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Ultrastructure and Microanalysis of Root Cementum in Diabetic Patients versus Healthy Patients with **Periodontitis**

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> *Abstract.* Diabetes itself is not a direct cause of periodontal disease, but rather it facilitates the development of gingivitis and periodontitis through local pathological changes in the oral cavity. The relationship between diabetes and hard dental structure, particularly root surfaces has received far less attention, despite the fact that root surfaces are exposed to multiple pathological factors. The aim of this study is to evaluate the effect of diabetes type 1 and 2 on the mineralization of periodontally diseased root cementum using scanning electron microscope and energy dispersive spectrometry. The sample of this study consisted of 30 periodontally diseased teeth obtained from healthy and diabetic patients' type $1 \& 2$, and was classified into three groups. The result of this study showed remarkable root cementum destruction in diabetic group versus control group. In addition to a significant decrease in the mineral contents, especially calcium ions in diseased root cementum of diabetic patients' type 1, this study concluded that the destruction of root cementum surface and the significant decrease in the calcium contents of the cementum of diabetic patients with periodontitis, may play an essential role of tooth looseness in diabetic patients. Moreover, it will assist to clarify the mechanism of periodontal destruction in diabetic patients.

> *Keywords:* Root cementum, Diabetes, Periodontitis, Energy dispersive X-ray analysis.

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S.O. Amro et al. 70

Introduction

Several trials have demonstrated a relationship between diabetes and tooth $loss^{[1-3]}$. The status of the periodontal disease was also examined in diabetics and a significant alveolar bone loss was observed $^{[4]}$. However, structural changes in the cementum of diabetics have not been evaluated adequately with regard to its relationship with mobility of the teeth and eventual, tooth loss. The increased frequency of tooth loss in diabetics has been associated with periodontitis, its accompanying tooth mobility, and deep pocket formation^[5]. Diabetes itself is not a direct cause of periodontal disease, but rather it facilitates the development of gingivitis and periodontitis through local pathological changes in the oral cavity $^{[6]}$. While periodontitis in diabetics is the subject of ongoing research, the structural changes in human teeth associated with diabetes have not been adequately studied. The relationship between diabetes and hard dental structure particularly root surfaces, has received far less attention, despite the fact that root surfaces are exposed to multiple pathological factors $[7,8]$. Root surface affected by periodontal disease may show various changes depending on the location of the root surface relative to the environment. When the exposed cementum comes into intimate contact with microbial dental plaque, changes occur in the diseased cementum, including hypermineralization of the cementum surface and a degeneration of the collagen matrix. In addition to a development of resorption lacunae due to penetration and/or absorption of bacterial endotoxins at the exposed cementum[9] .

Chemical analysis of the exposed cementum has shown an increase in calcium, magnesium, and phosphorus with a depth of penetration 50 um or less into the cementum. The crystals of the hypermineralized surface zone were observed to be larger than in the subjacent cementum $[10]$. A limited number of studies have used an electron probe to analyze the distribution of various elements in cementum. Hence, no consensus could be reached regarding the occurrence or, distribution of various elements and conflicting data were reported $[11-13]$.

Root surfaces have been evaluated for clinical changes due to the influence of periodontal diseases. The reported results from such teeth indicated a higher Ca and P content than non-diseased root surfaces. Similarly, it has been reported that when root surfaces became exposed to the oral cavity as a result of periodontal disease, the exchange of mineral at the cementum-saliva interface resulted in a more highly mineralized

surface zone approximately 40 microns in depth $[14]$. In the contrary to another study^[15], it was reported that exposed root structures did not show Ca and P differences to a depth of 60 microns when evaluated by scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis. They claimed that previous studies utilized preparative methods such as precipitating fixatives, embedding medium or solutions for extraction of organic matrix and dehydration, which altered the elemental content of the root surface.

Energy dispersive X-ray spectrometry (EDX) was carried out in combination with SEM. The EDX-analysis separates the x-ray spectrum by energy with enough sensitivity to show x-ray spectral data at lowbeam currents. The EDX-analysis was used to determine the chemical elemental content in the diseased cementum surface $^{[16]}$.

The primary composition of root cementum is of a mineralized nature, but the basic elements present, besides calcium and phosphorus, have not been verified. Opinions differ concerning the changes in cementum associated with periodontal disease. In order to understand the nature of this calcified structure in health and disease, knowledge of the elemental content of non-diseased as well as diseased root is required.

Objectives

Therefore, the present study was undertaken to evaluate the microanalysis of various elements, and assesses the surfaces characteristics of the diseased root surfaces among healthy and uncontrolled diabetic patients by using scanning electron microscopy and energy dispersive x-ray analysis.

