CREVICULAR FLUID LEVEL OF ELASTASE IN TYPE I AND TYPE II DIABETES MELLITUS PATIENTS

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ABSTRACT

Elastase activity in pocket fluid samples was taken from 120 patients divided into 4 groups. The groups were identified as Type I DM (Diabetes mellitus) metabolic uncontrolled, Type I metabolic controlled, Type II metabolic uncontrolled, and Type II metabolic controlled groups. The samples were evaluated with spectrophotometry. In our study we found that, in addition to the clinical data, elastase enzyme activity level and elastase enzyme concentration were found to be significantly higher in metabolic uncontrolled diabetic groups than in metabolic controlled groups (p<0.05). Furthermore, we found a relationship between total elastase enzyme activity and enzyme concentration in all of the four groups. The findings of the study support the relationship between elastase activity and the periodontal disease. We may further draw inferences regarding the effects of metabolic control levels on the periodontal tissues in diabetics patients.

Introduction

Diabetes mellitus is a chronic metabolic disease that affects 2-10% of general population and is characterized by increased blood glucose levels and glucose release in urine. Since periodontal disease is also common in diabetics, numerous studies have investigated the potential link between diabetes and periodontal disease (1).

Diabetes mellitus affects more than 100 million people worldwide and causes metabolic complications due to the absence, shortage or ineffectiveness of the insulin hormone combined with hyperglycemy. It is classified into two major types: juvenile (Type I) and adult (Type II) (1-3).

Diabetes cause complications throughout the body. Oral complications are also common in people with diabetes. These complications that accompany diabetes include xerestomia, loss of tooth, gingivitis, periodontitis, abces, and cavities. A conclusive link between diabetes and periodontal disease has not been established. Periodontal diseases are characterized by the infections of tissues that support teeth. In periodontal disease, tissue breakdown occurs in specific periods of time. Identification of these periods where the disease is active is important as it will help determine the current condition of the disease, identify those at high risk, and correctly determine the prognosis (4).

Most of the previous studies on the diabetics and periodontal disease based their findings on clinical parameters such as pocket depth measurement. In recent years, however, new methods are also widely being used including microbiological, immunological, genetic and biochemical methods, in order to determine the active area

where tissue breakdown occurs in periodontal disease. Therefore, there is a growing number of studies that investigate tissue breakdown by-products, infectious mediators, bacteria and their by-products in total saliva, blood, plaque, gingival tissue and gingival sulcus fluid (5).

The contents of GCF (Gingival crevicular fluid) primarily consist of enzymes, cellular elements, electrolytes, organic compounds, and antibacterial factors. Enzymes in GCF may be host or bacterial in nature. As a result of many studies on lysosom enzymes, PMN's (Polimorphonuclear leucocytes) have been shown to be the main source of a large majority of lytic enzymes in GCF. Studies on the development of periodontitis show a correlation between the active phases of tissue breakdown and PMN's infiltration towards the sulcus area. Higher PMN activity in the sulcus area is observed during the breakdown phase of the periodontal disease (6, 7). Recent studies have indicated that defects in their PMN function causes diabetics to be more prone to bacterial infections and that hyperglycemy and weak metabolic control increase the risks of the peridontal disease, both of which are attributed to the defects of PMN function and collagen synthesis in diabetes. The presence in GCF of indicators for primary granules of neutrophils shows the release of enzymes that cause protelytic tissue breakdown. One of the markers of primary granule release in GCF is elastase (8-11). Elastase is a cold protease that leads to collagene breakdown and that contributes to elastin turn over. It has been shown that, during periodontal disease, these enzymes are effective in the breakdown of building block proteins and plasma-based proteins that join the local defense reactions (12, 13).

The goal of this study was to collect clinical data that reflect the severity of the periodontal disease in Type I and Type II diabetics, as well as to determine activity levels of elastase enzyme which helps in tissue breakdown and to identify, in terms of elastase content in GCF, potential differences between metabolic controlled and metabolic uncontrolled patient groups with Type I and Type II DM.

