

Hnatiuk Mykhailo, Rubas Lesia. Morphometric evaluation of features of remodeling of chondrocytes of joint surfaces of temporomandibular joint of experimental animals in hyperglycemia. Journal of Education, Health and Sport. 2021;11(06): 137-142.
eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2021.11.06.014>
<https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2021.11.06.014>
<https://zenodo.org/record/5035110>

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation, § 8. 2) and § 12. 1. 2) 22.02.2019.

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The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 21.05.2021. Revised: 03.06.2021. Accepted: 25.06.2021.

MORPHOMETRIC EVALUATION OF FEATURES OF REMODELING OF CHONDROCYTES OF JOINT SURFACES OF TEMPOROMANDIBULAR JOINT OF EXPERIMENTAL ANIMALS IN HYPERGLYCEMIA

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Abstract

Aim: to study morphometrically the peculiarities of remodeling of chondrocytes of the articular surfaces of the temporomandibular joint in hyperglycemia.

Materials and methods: Quantitative morphological methods were used to study diameters of chondrocytes and their nuclei, nuclear-cytoplasmic relationship in these cells, relative volume of damaged chondrocytes, intercellular-chondrocytic relationship in mature chondrocytes of the articular surfaces of the temporomandibular joint of 45 laboratory mature white male rats with simulated diabetes.

Results: A comprehensive analysis of the morphometric parameters presented in the article showed that they did not differ in the articular surfaces of the temporal fossa and mandibular head of the intact temporomandibular joint. The expressed structural changes of chondrocytes were revealed at a monthly and a two-month diabetic arthropathy of this joint.

Conclusions: Proceeded studies and obtained results suggest that prolonged hyperglycemia leads to diabetic arthropathy of the TMJ, which is characterized by atrophy of chondrocytes, violation of nuclear-cytoplasmic relationships, an increase in the relative volume of damaged chondrocytes and volume of intercellular substance. The detected changes in quantitative morphological parameters depended on the duration of hyperglycemia and dominated in the cartilaginous tissue of the articular surface of the mandibular head.

Key words: **chondrocytes; temporomandibular joint; hyperglycemia; cartilaginous tissue.**

Diabetic arthropathy in hyperglycemia is a common complication. Thus, joint damage is diagnosed in 58 % of patients in type 1 diabetes, and in 28 % of patients in type 2 diabetes [5, 6]. This pathology disrupts carbohydrate, protein, mineral metabolism, causes hormonal disorders, angio- and neuropathies, which are complicated by bone resorption due to imbalance of osteoblast and osteoclast activity [4,6]. It should also be noted that the development of diabetic arthropathy is a complex and multifaceted process, which has been insufficiently studied. The temporomandibular joint (TMJ), which is formed by the mandibular fossa of the temporal bone, the head of the mandible and the intra-articular disc, is also affected by diabetes. It should be noted that the peculiarities of remodeling of cartilage chondrocytes in the articular surfaces of the TMJ in hyperglycemia remain unexplored. At the same time, the study of structural changes in chondrocytes in cartilage is important for explaining reparative chondrogenesis [6].

The purpose of the work is to study morphometrically the peculiarities of remodeling of chondrocytes of the articular surfaces of the TMJ of experimental animals with hyperglycemia.

Material and methods. Quantitative morphological methods were used to study mature chondrocytes of the articular surfaces of the TMJ of 45 laboratory mature white male rats, which were divided into 3 groups. The first group consisted of 15 intact practically healthy animals, the 2nd included 15 rats with monthly diabetic arthropathy of the TMJ, the 3rd had 15 animals with two-month specified pathology. Diabetes mellitus was simulated by a single intraperitoneal injection of streptozotocin «Sigma» at a dose of 50 mg/kg [8]. Euthanasia of animals was performed in terms of first and second months after the start of the experiment by bloodletting under thiopental anesthesia. Histological specimens were made from the temporal bone and mandible [3].

The diameters of chondrocytes (DC) and their nuclei (DNC), nuclear-cytoplasmic relationship in these cells (NCRC), relative volume of damaged chondrocytes (RVDC), intercellular-chondrocytic relationship (ICR) were determined in the cartilaginous tissues of the mandibular fossa of the temporal bone and the head of the mandible.

Chondrocyte morphometry was performed using a system of visual analysis of histological specimens, images of which were displayed on a computer monitor from a microscope MICROMed SEO SCAN and using a Vision CCD Camera. Morphometric studies were performed using Video Test-5.0, KAAPA Image Dase and Microsoft Excel on a personal computer.

All manipulations and euthanasia of rats were carried out in accordance with the basic principles of work with experimental animals in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986), "General Ethical Principles for Experiments on Animals" adopted by the First National Congress of Bioethics (Kyiv, 2001) [7], as well as the Law of Ukraine "On protection of animals from cruel treatment" (from 21.02.2006).

The obtained quantitative indicators were processed statistically. Processing of the obtained results was performed in the Department of System Statistical Research of Ternopil National Medical University in the software package STATISTIKA (Stat. Soft. Inc., USA). The difference between the comparative indicators was determined by the criteria of Student and Mann-Whitney [1, 2].