Materials and Methods

All patients were selected from dental patients attending King Abdulaziz University, Faculty of Dentistry Male department (all male patients). Thirty hopeless vital human teeth from diabetic patients and healthy volunteers were used. Diabetic state was determined by history of previous diagnosis of diabetes. A medical history was available for each person included in the study. Local ethics committee approval was gained for the project and the informed consent was obtained from all patients. The selected patients suffered from periodontal diseases and did not receive any antibiotics or periodontal therapy during the past 6 months. All the selected teeth had periodontal pockets that ranged from

7-9 mm probing depths and minimum 35% bone loss as determined from the radiographs. They were indicated for extraction due to advanced adult periodontitis. The collected teeth were categorized into three groups:

Group I: 10 periodontally diseased teeth from healthy volunteers (Control group).

Group II: 10 periodontally diseased teeth from diabetic patients type 1.

Group III: 10 periodontally diseased teeth from diabetic patients type 2.

During extraction, care was taken to avoid instrumentation to the areas of the root to be studied. The teeth were collected in deionized water and stored in the refrigerator. Cross root sections were cut using diamond saw at more than 5 mm apical to the cementoenamel junction. The root surface opposite the surface to be evaluated was marked with shallow groove for proper identification of the examined surface. Areas for electron microscopic examination were selected to correspond to areas examined in the EDX-analysis. All tooth samples were mounted on specimen stubs and sputtered with a 15 nm thick gold layer§ . The specimens were then examined with a scanning electron microscope[¶]. The microscope was operated at an accelerating voltage of 20 kV. The specimen was analyzed by using energy dispersive analyzer unit attached the scanning electron microscope at the electron microscopic unit, Faculty of Science, King Abdulaziz University (KAU).

Statistical Analysis

Data were collected and tabulated using Microsoft Office Spreadsheet version 3.2 (Microsoft Corp., Washington DC, USA). They were also subjected to statistical analysis with ANOVA test using R (R Development Core Team, Bell Laboratories, New Jersey, USA)[17]. The significant level was set at 0.05.

Results

The mean age in the diabetic groups was 56.1 ± 13.1 years versus 55 ± 14.2 years in the control group.

§ *JEOL JFC- 1600 Auto Fine Coater* ¶ *JSM-6360LA, JEOL, Tokyo, Japan* ║*EX-23000BU*

Scanning Electron Microscope Examinations

Periodontally diseased cementum from diabetic patients and healthy volunteers showed different morphological features. The periodontally diseased root cementum of healthy volunteers (Group I), showed an irregular, uneven surface with multiple superficial defects (Fig. 1). The diabetic patients' type 1 with periodontally diseased teeth (Group II) showed distinct features. The cementum surface was severely damaged with the presence of numerous circular domes giving a pebbly appearance with complete absence of periodontal ligament fibers. In addition to the presence of multiple deep crack lines, also, numerous resorption areas extended deeply into the underlying dentin (Fig. 2). In group III, the resorption defect areas widely covered the

Fig. 1. Scanning electron micrograph of Group I (control) surface view of periodontally diseased cementum. It appears irregular, uneven and with superficial defects (X 16000).

Fig. 2. SEM image of Group II, (type 1 DM), shows severe destruction of the cementum surface, multiple crack lines and exposure of the underlying dentin (X 25000).

diseased cementum surface with variable depths of penetration into the underlying dentin, in addition to the presence of multiple crack lines (Fig. 3).

Fig. 3. SEM image of Group III, (type 2 DM). The root surface appears widely covered by diseased damaged cementum with the presence of multiple deep defects of variable depths into the underlying dentin and absence of periodontal ligament fibers. (X 10000).

Electron Dispersive X-ray Analysis

The elements analyzed and compared in both groups of the energy dispersive spectrometry study were those with an atomic number of 5 or higher, having enough intensity that the EDX could discern them from background or scatter radiation. The minerals most often detected in the specimens were calcium (Ca), fluoride (F), sodium (Na), magnesium (Mg), phosphorus (P), sulfur (S) and chloride (CL) (Table 1),

		Mean	SD	$\mathbf n$	Groups	P value
Ca	Group I	61.75	12.89	5	$G2-G1$	0.04
	Group II	43.72	7.16	5	$G3-G1$	0.56
	Group II	55.09	9.04	5	$G3-G2$	0.21
C1	Group I	0.46	0.34	5	$G2-G1$	0.07
	Group II	1.54	0.93	5	$G3-G1$	0.17
	Group III	1.31	0.67	5	$G3-G2$	0.85
F1	Group I	13.08	3.80	5	$G2-G1$	0.46
	Group II	10.73	2.76	5	$G3-G1$	0.46
	Group III	8.72	2.31	5	$G3-G2$	0.56

Table 1. Descriptive statistics for the different elements used in the current study.

