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Histological Analysis of the Mandible in Patients with Type 2 Diabetes Mellitus for Implant-Prosthetic Rehabilitation. A Pilot Case-Control Study

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Abstract

Diabetes mellitus has a major impact on the metabolic activity of a significant number of tissues. Its impact on the jaw bones cannot be neglected, especially if rehabilitation using prosthetic restorations supported on dental implants is intended. The aim of this study is to comparatively analyze vascularization, the degree of mineralization and the cellular component of the mandible in type 2 diabetes mellitus patients undergoing dental implant placement. For this study, eight patients assigned to two groups were selected. The study group included four patients with type 2 diabetes mellitus in whom dental implants were placed, and the control group comprised 4 patients without systemic pathology. The bone debris obtained after dental implant placement were collected and analyzed using hematoxylin-eosin and PAS–Alcian Blue staining. The results obtained indicated the presence of diabetic angiopathy in the mandible, a higher cellular density in the diabetic bone, and a lower degree of mineralization in the bone taken from patients of the study group. In conclusion, histological changes can be detected in the mandible of patients with type 2 diabetes mellitus compared to those without systemic disease, but their effect on bone healing cannot be quantified.

Keywords: diabet mellitus; implant-prosthetic rehabilitation; mandible; histology.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a major metabolic disorder with impact on many human tissues, including the jaw bones. Its effect on the quality of bone is still a debated subject in the literature. Some authors maintain that patients with diabetes mellitus (DM) have a more fragile bone structure [1, 2], regardless of the type of diabetes, while other authors indicate an increase in bone density in patients with T2DM [3, 4]. Paradoxically, an increase in the degree of bone mineralization is observed in T2DM patients [1, 2, 4], which suggests that diabetic patients present a deficiency of the non-mineral component that might reduce bone quality. The role of bone status in patients with T2DM becomes increasingly important in the case of oral function rehabilitation, because one of the best rehabilitation methods uses prosthetic restorations supported on endosseous dental implants [5]. Some authors have identified a lower rate of osseointegration in patients with T2DM compared to non-diabetic patients, with a rate of failure ranging between 4% and 14% [5, 6]. However, dental implant rehabilitation remains without any doubt a very good method for the restoration of oral cavity functions in T2DM patients, but an improvement in the success rate of dental implant osseointegration is required. The aim of this study is to evaluate jaw bone quality in patients with T2DM in order to determine their characteristics. In this way, the elements directly involved in dental implant osseointegration can be identified.

2. Material and method

For the current study, four patients were selected from the authors' clinical patients, who required treatment of missing teeth using dental implants. The patients were selected in the period December 2015 – August 2016. In parallel, the same number of patients were recruited in the control group. The study inclusion criteria were: adult patient, patient with mandibular missing teeth, patient with type 2 diabetes mellitus, absence of other systemic diseases or drug therapies altering bone metabolism, patient having signed an informed consent for participation in the study. The control group was selected to match the general characteristics of the patients in the study group, the only difference between the two groups being the presence or absence of T2DM. Biological samples were collected from the bone debris obtained at the time of dental implant placement. The resulting bone debris were introduced in containers with formol and sent for histological evaluation. The variables monitored for each histological sample were the following: presence/absence of angiopathy in the bone structure, bone cellularity (of three degrees, high, moderate and low), and bone mineralization (low, moderate and high). Histological analysis was performed in eight samples, four for each group. The histological protocol was as follows: fixation of the samples in formol for at least 24 hours, washing of the samples with water and their immersion in trichloroacetic acid until the tissue samples were soaked, washing of the samples followed by their treatment with sodium sulfate aqueous solution. The next stage was dehydration using three alcohol solutions of different concentrations (70° alcohol, 90° alcohol, absolute alcohol). The third stage was decalcification in xylene bath solution, followed by paraffin embedding. The samples were cut with the microtome in sections between 3 and 5 microns. Using a float bath, the samples were displayed on glass slides. Subsequently, the sections were deparaffinized and rehydrated for staining. Hematoxylin-eosin staining was used according to the following protocol: immersion of the sections in distilled water, hematoxylin staining of the nuclei (5 minutes), differentiation with 0.3% acid alcohol, washing with tap water, eosin staining (2 minutes), dehydration, clarification. Subsequently, the tissues were mounted between two microscope slides. Concomitantly, PAS-

Alcian Blue staining was used as follows: deparaffinization, immersion in distilled water, Alcian Blue staining (15 minutes), washing with tap water (2 minutes), washing with distilled water, Schiff reagent staining (10 minutes), energetic washing with tap water (5 minutes), hematoxylin staining of the nuclei (1 minute), acid alcohol differentiation, washing with Scott solution, dehydration, clarification, mounting. Images were captured using an HD camera attached to a Leica DM 300 microscope and were processed with the software provided by the manufacturer. The results were centralized using Microsoft Excel.

