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Histomorphometric study regarding the evolution under treatment of the changes appearing at the level of the gingival mucosa in diabetic children

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Abstract

The values of the glucose influence the status of the periodontium, but also the periodontitis influences the glucose balance by increasing the resistance to insulin. In the case of children in the first step the gingivitis appears, than if the glucose control is not made and the dental hygiene is defective the evolution is towards advanced periodontal disease. The present histomorphometric study wants to emphasize the eventual changes that appear at the level of the gingival epithelium before and after starting a correct treatment of the periodontal disease. The histomorphometric study indicates an average nuclear area of the keratinocytes of 211.65 μm^2 in the batch studied before the application of the treatment and an increase of the average nuclear area to 234.88 μm^2 after the treatment (p=0.010538). The nuclear volume before the treatment has an average value of 2396.61 μm^3, after the treatment it reaches the value of 2996.924 μm^3. The area of the keratinocytes has an average of 495.43 μm^2, after the treatment it reaches the value of 724.89 μm^2, noticing a 14.6% (p=0.004) increase of the cellular area. Before the treatment, we notice a ballooning of the cells from the intermediary level, the existence of some pyknotic nuclei and the disappearance of the nucleoli. The associated gingival pathology *diabetes mellitus* type 1 in the case of children and teenagers is treatable within six months, macroscopically and microscopically the gingival mucosa approaching to normal conditions. In the case of children and teenagers diagnosed with type 1 diabetes, the dental check is mandatory in order to prevent the gingival and periodontal diseases.

***Keywords*:** type 1 diabetes, gingivitis, periodontitis, child.

Introduction

The susceptibility regarding the periodontal disease, many types called "the sixth complication of diabetes" [1], represents the most frequent complication of diabetes at the level of the oral cavity. The patient with badly managed diabetes has a higher risk of developing periodontal diseases. Initially, in the case of children, the gingivitis appears, then if the glucose control is not made, and the dental hygiene is defective the evolution is towards advanced periodontal disease [2]. Other studies have evaluated the loss of teeth and the presence of the total edentulous in the case of persons with type 1 diabetes [3]. When the persons suffering from diabetes are also smokers, the chances to develop the periodontal disease accompanied by the loss of the bone attachment are 20 times higher compared to the persons without diabetes [4].

The oral complications in the cases of the patients with uncontrolled diabetes are most likely connected to the modified answer towards infections, the micro-vascular changes and the increase of the glucose concentration in the saliva (salivary hyperglycemia). The salivary hyperglycemia can represent an important factor that contributes to the periodontal disease [5]. The periodontal prevention therapy must be included in the complete therapy of the patient suffering from diabetes. The therapy includes an initial evaluation of the progression risk of the oral disease, an explicit oral hygiene instruction, the food evaluation and making periodical dental checks for prevention.

The present histomorphometric study wants to emphasize the eventual changes that appear at the level of the gingival epithelium before and after starting a correct treatment of the periodontal disease.

Materials and Methods

We have included in our study 20 children of both sexes, aged between 10 and 18-year-old, selected from a batch of 98 patients from the records of the Diabetes Section of the "Sf. Ioan" Children Emergency Clinical Hospital from Galaţi.

All the patients have manifested the starting of the disease more than 10 years ago.

For each patient, the acceptance for including them in the study was signed; an individual file was made for appreciating the periodontal status.

After establishing the initial status, for each patient there was made an individual treatment protocol made up from: removing the dental plaque, scaling, the treatment of the caries, antimicrobial medical treatment

according to the bacteriological examination, in the case of partial edentation, occlusal rebalancing. All the subjects were called in for a check after six months.

In the moment when the patients were included in the batch and after six months from starting the treatment, each patient made a simple gingivectomy, at the level of 4.6, from the vestibular gum, using the circular knife. The samples were immediately put in a 10% neutral formalin solution for 24 hours at the lab temperature, than they were processed using paraffin inclusion technique, sectioned, numbered and colored Hemalaun–Eosin and Masson's trichrome.

The samples obtained were examined using a Nikon E600 microscope and put into a computer using a Sony video camera. The obtained images were processed using the image analysis software Lucia G 3.52 from the Laboratory of Histology, Faculty of Medicine, "Ovidius" University of Constanţa.