		Mean	SD	$\mathbf n$	Groups	P value
Mg	Group I	1.68	0.35	5	$G2-G1$	0.61
	Group II	1.42	0.17	5	$G3-G1$	0.93
	Group III	1.77	0.62	5	$G3-G2$	0.41
Na	Group I	8.72	4.28	5	$G2-G1$	0.97
	Group II	8.03	3.25	5	$G3-G1$	0.86
	Group III	10.17	5.26	5	$G3-G2$	0.72
P	Group I	20.06	1.51	5	$G2-G1$	0.68
	Group II	20.65	0.75	5	$G3-G1$	0.99
	Group III	20.04	0.85	5	$G3-G2$	0.66
S	Group I	1.38	0.35	5	$G2-G1$	0.040
	Group II	2.20	0.45	5	$G3-G1$	0.71
	Group III	1.14	0.57	5	$G3-G2$	0.01

Table 1. (Continuation) Descriptive statistics for the different elements used in the current study.

F -test p < 0.05

Group I = G1, Group II = G2, Group III = G3

In the three groups, the content of phosphorus and calcium represents the main essential components of the diseased cementum of healthy and diabetic patients. Calcium contents of the diseased Calcium contents of the diseased cementum surface of healthy controls (Group I) were higher in comparison to the diseased cementum of diabetic patients (Group II). The difference between them was statistically significant (p-value $= 0.036$) (Fig. 4).

The influence of diabetes on calcium (Ca) contents was variable among the two diabetic groups (II $&$ III). The type 1 diabetic patients showed remarkably decreased calcium in comparison to type 2 diabetic patients, but the difference was not significant. The influence of diabetes on fluoride (Fl) seemed to be much less dramatic since the difference between the three groups was not significant.

The sulfur (S) contents of the diseased cementum of group II was the highest. The difference was significant compared with controls (p value = 0.04) and with other diabetic (Group III) (p value = 0.01) (Fig. 5).

The difference for the other elements magnesium (Mg), sodium (Na), chloride (Cl) and phosphate (P) among the three groups was not significant (Table 1).

S.O. Amro et al. 76

Fig. 4. Error bars showing the mean and standard errors of calcium level in the 3 studied groups.

Fig. 5. Error bars showing the mean and standard errors of sulfur level in the 3 studied groups.

Discussion

This study was primarily concerned with ultrastructural and elemental changes within periodontally diseased cementum of diabetic patients. The effects of metabolic changes in diabetes are multifaceted including plaque microbiology, vascular changes, and alterations in the metabolism of collagen tissues and in immunological responses^[18].

The results of the scanning electron microscope of the diseased cementum surface of non diabetic patients revealed the presence of rough irregular surface with multiple resorption lacunae of variable depths.

This is in agreement with Adriaens *et al.*^[19] who explained that these lacunae may display a route of entry for bacteria into root cementum and radicular dentine. Daly *et al.*^[20] also showed cracks within cementum from periodontally - involved root surfaces. These surface topographical features may be of importance in therapeutic interventions aimed at rendering the root surface biologically acceptable. Eide *et al.*^[21] have observed a mineralized surface coating on dental cementum incident to periodontal disease. They stated that this coating is derived from components of inflammatory exudates within periodontal pockets and
that this might be a reservoir of cementum-associated that this might be a reservoir of cementum-associated lipopolysaccharides.

The strongest ultrastructural destructions were observed in type 1 diabetic patients (Group II); where large areas of cemental surface were destroyed, while in type 2 diabetes the cemental destruction was mild. This is in agreement with Atar et al ^[22] who found that diabetic patients show more destructive cementum than the healthy controls. The extent of these destructions is decreasing in the order, with the least in the shorter duration of diabetes. From the SEM micrographs shown in this study, it was evident that all patients affected by a genetically determined diabetes show markedly stronger defects than the acquired diabetes. From these findings, the conclusion was drawn that genetically induced diabetes, like type 1 diabetes may lead to much more destruction than type 2 diabetes, which is normally acquired during middle-age. Oliver and Tervonen^[23] stated that, in addition to periodontitis, type 2 diabetes is related to other complications in the oral cavity including tooth decay, dry mouth, fungal infections, as well as oral and peripheral neuropathies. The incidence of tooth loss is 15% higher in type 2 diabetic subjects compared to healthy controls. It has been suggested that this difference can be accounted for alveolar bone resorption, loss of attachment of the periodontal ligament to the cementum, and alterations in the structure and thickness of the cementum layer. The work of El-Bialy et al ^[24] unequivocally supports our suggestions concerning stronger defects in type 1 diabetes mellitus (DM). They found a decreased skeletal maturation and cephalometric measurements in diabetic patients. Their results may possibly be transferable to dental cementum, since bone tissue and cementum show similarities in their development and function as mineralized tissues.