Materials and Methods

Study Population

This study was conducted at Department of Endrochronology, Faculty of Medicine at Dicle University. A total of 120 patients participated in the study, including 62 women and 58 men who were diagnosed with Diabetes mellitus. Patients were admitted to the study if they were not taking medication affecting periodontal status and had received no periodontal therapy in the preceding 6 months. The classification of the subjects with respect to their diabetic state was based on the long-term metabolic control measured as percentages of glycosylated hemoglobin (HbA1c) level. HbA1c values were classified as follows: $\leq 8.5\%$ (good), 8.6-9.9 % (moderate) and $\geq 10.0 \%$ (poor) (14).

Participants were divided into the following four groups according to HbAlc levels :

1. Type I diabetic metabolic uncontrolled group: In this group were 30 participants, including 16 females and 14 males aged 12-21. The average age of the group was 15.2±2.36.
2. Type I diabetic metabolic controlled group: This group consisted of 30 participants including 22 females and 8 males, aged 12-21. The average age of the group was 14.9 ±2.59.
3. Type II diabetic metabolic uncontrolled group: This group consisted of 30 participants including 12 females and 18 males, aged 37-67. The average age of the group was 52.4±9.46.
4. Type II diabetic metabolic controlled group: This group consisted of 30 participants including 12 females and 18 males, aged 38-67. The average age of the group was 50.03±8.99.

Determination of Clinical Periodontal Status

During the course of the study, the following measurements from the individuals in each group were recorded: the Silness-Löe plaque index, Löe-Silness gingival index (15), and pocket depth measurements .

Sampling of Gingival Crevicular Fluid

Pocket fluid samples were taken during the morning hours.In order to eliminate the risk of contamination with saliva, sites for GCF sampling were chosen among maxillary teeth in every case. The selected sites was isolated with cotton rolls and gently air dried. Crevicular fluid was collected onto pre-weighed 2X8 mm filterpaper strips of Whatman 3MM chromatography paper (Whatman Industries, Dartford, Kent, UK), according to the orifice method described by Rüdin et al.(16). The paper strips were inserted 1mm. into the sulcus/ pocket and left in place for 30 seconds. We discarded the paper strips that were contaminated with blood. The strips were then immediately weighed with a scientific scale, and the minimize the risk of evaporation, paper strips placed into Eppendorf tubes were firmly wrapped and stored at -20°C. (17).

Determination of Elastase-like Enzyme Activity in GCF samples

Three hundred (300) μl of saline was added to each Eppendorf tube. The tubes were vortexed and kept at -4 °C to maintain the extraction of GCF into the saline. Elastase activity of GCF was determined using succinyl-(ala)3 -p-nitroanilide as substrate. The activity was measured in 50mM Tris/ HCL buffer containing 5 mM $CaCl_2$, 200 mM NaCl, and 1 mM substrate. The reaction was started by the addition of 0.1 ml of GCF extract, and the initial absorbance values were measured at 405 nm. The mixtures were then incubated 37 ºC for 3 hours. At the end of the incubation period, the absorbance of the assay mixture was determined, and absorbance changes were calculated using the initial and final readings. Elastase- like activity of GCF samples was calculated as the amount of enzyme that hydrolyzes 1 nmol of substrate in 1 minute under the conditions given above. Enzyme activity was expressed as enzyme concentration and total enzyme activity. Enzyme concentration standards for the enzyme activity per μl of GCF, and total enzyme activity refers to the total activity collected onto the paper strips in 30 seconds (17).

Statistical Analysis

Student test was used in analysis and comparison of the groups based on clinical and laboratory parameters. Pearson Corelation analysis was used in evaluating the relationships between GCF elastase levels and clinical parameters, and between concentration levels and total enzyme activity (18).

Results and Discussion

Clinical findings: All mouth PD (Probing deep), GI (Gingival index) and PI (Plaque index) average values in Type I and Type II diabetic metabolic uncontrolled groups were found to be higher than metabolic controlled groups ($p < 0.05$). Results are shown in **Table 1** and **Table 2**.

