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PROPERTIES OF THE STEM CELLS OF THE PRIMITIVES OF THIRD MOLARS

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Summary.

Regenerative medicine becomes an interesting field of research that solves the problem of the treatment of severe illnesses by replacing the affected structures with cell-based therapy or tissue engineering using autogenous stem cells. Due to these researches over the past decade, scientists were able to find new sources of stem cells in the adult body, improve treatment methods of various diseases, regenerate and replace tissues of many organs, and even correct some congenital defects. Systematization of theoretical knowledge and clinical research in the field of regenerative medicine is the basis for further selection of the proper stem cells sources and development of the best methods of regeneration and their successful application in practical medicine.

The dental germs of the third permanent molars were used as research objects. To obtain the materials, the germs of the third molars were taken from 20 patients aged from 12 to 25 years old during the surgical stage of orthodontic treatment. Dental manipulations were performed after the signing of the voluntary information consent on conducting clinical trials by patients. It was decided to create three observation groups, taking into account the stage of formation of the third molars germs and the age of patients. The first group consisted of patients with the third molars germs at the stage of unformed root; the second – patients with the third molars germs with formed root and unformed apex; the third one – patients with formed root and formed apex of the third molars germs. Methods of the research: radiological – to assess the stage of development of the third molar germs; histological – for the morphological characteristics of the third molar germs tissues; immunohistochemical – to characterize the degree of maturity of the third molar germs tissues.

The unformed root stage contains more reserve of poorly differentiated cells of mesenchymal origin. The light color of the preparation indicates on a low concentration of protein Vimentin in the early stages of development, which indicates on low cell differentiation. At the same time, histological examination of third molars germs of the third observation group showed the presence of highly differentiated cells such as fibroblasts and fibrocytes, as well as a significant content of vimentin protein, which confirms the maturity of these cells.

The obtained results allow us to identify the most suitable collection periods of the development for the third molars germs. It gives the opportunity to expand the scientific data on the properties of mesenchymal stem cells of odontogenic origin and to investigate further use of mesenchymal stem cells of odontogenic origin in practical medicine.

Introduction.

Regenerative medicine is an interesting and developing branch of science nowadays, covering many areas of stem cells usage in diseases treatment of various origin [1].

Mesenchymal stem cells derived from the bone marrow and adipose tissue, are actively used in diseases treatment of cardiovascular, gastrointestinal, musculoskeletal, nervous systems, malignant blood tumors, diabetes mellitus, liver cirrhosis [2]. There are studies that showed successful results of using stem cells to regenerate and replace tissues of the skin, heart, kidneys, liver [3].

A full-fledged competitive source of these cells are odontogenic mesenchymal stem cells, which in their anti-apoptotic, anti-inflammatory, angiogenetic and high proliferative indicators are not worse than those of red bone marrow and adipose tissue stem cells. Furthermore, their availability of collection is much higher. The literature describes the use of mesenchymal stem cells ofodontogenic origin for the diseases treatment of mentioned above systems and dental illnesses [3-7], which determines the relevance of our research.

The aim of the research: to study the properties of mesenchymal stem cells of odontogenic origin for further use in practice..

Research methods: radiological – to assess the stage of development of the third molar germs; histological – for the morphological characteristics of the third molar germs tissues; immunohistochemical – to characterize the degree of maturity of the third molar germs tissues.

Materials and methods

The sources of mesenchymal stem cells of odontogenic origin were chosen to be the dental germs, namely of the third permanent molars, which were removed during dental manipulations in orthodontic patients. To obtain the materials, the

germs of the third molars were taken from 20 patients aged from 12 to 25 years old during the surgical stage of orthodontic treatment. Patients underwent a complex orthodontic treatment, including surgery on extraction of the germs of the third molars, on the basis of the Department of Pediatric Dentistry of the University in the period from 2020 to 2021.

Any dental manipulations, as well as the collection of biomaterial for research, were carried out after signing by parents or patients (14 years and older), voluntary information consent to conduct clinical trials in compliance with the basic provisions of GSR (1996), Council of Europe Convention on Human Rights and Biomedicine (dated 04.04.1997), Helsinki Declaration of the World Medical Association on ethical principles of scientific medical research with human participation (1964-2013), orders of the Ministry of Health of Ukraine № 690 dated 23.09.2009.

In the course of the study, it was decided to share patients in 3 groups, taking into account the stage of development of the third molar germs and the age of the patients. The first group consisted of patients withthe third molar germs at the stage of unformed root; group II – patients with the third molar germs with formed root and unformed apex; group III – patients with formed root and formed apex. Age and gender characteristics of patients in the observation groups are given in table 1.