Results and discussion. The data obtained as a result of the study are shown in Table 1. A comprehensive analysis of the morphometric parameters presented in this table showed that they did not differ in the articular surfaces of the temporal fossa and mandibular head of the intact TMJ. The expressed structural changes of chondrocytes are revealed at a monthly and a two-month diabetic arthropathy of a TMJ. Thus, the diameter of chondrocytes in the cartilaginous tissue of the articular surface of the fossa of the temporal bone was statistically significantly ($p < 0.05$) decreased from $(11.20 \pm 0.12) \mu\text{m}$ to $(10.75 \pm 0.12) \mu\text{m}$, that is 4.0 %, and the diameter of their nuclei lowered by 2.3 % in a monthly experiment. Uneven changes in the spatial characteristics of the nucleus and cytoplasm of chondrocytes led to violations of the relationship between them, which determined the nuclear-cytoplasmic relationship. Thus, the nuclear-cytoplasmic relationship in chondrocytes increased from (0.256 ± 0.002) to (0.268 ± 0.002) . A statistically significant difference ($p < 0.01$) was found between the given morphometric parameters. The last index exceeded the previous one by 4.7 %. Some

researchers claim that changes in nuclear-cytoplasmic relationships in cells indicate a violation of structural cell homeostasis [1].

Table 1. Morphometric parameters of chondrocytes of the articular surfaces of the temporomandibular joint of experimental animals ($M \pm m$)

Indicator	Observation group		
	1-a	2-a	3-я
Articular surface of the mandibular fossa of the temporal bone			
DC, μm	11,20 \pm 0,12	10,75 \pm 0,12*	9,82 \pm 0,09***
DNC, μm	5,67 \pm 0,04	5,56 \pm 0,03**	5,20 \pm 0,02***
NCRC	0,256 \pm 0,002	0,268 \pm 0,002**	0,280 \pm 0,003***
RVDC, %	2,08 \pm 0,02	9,80 \pm 0,12***	13,50 \pm 0,15***
ICR	0,144 \pm 0,001	0,155 \pm 0,002***	0,166 \pm 0,002***
Articular surface of the mandibular head			
DC, μm	11,22 \pm 0,12	10,82 \pm 0,12*	9,75 \pm 0,09***
DNC, μm	5,65 \pm 0,05	5,62 \pm 0,04	5,20 \pm 0,02***
NCRC	0,255 \pm 0,003	0,270 \pm 0,003*	0,284 \pm 0,003**
RVDC, %	2,10 \pm 0,18	13,65 \pm 0,15***	15,20 \pm 0,15***
ICR	0,145 \pm 0,001	0,158 \pm 0,002**	0,172 \pm 0,002***

Note. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$, compared with the 1st group

The relative volume of damaged chondrocytes increased in these experimental conditions with a pronounced statistically significant difference in 4.7 times ($p < 0.001$), and intercellular-chondrocyte relationship lowered by 7.6 % ($p < 0.001$).

The studied morphometric parameters changed more markedly in two-month diabetic arthropathy. Thus, the diameter of chondrocytes of the articular surface of the fossa of temporal bone was statistically significantly ($p < 0.001$) decreased by 12.3 %, and the diameter of their nuclei lowered by 8.3 % ($p < 0.001$). Nuclear-cytoplasmic relationships increased in the studied cells with a pronounced statistically significant difference ($p < 0.001$) by 9.4 %, the relative volume of damaged chondrocytes grew up in 6.5 times ($p < 0.001$), intercellular-chondrocyte relations increased by 15.3 % ($p < 0.001$). Changes of the last indicator showed an increase in the cartilaginous tissue of the articular surface of the intercellular substance.

The studied morphometric parameters of chondrocytes changed similarly, but more pronounced, in the cartilaginous tissue of the articular surface of the mandibular head of the TMJ in experimental hyperglycemia.

Thus, the diameter of chondrocytes was statistically significantly ($p < 0.05$) decreased by 3.56 % and the diameter of their nuclei lowered only by 0.53 % in monthly diabetic

arthropathy of the TMJ. Nuclear-cytoplasmic relationships in chondrocytes increased by 5.9 % ($p <0.001$), the relative volume of damaged chondrocytes grew up in 6.5 times ($p <0.001$), intercellular-chondrocyte excitations increased by 8.9 % ($p <0.001$). The studied morphometric parameters of chondrocytes changed accordingly by 13.1%; 7.9%; 11.3%, in 7.2 times and by 18.6% ($p <0.001$) at two-month diabetic arthropathy of the TMJ. The presented and analyzed morphometric parameters of chondrocytes indicate that the most pronounced degree of their remodeling was in two-month diabetic arthropathy and dominated the cartilaginous tissue of the articular surface of the mandibular head of the TMJ.

Conclusions. Thus, proceeded studies and obtained results suggest that prolonged hyperglycemia leads to diabetic arthropathy of the TMJ, which is characterized by atrophy of chondrocytes, violation of nuclear-cytoplasmic relationships, an increase in the relative volume of damaged chondrocytes and volume of intercellular substance. The detected changes in quantitative morphological parameters depended on the duration of hyperglycemia and dominated in the cartilaginous tissue of the articular surface of the mandibular head.

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