In this study, a remarkable decrease in calcium and an increase in sulfur contents in diabetics than control were found. Other elements

including F, Na, Mg, P, Cl, were also analyzed. No statistic difference was observed in relative content of these elements between diabetic and control. These results are consistent with the report conducted by Yurong, et al.^[25]

Our findings support the hypothesis that a decrease of calcium levels in the blood or diminished calcium incorporation into the cementum caused by reduced cellular activity of the cementoblast may be directly responsible for the cemental defects in diabetic patients. This is in α accordance with Balint *et al.*^[26] who demonstrated that a glucose concentration similar to those observed in patients with poorly controlled diabetes causes significant inhibition of osteoblastic calcium deposition.

The recent investigation of Gunczler *et al.*^[27] also referred to a decreased bone mineral density and bone formation markers shortly after diagnosis of clinical type 1 DM. It's believed that significant loss of calcium accounts for the marked destruction in cemental layers of diabetic patients. Sulfur exists in many substances that are essential to bone metabolism^[28] Therefore, obvious increase of sulfur in the current study probably contributed to diabetic changes in the cemental tissue in accordance with Yurong, et al ^[25], who found an increase of sulfur component in bone of diabetic patients. In this study a significant increase in the sulfur content in type 1 diabetic patients was found. This is in agreement with Kodaka and Debari^[29] as they reported that high sulfur and inorganic sulfate as a result of hyperglycemia. There is no physical explanation for an increasing sulfur value in our research, but it could be due to an increased sulfur concentration in serum leading to its precipitation in bone and cementum. However, elemental analysis in the cementum of diabetic patients has not been previously evaluated and this research may be considered as a first study concerned regarding this point.

Further investigations on a large number of samples would be necessary to clarify the role of elemental changes in diabetic cemental destruction and to confirm this finding.

Conclusion

Our study concluded that the severe destruction of root cementum surface, and the significant decrease in the calcium contents of the cementum of diabetic patients with periodontitis, may play an essential

role of tooth looseness in diabetic patients, moreover, will assist to clarify the mechanism of periodontal destruction in diabetic patients.

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S.O. Amro et al. 80

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التركيب الدقيق والتحليل المجهري لملاط جذور الأسنان المصابة بالتهاب النسيج السمحاقى عند مرضى السكرى و الأصحاء

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المسـتخلص. الغـرض مـن هـذا البحـث كـان لتقيـيم تـأثير داء السـكري من النوع الأول والثـاني علـى تركيـب و تمعـدن طبقـة المـلاط فـي جـذور الأسـنان المصـابة بالتهـاب النسـيج السـمحاقى وذلـك باسـتخدام المجهـر الإلكترونـي الماسـح، وجهـاز تحليـل الأشـعة السـينية باسـتخدام الطاقـة المتفرقة. استخدم في هذه الدراسة ثلاثين سنًا مصـابة بالتهاب النسيج .
أ السـمحاقى، تـم الحصـول عليهـا مـن أشـخاص أصـحاء، و مـن مرضـى مصـابون بـداء السـكري مـن النـوع الأول والثـاني، وقـد تـم تقسـيمهم إلـى ثــلاث مجموعــات. أســفرت نتــائج هــذه الدراســة عــن وجــود تصــدعات ملوحظـة يفـ مـلاط جـذور ا لأسـنان لـدى مرضـى السـكري، مقارنـة إلـى الأصـــحاء. هـــذا إلـــى جانـــب وجـــود انخفـــاض ملحـــوظ فـــى العناصـــر المعدنيــة لمــلاط الأســنان، خاصــة الكالســيوم، عنــد مرضــى الســكرى، وعلــى نحــو خــاص مــن النــوع الأول. ويســتنبط مــن هــذا البحــث، أن التدمير الشـديد لمـلاط سـطح جـذور الأسـنان المصـابة بإلتهـاب النسـيج السمحاقي، وانخفاض نسبة المعادن به، وخصوصًا عنصر الكالسيوم،

S.O. Amro et al. 82

ً قد يلعب دورًا أساسيًا في ضعف الأسنان عند مرضىي السكرى، وأيضًا **ٍ** ً ســوف يســاعد علــى توضــيح آليــة تــدمير الأنســجة الداعمــة للأســنان عندهم .