The stage of development of the third molar germs was determined on the basis of X-ray examination of the maxillofacial area using a digital panoramic dental Xray machine. The degree of development of the root and the crown of the third molar germs was studied on panoramic images using the X-ray classification of the stages of tooth development according to Demirjian [41].

In most cases, the biomaterial was removed in a block, which allowed to save the macroarchitectonics. Macroscopic analysis was a description of the consistency, color, nature of the surface. Samples of 0.7-0.5 cm were cut out for microscopic examination. The test material was immediately fixed in 10% neutral buffered formalin solution, 1 part 40% formaldehyde (100% formalin) was diluted with 9 parts water, adding disubstituted and monosubstituted sodium phosphate in the proportion that provides (pH=7.0). The obtained samples were washed in running water to prevent artifacts associated with incomplete removal of formalin. Then the pieces were dehydrated in alcohols of increasing concentration. According to the generally accepted method, paraffin blocks were made from tissue samples at a temperature of 64°C. As it's necessary for histological techniques, we used sections with a thickness of 5-7 μm, which were made on a sled microtome.

Histological examinations were performed on 1-2μm-thick sections with pre-treatment of slides with poly-L-lysine, which was used for stronger fixation of sections.

For review microscopy, dewaxed preparations were stained with hematoxylin and eosin.

The obtained results were investigated using an image analyzer using computer morphometric programs. The evaluation was performed using an image analyzer consisting of an Olympus BX51 microscope with a C-4040zoom digital camera and an Athlon XP 2.0 personal computer. For metric characteristics, UTHSCSA Image Tool ® for Windows ® version software was used in an interactive mode using a × 20, × 40 lens and a × 10 eyepiece. E.LEITZ WETZIAR micrometer object was used for calibration in image analysis.

Results and discussions

Our research included the evaluation of orthopantomographic images of orthodontic patients of the observation groups before the operation to remove the third molar germs. After the operation, the soft tissue of the third molar germs was subjected to histological examination and immunohistochemical analysis according to three criteria - the content of vimentin protein, Ki67 and CD-34.

Detailed analysis of panoramic images made it possible to clearly determine the stage of development

Table 1.

Age and gender characteristics of patients in the observation groups

Group	Age of patients (n)	Sex of patients		Total
		male (n)	female (n)	
I	12-15	3	3	6
II	16-19	5	4	9
III	20-25	3	2	5
Total	12- 25	11	9	20

n – the number of patients.

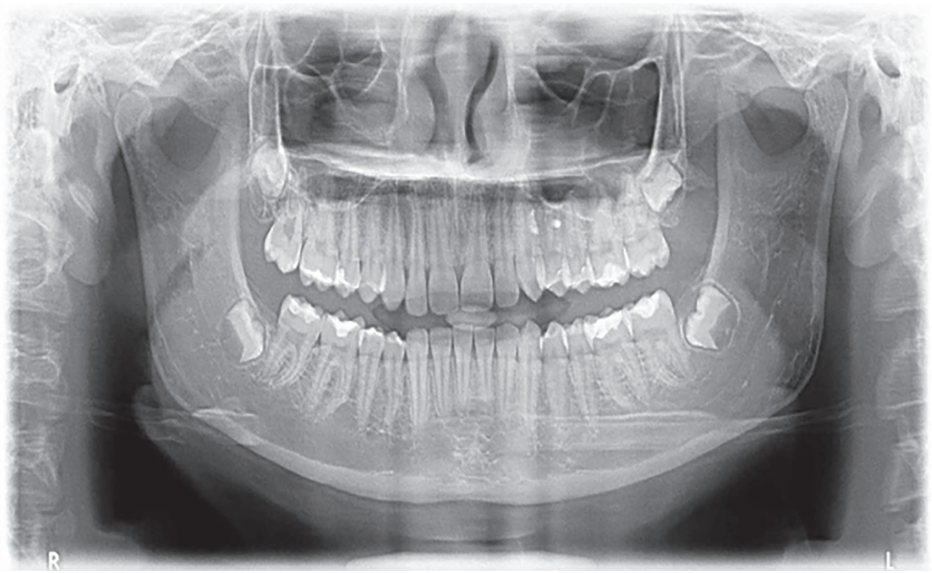

Figura 1. Orthopantomogram, orthodontic patient, X, 13 years old.