Before starting the histometric measurements using the analysis software, we have calibrated the camera objective that we used. We used for the histometric analysis the 20× camera objective, and its calibration was made using a Carl Zeiss Jena standard blade on which it is engraved a standard micrometric scale. On the image taken and acquired on the computer there was defined the number of pixels equivalent for 1 μm. The next step in the histometric examination was to define the color threshold. On the images acquired on the computer there was defined the color threshold adequate for the examined structure (cytoplasm, nucleus) definition that is the same for all the examined samples. The result of this initial calibration of the color threshold was the obtaining of a "binary" image in which conventionally "0" corresponds for the black color and "1" corresponds for the white color. Based on these binarized images, the processing software Lucia G 3.52 using the matrix calculation, can accurately measure the parameters required by the user. Thus, we could determine:

- At the level of the nucleus: the equivalent diameter, the nuclear area and the nuclear volume;
- In the case of the cells: the equivalent cellular volume, the perimeter and the fraction area.

For each subject, there were studied 100 contiguous microscopic fields during the examination, moving the section as in the case of the leukocyte formula. The quantitative data obtained were put into charts on batches on which the average was made. There was calculated according to the batches the dynamics and the density of the cells, on two batches using the Student–Fisher method (the *t*-test for the populations with unequal dispersions), when a statistical coefficient $p<0.05$ is considered significant.

Results

The histological study of the gum fragments of the patients with type 1 diabetes, samples taken before the treatment has emphasized in the majority of the cases changes at the level of all the covering epithelium layers. Being considered the purest tissues, the epitheliums are made up from own cells, powerfully connected. The penetration of the inflammatory cells at the level of the epithelium, leads to the destruction of the intracellular connections and the manifestation of ulcerations (Figure 1).

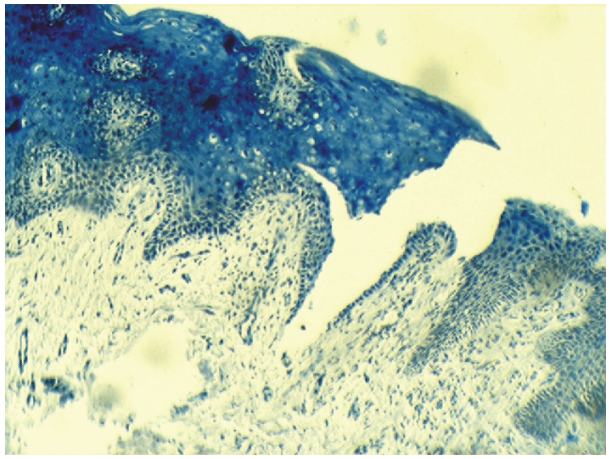

Figure 1 – ***The covering epithelium showing the destruction of the cellular connections and ulceration, batch before the treatment (Masson's trichrome stain, ob. ×20).***

At the level of the intermediary layer it can be noticed the presence of acanthosis and the elongation of the delomophic and allomorphic papillae (Figure 2).

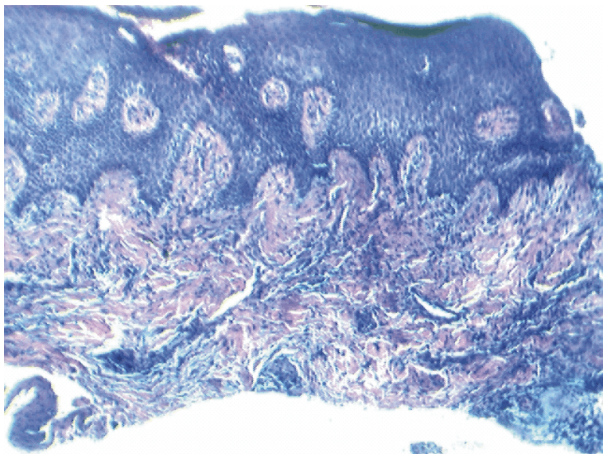

Figure 2 – ***The emphasis of the delomophic and allomorphic papillae, batch before the treatment Masson's trichrome stain, ob. ×20).***

The cells mainly keep their polyhedral shape, but ballooning can sometimes appear. The nuclei have smaller sizes and loose their nucleoli. In the superficial layer ortho-keratinisation and para-keratinisation phenomena can appear. After applying the treatment, the inflammatory infiltration and the ulcerations disappear. Mainly the intercellular connections are remade. In the thorny layer, the cells maintain their polyhedral shape; the nuclei are large and centrally disposed with evident nucleoli.

After the treatment, the epithelium has an aspect similar with the normal one.

The average values before and after the treatment of the nuclear area, the nuclear volume and the cellular volume are shown in Figures 3 and 4.