Laboratory findings: GCF total elastase activity and elastase concentration levels were found to be significantly higher in metabolic uncontrolled patient groups. After the groups were compared using the one-way variance analysis, differences among groups were found to be unimportant for total elastase activity and elastase concentration at Type I and Type II metabolic uncontrolled and Type I and Type II metabolic controlled groups (**Table 3** and **Fig. 1**).

Relationships between GCF enzyme levels and clinical data: we used Pearson Correlation factor to determine relationships between enzyme activity in gingival sulcus fluid and clinical parameters. After the analysis, in Type I metabolic uncontrolled group, a significant relationship was found between pocket depth and total

TABLE 1

Averages and standard deviation of clinical and GCF parameters per group

GROUPS	PD		PI		GI		DOS	
	X	SD	X	SD	X	SD	X	SD
1	2.668	0.256	2.140	0.330	1.956	0.443	0.998	0.313
2	2.178	0.258	1.804	0.349	1.569	0.432	0.737	0.101
3	3.674	0.757	2.470	0.469	2.318	0.437	1.075	0.441
4	2.656	0.452	2.128	0.376	1.857	0.410	0.728	0.111

TABLE 2

Differences among groups per clinical parameters

GROUPS	PD		PI		GI	
	T	p	t	P	t	p
1 & 2	0.490	0.001*	0.335	0.006*	0.387	0.004*
1 & 3	1.006	0.000*	0.330	0.007*	0.363	0.008*
1 & 4	0.012	1.000	0.012	0.999	0.099	0.810
2 & 3	1.496	0.000*	0.665	0.000*	0.750	0.000*
2 & 4	0.478	0.001*	0.324	0.008*	0.288	0.052
3 & 4	1.018	0.000*	0.342	0.005*	0.462	0.000*

TABLE 3

Elastase-like enzyme activity in gingival crevicular fluid

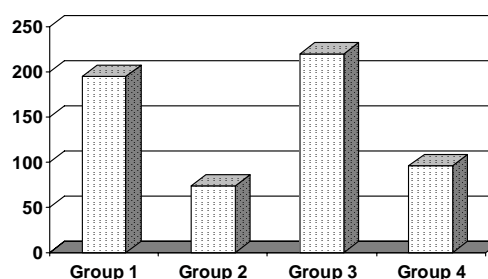| GROUPS	ELA Concentration (nmol/min/ml)	ELA activity (U)
1	195.764 ± 64.170	0.269 ± 0.300
2	74.035 ± 34.683	0.090 ± 0.148
3	220.430 ± 61.257	0.231 ± 0.088
4	96.757 ± 57.658	0.093 ± 0.137

Fig. 1. Elastase concentration in GCF (nmol/min/ml).

elastase activity ($p<0.05$). Also in the same group, another significant relationship was found between GCF volume and total elastase enzyme activity level ($p<0.05$). In Type II DM metabolic uncontrolled group, a particularly strong relationship was found between GCF volume and total elastase activity ($p<0.001$). (**Table 4**). Total elastase like enzyme activity was found to be higher metabolic uncontrolled groups than metabolic controlled groups (**Fig. 2**).

The above-average presence of PMN in GCF occurs simultaneously with the loss of clinical attachment along the root surface.

In many studies, it is shown that periodontal pathogens activate PMN's and that in conjunction with oxygen radicals, they start the release of granule contents. The presence of neutrophils indicators in GCF that are specific to primary granules is the main evidence that enzymes that cause tissue breakdown have been released. Elastase is one of the indicators of primary granule release in GCF (8, 17, 18).