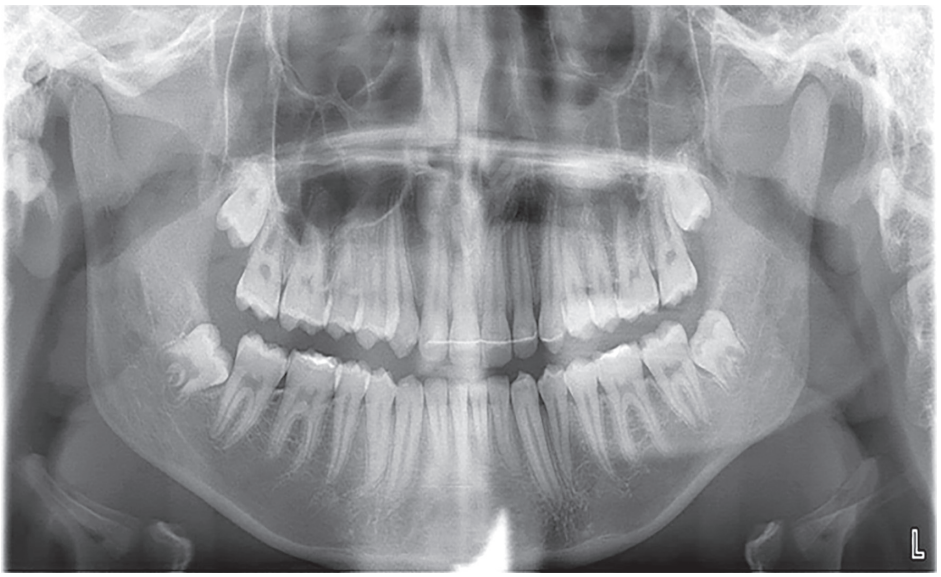

Figura 2. Orthopantomogram, orthodontic patient, Y, 18 years old.

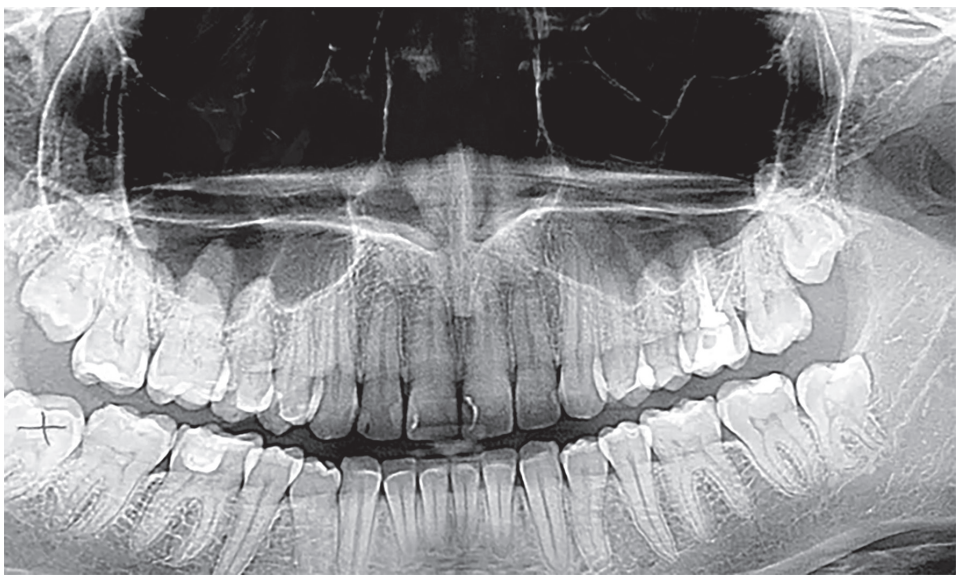

Figura 3. Orthopantomogram, orthodontic patient, Z, 23 years old.

of the rudiment of the third molar according to the Demirjian classification. The orthopantomographic images of patients of the first group revealed the third molar germs characterized by the formed crown part of the tooth up to the boundaries of the cement-enamel joint (Fig. 1).

The beging of the growth and development of the root is seen by the formation of its pulp chamber the walls of which form a spear-shaped cavity. This radiological picture of the third molar germs corresponds to the stage of unformed root, which is indicated as stage "D" according to the Demirjian classification

While analysing the orthopantomographic images of orthodontic patients of the second group of observation, it was found that the third molar germs correspond to the stage of the formed root and unformed apex, as illustrated in Figure 2.

