. Morphology and size cell of chondrocyte and pericellular matrix (9000x) by Scanning Electron Microscope (SEM). C = control; SI: Sugar intake (0,5mg/kg BW; 0,75mg/kg BW; 1mg/kg BW; 1,5mg/kg BW; 2mg/kg BW).

of group SI1,5 and SI2 was increasing significantly that show the cell is rarely along with increasing blood sugar level (Figure 3B).

The result showed that blood sugar level was increased, according to carbohydrate diet in the SI 0.5 group with BSL: 142 mg/dl; SI 0.75 group with BSL: 160.5 mg/dl and SI 1.0 group with BSL: 127.0; SI 1.5 group with BSL: 200.3 mg/dl and SI 2 group with BSL: 184.0 mg/dl, all of five groups has BSL above the control group. The result of cell diameters was decreasing according to BSL increasing, but cell distances were increasing. The most significant change of the longest diameter at SI 1.5 group: 9.69 μM and at SI 2 group: 8.51 μM comparable to control group: 11.40 μM. The most significant of cell distances changing was increased in SI 1.5 group: 5.25 μM and 10.29 μM. This condition is shown that increasing of blood sugar level of animal model influence to chondrocytes shape and formation.

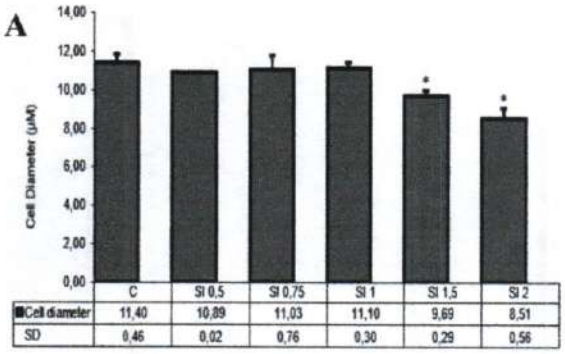
5. DISCUSSION

The previous studies result that was a link between hyperglycemia in type 2 diabetes with osteoarthritis disease (7). Hyperglycemia induce chondrocytes to produce pro-inflammatory mediators, such as: advanced glycation end products (AGEs); known as chondrocytes activation (8). This condition increasing local toxicity to joint tissue which caused chondrocytes dedifferentiation and goes apoptotic (9). Above condition has not explained cellular and morphological changes of chondrocytes formation at articular cartilage. Previous literature stated that chondrocytes will become hypertrophic (10) during osteoarthritis. Current conditions occur in the articular cartilage and have not been explained how the morphology chondrocytes changing, especially at the superficial layer of cartilage. The present study has an answer about this morphological changing of chondrocytes at the superficial layer of articular cartilage.

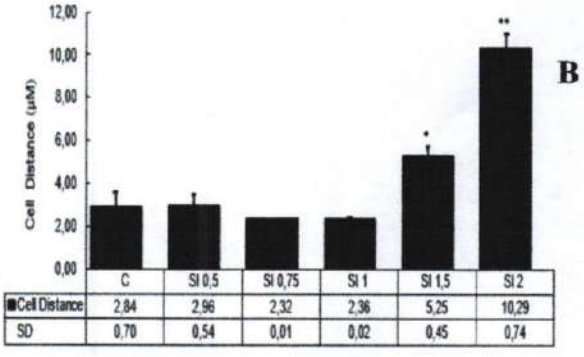
The result of this research was representative of the morphological condition of chondrocytes, which being influenced by hyperglycemia at five groups of treatment. The impact of excess carbohydrate diet was increasing of blood sugar level of animal models which caused the changing of cell diameters and cell distances. Cell diameters at this study were measurement of the longest diameter of each chondrocyte, cell distances was representative of chondrocytes anatomical form. It represents that excess carbohydrate diets influence to chondrocytes at the superficial layer of articular cartilage of animal model. The long diameters of chondrocytes were increasing, it means that chondrocyte has rounder shape and less flat form, at normal condition the chondrocyte form of superficial layer must be flat.

Shape changing of decreasing of long diameters of chondrocytes at this study as a sign which chondrocytes entering a hypertrophic phase from proliferation phase. This condition will give no good impact to cartilage health in the future, because hypertrophic chondrocytes produce more catabolic factor such as: IL-1ß (Interleukin 1 beta), FGF-2 (Fibroblast Growth Factor 2) which increasing of cartilage matrix degradation (11). Changing of chondrocytes distance as a sign that hyperglycemia of animal model at present study showed the negative impact to the chondrocytes population at the superficial layer of cartilage layer. It means that hyperglycemia influence to chondrocytes formation, more rarely than normal condition. Changing of chondrocytes diameter and cell distances as representing the condition of chondrocytes formation, which two parameters indicate of changing of intracellular matrix strain fields changing at the superficial layer of articular cartilage.

When chondrocytes formation at superficial layer was changed, it can be followed by changing of chondrocytes intracellular matrix strain fields. Chondrocytes are the only permanent residence at articular cartilage (12). It produces many major and minor components of articular cartilage, such as: collagen type II, glicosaminoglican and hialuronan, woven around condrosit as a major component (13),(14), and collagen type V, VI, IX, X, XI,



	C	SI 0,5	SI 0,75	SI 1	SI 1,5	SI 2
Cell diameter	11,40	10,89	11,03	11,10	9,69	8,51
SD	0,46	0,02	0,76	0,30	0,29	0,56



	C	SI 0,5	SI 0,75	SI 1	SI 1,5	SI 2
Cell Distance	2,84	2,96	2,32	2,36	5,25	10,29
SD	0,70	0,54	0,01	0,02	0,45	0,74

Figure 3. A) Cell diameters and B) Cell distances of chondrocyte and pericellular matrix. C = control; SI: Sugar intake (0,5mg/kg BW; 0,75mg/kg BW; 1mg/kg BW; 1,5mg/kg BW; 2mg/kg BW). * = significantly different level (p<0,05)

XII, XIV, decorin, biglican, fibromodulin, perlecan; non colagenus protein: matrilins, trombospondin-5/ COMP (15),(16),(17). All of the components above need to support matrix integrity, if chondrocytes distance was increased by the influence of hyperglycemia at animal model, it increases risk to lowering of matrix density, which can decrease intracellular matrix strain fields by the dynamical movement of the joint (18). It can increase the risk of articular cartilage damage, beginning at superficial layer.

6. CONCLUSION

This study concludes that hyperglycemia can be act as a trigger factor of osteoarthritis disease, which it can be worse by chondrocytes senescence as a pathogenic factor. Present as basic to continue with next research which can measure of cartilage pericellular matrix strain fields and counting of the chondrocyte's population at the surface, middle and deep layer of articular cartilage of animal model, as complement to the pathophysiology of correlation between diabetic with osteoarthritis diseases. Present study shown that hyperglycemia by excess carbohydrate diets already influence to articular cartilage, so the suggestion was controlling carbohydrate diets need to support articular cartilage in the future.

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- **Authors' contributions:** All the authors were involved during the investigation process in all stages of this study including a primary data collection, analysis and the documentation of the collection.
- **Conflict of interest:** none declared

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