The histomorphometric study indicates an average nuclear area of the keratinocytes of 211.65 μm^2 in the batch studied before the treatment and an increase of the average nuclear area to 234.88 μm^2 after the treatment, (p=0.010538). The nuclear volume before the treatment has an average value of 2396.61 μm^3, after the treatment it reaches the value of 2996.924 μm^3. The area of the keratinocytes has an average of 495.43 μm^2, after the treatment it reaches the value of 724.89 μm^2, noticing a 14.6% (p=0.004) increase of the cellular area.

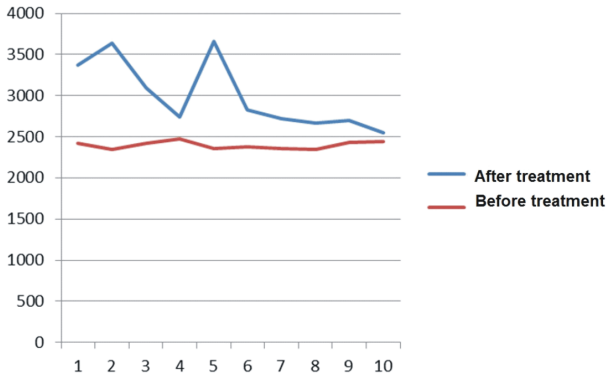

Figure 3 – ***The graphical representation of the nuclear area dynamics within the batch, before and after the treatment.***

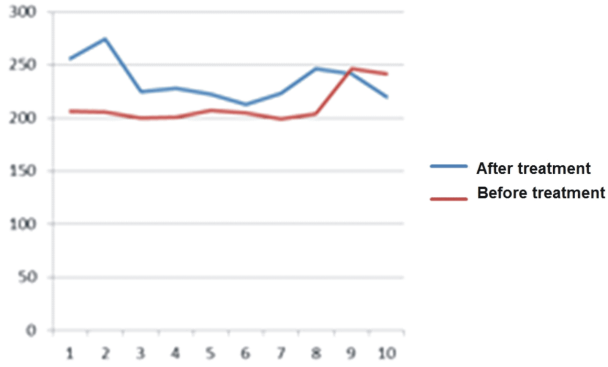

Figure 4 – ***The graphical representation of the nuclear volume dynamics within the batch, before and after the treatment.***

Discussion

The *American Diabetes Association* when it takes into account the diagnosis, the management and the treatment for the patients under the age of 18 years says "children are not simply small adults". In the case of children and teenagers having type 1 diabetes, the symptoms and also the starting of the disease significantly differs from the adult patients [6].

The studies published in the *Journal of American Dental Association* made on large batches of subjects diagnosed with type 1 diabetes, indicates the fact that until the age of 12 years, the periodontitis does not appear, between 13 and 18-year-old, the periodontitis is identified in 13% of the patients, these usually having an uncontrolled glucose and a poor oral hygiene.

The incidence of periodontitis suddenly increases to 39% at the age of 19 years. The studies have shown that in the case of the patients under the age of 18 years having diabetes the appearance of gum inflammation is twice as frequent, compared with the control group [7]. Also, in our study we have noticed the presence of the gum inflammation, the macroscopic aspect being correlated with the microscopic one, the poor oral hygiene increasing the severity of the gum inflammation [6, 7].

The morphometric study shows us degenerative changes of the epitheliocytes that imply the nuclear component as well as the cytoplasmic component. Within our batch, we did not encounter the epithelial atrophy phenomenon, with the reduction in the number of layers of the squamous cells, with the erasing of the interpapillary ridges and the tendency to straighten the epithelium–chorion limit. Similar to other studies there appear changes at the level of the thorny layer, with significant acanthosis at the level of the gum epithelium, epithelial discontinuity, the elongation of the epithelial processes ballooned cells and pyknotic nuclei [8–10]. These changes disappear after applying a correct dental treatment. The histomorphometric studies showed that the diabetes leads to cellular transformations in the different levels of the gum epithelium, changes that could be used in the early diagnosis of this disease [10].

Conclusions

The associated type 1 diabetes gingival pathology in the case of children and teenagers is treatable within six months, macroscopically and microscopically the gingival mucosa approaching to normal conditions. The untreated gum inflammation in the case of a teenager leads to cellular and nuclear changes, evolving towards periodontal disease, affecting the image and the wellbeing of teenagers. In the case of children and teenagers diagnosed with type 1 diabetes, the dental check is mandatory in order to prevent the gingival and periodontal diseases.

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