3. Results

Diabetic microangiopathy was evidenced in 3 of the 4 samples taken from patients of the T2DM group. The angiosclerosis appearance is specific to patients with T2DM in PAS-Alcian Blue staining, where acid glycoproteins stain blue, neutral glycoproteins stain purple and cellular nuclei stain blue (**Figure 1**).

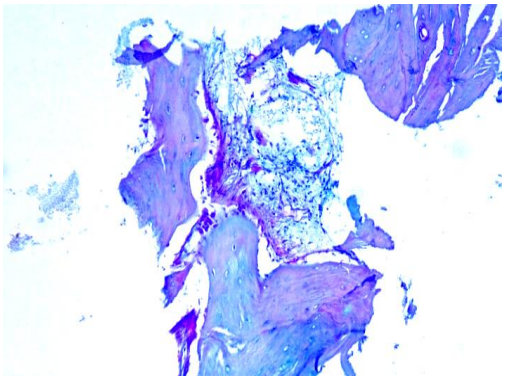

Figure 1: Diabetic angiosclerosis appearance. PAS-Alcian-Blue 100X. In the connective vascular tissue, small size blood vessels can be seen, which show homogeneous amorphous material deposits (PAS+). Thickened basal membrane.

Differences between the two groups also occurred when analyzing bone cellularity. Thus, in the case of the T2DM group, different degrees of cellularity were observed, two patients showed increased cellularity (**Figure 2**), one patient exhibited moderate bone cellularity (**Figure 3**), and one patient had low cellularity (**Figure 4**). Unlike the study group, all patients in the control group presented low cellularity.

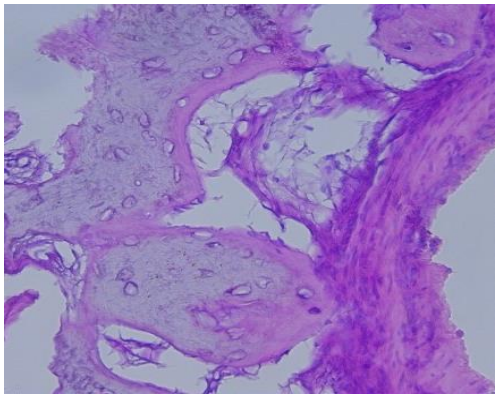

Figure 2: Highly cellular connective vascular tissue and cartilaginous tissue. HE 200X

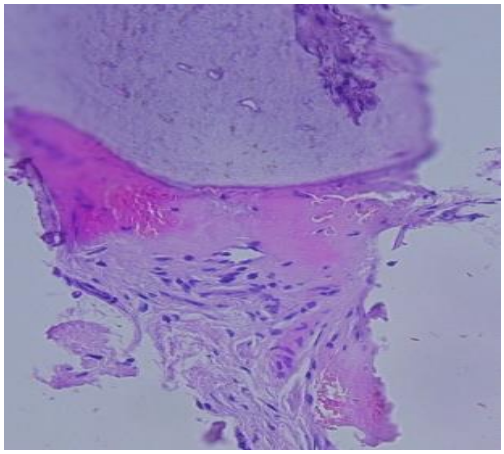

Figure 3: Bone tissue with moderate cellularity. HE 100X

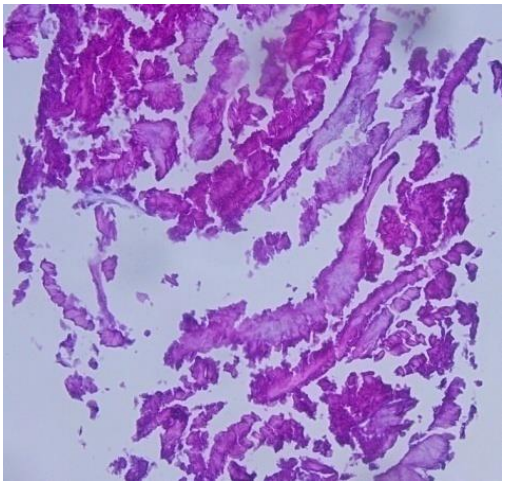

Figure 4: Bone tissue with low cellularity. HE100X

Bone mineralization was low for three patients (**Figure 5**) in the T2DM group and moderate in the case of one patient, while in the control group, a high degree of mineralization was observed for all 4 patients (**Figure 6**).