The root of third molar germs is already visible on the images of the second group of observation. The pulp chamber of the root of these third molar germs from the spear-shaped transformed in an isosceles triangle. However, the top of the root ends in the form of a funnel, which indicates the unformed apex of the tooth. The length of the root is equal to or slightly exceeds the height of the crown. Demirjian attributes this radiological description of the third molar germs to the stage "F" of his classification.

Panoramic images of the third group of patients in addition to the above-mentioned radiological changes, namely the formed crown of the tooth and the formed root, were characterized also by a fully formed apex of the root of the third molars. The apical end of the root is completely closed and the periodontal fissure has the same width along the entire apex of the tooth. This characteristic corresponds to the formed root and the formed apex of tooth development stages. X-ray classification according to Demirgian refers such radiological description of the third molar to the last "H" stage (Fig. 3).

There were interesting results of histological examination of the third molar germs at different stages of their development. Histological preparations of the pulp of the third molar germs of the first observation group were characterized by chaotic placed oxyphilic collagen fibers which indicates the presence of loose fibrous connective tissue in the field of view. Lymphoid cells (6,8 ± 0,12)% and mesenchymal cells (18,1 ± 0,28)% were visualized among stromal ones which were mainly localized along the course of the blood vessels. The fibroblasts (1,5 ± 0,02)% had a rounded or spindle-shaped shape and at this stage of tooth development (unformed root stage) are less specialized cells. Several disordered odontoblasts were located on the periphery of the pulp tissue. The phenomenon of neoangiogenesis in the form of hematogenous islets was verified in the central layer of the pulp. The specific volume of venous vessels is bigger (4,1 ± 0,13)% than the specific volume of arterial vessels (2,4 ± 0,11)%.

Subsequent histological examinations of the third molar germs' pulp of the second observation group (stage of formed root and unformed apex) show that in the stroma of loose fibrous connective tissue replacement of young fibroblasts by more specialized fibroblastic cells occur (6,9 ± 0,09)% which form collagen bundles. The specific volume of polypotent lymphoid cells decreases to (4,3 ± 0,08)% and mesenchymal cells to (11,2 ± 0,18)% which indicates their differentiation in the extracellular matrix. Odontoblasts acquire a characteristic prismatic shape and form a peripheral layer of the tooth pulp. The microcirculation is enriched with new venous plexuses (4,8 ± 0,14)% and arterial vessels (2,7 ± 0,12)%. The total specific volume of the microcirculation increased to (3,2 ± 0,12)%. At the same time, the ratio of venous to arterial vessels remained unchanged.

The pulp of third molars of the third group of observation is the most mature in terms of quantitative and qualitative composition according to the results of histological investigation. Collagen fibers were located more densely in the form of bundles of I, II, and III orders. Fibroblastic cells at this stage accounted for a specific volume of (17,9 ± 0,24)% and structurally resemble mature fibroblasts, having an elongated shape and a relatively large number of thick processes, which indicates their high functional activity. There was a sharp decrease in the specific volume of lymphoid cells to (2,2 ± 0,03)% and of mesenchymal cells to (3,4 ± 0,07)% which indicates a qualitatively different regenerative potential. The peripheral layer of the pulp is supported by mature prismatic odontoblasts, the intermediate layer is formed by cell-free and cell-riched zones. The last one contained immature collagen fibers and spindle-shaped fibroblasts. In the central layer of the pulp there were blood vessels and islets of neoangiogenesis in addition to the above mentioned cells

Conclusions

1. A detailed histological evaluation of the three key stages of development of the third molars rudiments in accordance with their radiological picture is conducted. An increase in the total specific volume of the bloodstream from (1,4 ± 0,07) to (5,6±0,14)%, arte-

rial vessels from (2,4 ± 0,11) to (3.2 ± 0,13)%, vessels of venous type from (4,1 ± 0,13) to (6,4 ± 0,15)%, fibroblasts from (1,5 ± 0,02) to (17,9 ± 0,24)%, decrease in the specific volume of lymphoid cells from (6,8 ± 0,12) to (2,2 ± 0,03)% and mesenchymal cells from (54,8 ± 0,95) to (3,4 ± 0,07)% with the transition of the third molars germs to a later stage of development.

2. The obtained results provide an opportunity to develop practical recommendations for the optimal time of surgery to remove the rudiments of third molars on orthodontic indications in order to combine the positive effect for the formation of the maxillofacial area and for the collection of biomaterial for further use in regenerative medicine.