Our objective in this study was to identify the clinical parameters and determine the GCF elastase enzyme levels that reflect the severity of the disease in Type I and

TABLE 4

Relationship between GCF enzyme levels and clinical data

|	GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	r	p	r	p	r	p	r	p
PD-ELA activity	0.557	0.039*	0.206	1.000	-0.271	1.000	0.313	1.000
PD-ELA concentration	0.603	0.012*	0.142	1.000	-0.426	0.531	0.303	1.000
PI-ELA activity	0.166	1.000	0.273	1.000	-0.331	1.000	0.291	1.000
PI-ELA concentration	0.082	1.000	0.243	1.000	-0.468	0.257	0.337	1.000
GI-ELA activity	0.243	1.000	0.383	1.000	-0.102	1.000	0.422	0.562
GI-ELA concentration	0.278	1.000	0.350	1.000	-0.192	1.000	0.426	0.533
DOS-ELA activity	0.553	0.043*	0.425	0.538	0.618	0.008*	0.595	0.015*
DOS-ELA concentration	0.205	1.000	0.400	0.801	-0.299	1.000	0.510	0.111
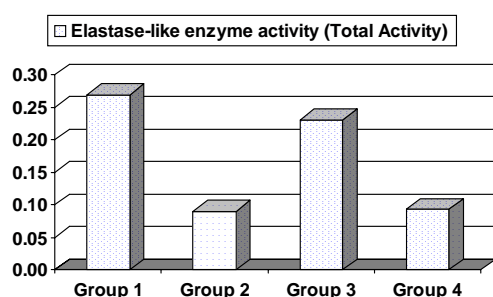
Fig. 2. Total ELA (U) observed in GCF.

type II controlled and uncontrolled diabetic patients, and their relationship to one another.

There are a number of methods used to collect GCF. Paper strips, capillary tubes, and gingival washing apparatus can all be used to obtain GCF samples. In this study, we used the Orifice technique described in Rudin et al. whereby paper strips are placed at the sulcus entry (16). We first grouped the 120 Type I and Type II diabetes patients based on their metabolic controls. We then measured the periodontal conditon, pocket depth, plaque index, and gingival index of each group to clinically establish their peridontal condition. At the conclusion, we found the clinical data to be higher in metabolic uncontrolled groups than in metabolic controlled groups.

Tervonen and Knuuttila have not found statistically significant differences between the formations of pockets in diabetics and nondiabetics (19). However, they report that decreases in the metabolic control in diabetics result in increases in the number of pockets. A quick survey of the literature including Taylor et al. reveals that weakness of the metabolic control in diabetics constitutes a risk factor in periodontal diseases (20).

The formulation of the enzymatic content of GCF varies in different studies. Total enzyme activity (U) refers to the total enzyme activity of GCF obtained within a well-defined and constant period of time. Enzyme concentration is GCF enzyme activity per unit volume (17). A 1988 report by Lamster et al. found total unit activity of gingival pocket fluid enzyme to be a better indicator of periodontal disease (21).

In our study, after comparing elastase enzyme to total enzyme activity in Type I and Type II diabetics, GCF elastase levels were found to be high in metabolic uncontrolled groups and the difference was found to be statistically significant. Considering the fact that metabolic uncontrolled groups would have higher clinical index values than other groups, total elastase activity level can be said to be an indicator of periodontal pathology.

In their 1992 paper, Eley and Cox found the total elastase activity to be a major indicator in determining periodontal conditions (22).

An analysis of our study reveals that the

groups with the highest elastase concentration levels are the metabolic uncontrolled groups. In these groups, the highest elastase concentrations were found in Type II DM metabolic uncontrolled group, with Type I DM uncontrolled group showing the second highest levels.

In their studies of elastase concentration levels in 11 volunteer patients, Meyle et al. determined that the elastase concentration levels were affected by oral hygiene (23). Similarly in our study, we have found a correlation between lower elastase concentration levels and deterioriating periodontal conditions in the metabolic control groups.

The findings of our study sheds light on the effects of metabolic control levels on periodontal tissues and hints that low metabolic control is a potential risk factor for the aggressiveness of the disease. Furthermore, our study highlights the need to more thoroughly study the enzymatic structure of GCF, a resource that is easy to obtain and extremely valuable for the timely and sufficient treatment of the periodontal disease.

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