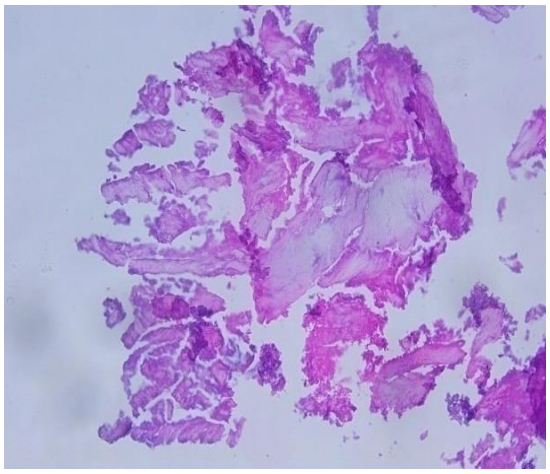

Figure 5: Low mineralization bone tissue slides. Low cellularity. HE100X

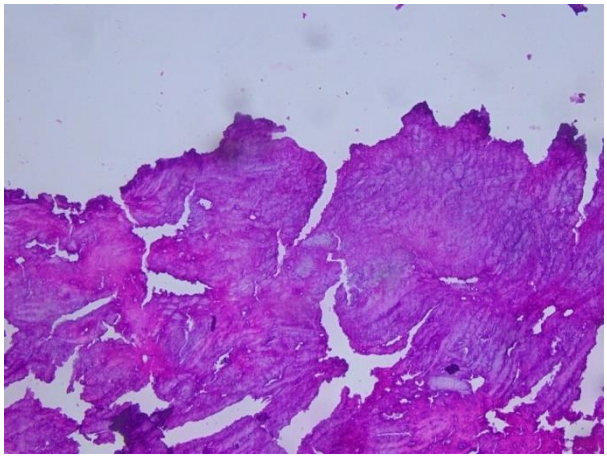

Figure 6: High mineralization compact bone tissue slides. HE 200X

4. Discussions

The histological results obtained evidenced significant differences between the bone of diabetic and non-diabetic patients. The mandible is affected by diabetic angiopathy similarly to the rest of human tissues. This is not surprising considering the multitude of tissues affected by this complication of T2DM [7]. However, some authors maintain that the impact of angiopathy on dental implant osseointegration is not major, which is proved by the high success rate of implant-prosthetic rehabilitation in patients with T2DM [1, 2, 8]. Most probably, angiosclerosis explains the frequent development of periodontal disease in T2DM patients [9, 10] and possibly, in the peri-implant areas. One of the main limitations presented by this study is the relatively small number of biological samples that can statistically confirm angiosclerosis in the maxillary bones of patients with T2DM. To clarify this problem, a prospective study in a larger group of patients is required; the data obtained in the current study are not sufficient to validate this hypothesis. Surprisingly, the degree of bone cellularity in patients with T2DM is high, which indicates a good postoperative healing potential [11]. The increase in cellularity does not mean an adequate metabolic activity of bone cells. Thus, as evidenced by a series of literature studies, the development of metabolic angiopathy is associated with an impairment of cellular functions in tissues such as retinal or brain tissue [12, 13]. This can explain why, despite the increased cellularity present in the bone of diabetic compared to non-diabetic patients, bone healing outcomes are not so good. A limitation of the current study is the fact that bone cellularity was evaluated without evaluating the type of cells present at the bone level. A more accurate histological evaluation of the type of cells present in the alveolar bone of the patient with T2DM is required to determine their main activity. Increased cellular density in the samples of T2DM patients is inversely proportional to the degree of bone mineralization in patients of the study group. The results obtained indicate a significant diminution of the mineral component in patients with T2DM; only one patient had moderate bone mineralization. This diminution of the mineral component was confirmed by imaging studies [14] as well as in vitro experimental studies [3]. The results obtained by us in this regard are relatively clear, while the subject is extensively debated in the literature. Some authors show an association between diminished bone mineralization, a reduced cell division rate and the alteration of the collagen matrix in patients with T2DM [3]. On the other hand, there are authors who maintain that a metabolic acceleration and increased remodeling occur in the bone [15]. It is quite difficult for us to support the second variant, especially when considering that

there is a lower rate of bone osseointegration and implicitly of bone healing in patients with T2DM, even if the difference is not statistically significant. Recent studies show a diminution of the bone healing rate in the context of T2DM, without this contraindicating implant-prosthetic rehabilitation in diabetic patients [16, 17]. The results obtained in this pilot study evidence the need for further research in order to determine the degree of metabolic activity in the jaw bones of T2DM patients.

5. Conclusion

The presence of T2DM has an obvious impact on the structure of the jaw bones in which dental implant placement is intended, without influencing their implant osseointegration capacity.

6. Recommendations

Data obtained so far indicate that dental implants can be used in patients with T2DM with a good prognosis. Compliance with the standard protocol for insertion and prosthesis of dental implants in the patient with T2DM seems to be sufficient for having a favorable postoperative evolution.

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