Comparative Analysis of the Blood Flow Values of Patients with Type 2 Diabetes Mellitus Presenting with Chronic Periodontitis, Patients with Chronic Periodontitis Only and Healthy Individuals
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ABSTRACT

Objectives: Laser Doppler flowmetry (LDF) is a non-invasive technique used for various reasons in medicine and dentistry. There are many reports of vascular and microcirculatory changes in patients with diabetes mellitus. The aim of the study was to assess the gingival blood flow (GBF) measurements in patients with chronic periodontitis (Group 1), patients with chronic periodontitis and Type 2 diabetes mellitus (Group 2) and healthy controls (Group 3).

Methods: Forty-eight individuals were included in the study and divided into three groups. Gingival blood flow measurements were taken from 16 points in the upper vestibular aspect of six anterior teeth using LDF probe. In addition, plaque index (PI), gingival index (GI), probing depth (PD) and attachment loss (AL) were registered to determine the periodontal status.

Results: Results showed a significant correlation (p < 0.05) in clinical indices and GBF among the groups. Moreover, in Group 1, there was a correlation between GI and AL and between PD and AL. In Group 2, a correlation was observed between PI and AL and between GI and GBF.

Conclusion: The results revealed an increase in GBF value in the chronic periodontitis group with Type 2 diabetes mellitus compared with the group with chronic periodontitis only. Diabetes can also affect GBF due to the nature of the disease, which affects the microcirculatory status.

Keywords: Chronic periodontitis, gingival blood flow, Type 2 diabetes mellitus

Análisis Comparativo de los Valores de Flujo de Sangre en Pacientes con Diabetes Mellitus Tipo 2 con Periodontitis Crónica, pacientes con Periodontitis Crónica, e Individuos Sanos
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RESUMEN

Objetivos: La flujometría con láser Doppler (FLD) es una técnica no invasiva, usada en medicina y odontología por varias razones. Se han reportado muchos cambios vasculares y microcirculatorios en pacientes con diabetes mellitus. El objetivo del estudio fue evaluar las mediciones de flujo de sangre gingival (FSG) en la periodontitis crónica (Grupo 1), la periodontitis crónica en pacientes con diabetes mellitus tipo 2 (DT2) (Grupo 2), y controles sanos (Grupo 3).

Métodos: Cuarenta y ocho personas fueron incluidas en el estudio y divididas en tres grupos. Se tomaron medidas del FSG de 16 puntos en la cara vestibular superior de seis dientes anteriores mediante una sonda de flujometría con láser Doppler. Además, se registraron el índice de placa (IP), el índice gingival (IG), la profundidad de sondaje (PS), y la pérdida de anclaje (PA) a fin de determinar la condición periodontal.

Resultados: Los resultados mostraron una correlación significativa (p < 0.05) en los índices clínicos y FSG entre los grupos. Por otra parte, en el grupo 1 se observó una correlación entre IG y PA así como entre PS y PA. En el grupo 2, se observó una correlación entre IP y PA, e igualmente entre IG y FSG.
INTRODUCTION

Periodontal diseases represent chronic inflammatory responses to a bacterial challenge. One of the earliest findings of inflammatory process is the alterations in the vascular structure and microvasculature. Healthy gingiva is observable at the subepithelial vascular plexus including a capillary network with loops arching toward the epithelium (1). Gingival inflammation results in an enhanced vascularity with more capillary loops (2), large vessel size and slower blood flow (3); all of these symptoms have been found in the gingiva of experimental animals. The capillary units in the crestal gingiva are among the first vessels affected by inflammation (4). When the changes of the vascular morphology in inflammation are related to blood flow changes, these changes may be the first signs to estimate the onset of pathological events in gingival tissue. Thus, gingival blood flow (GBF) may serve as a prognostic marker (5).

Laser Doppler flowmeter was used to estimate gingival blood flow (6). The beam from a helium-neon laser was directed at the free gingival margin. The light was scattered by moving blood cells to a depth of 1.0 mm in the tissue. A shift in frequency was found according to the Doppler rule. The frequency difference is a measurement of the velocity of blood cell movements. The output signal from the laser Doppler flowmeter instrument was proportional to the mean frequency shift multiplied by the number of blood cells in the volume under study. The laser Doppler flowmetry (LDF) is non-invasive and allows the continuous monitoring of GBF in humans (7). This technique has been used to evaluate blood flow in intact microvascular system such as retina, gut mesentery, renal cortex, skin and mucous membranes (8). Moreover, some researchers reported blood flow in human dental pulp (9), periodontal ligament (10), gingival or sulcular region (11), the effect of orthodontic therapy (12) and the injection of vasoconstrictive anaesthetics on blood flow (13).

Type 2 diabetes mellitus (DM) is a disorder characterized by chronic hyperglycaemia resulting from impaired insulin hormone secretion and action. People are increasingly suffering from diabetes mellitus around the world (14). Periodontitis, as an inflammatory process initiated by the plaque biofilm, leads to the loss of periodontal attachment to the root surface and adjacent alveolar bone, which ultimately results in tooth loss (15).

The present investigation aims to evaluate the comparative results of the blood flow measurements from Type 2 DM patients with chronic periodontitis, patients with chronic periodontitis only and healthy individuals.

SUBJECTS AND METHODS

Forty-eight study participants were divided into three groups: 1) chronic periodontitis (n = 16, aged 65.31 ± 5.98 years), 2) chronic periodontitis with Type 2 DM (n = 16, aged 51.62 ± 9.56 years) and 3) healthy controls (n = 16, aged 23.06 ± 4.44 years). All participants were selected at the Department of Periodontology, Faculty of Dentistry, Cumhuriyet University and each participant was informed and consented to the study.

The diabetic patients were diagnosed by a specialist with the measurement of the glycated haemoglobin (HbA1c) in the peripheral blood (16). Type 2 DM patients were poorly controlled and HbA1c was > 8.5%. All types of periodontitis showed clinical inflammation, gingival index (GI) > 1; more than 30% of sites had periodontal pocket depth (PPD) ≥ 5 mm and clinical attachment loss (CAL) ≥ 3 mm, with radiographic evidence of bone loss on their six upper anterior teeth. Healthy controls showed GI = 0 (absence of clinical inflammation), PPD ≤ 3 mm and CAL = 0, without any evidence of bone loss on radiographs. All patients were healthy non-smokers. Pregnant patients, patients with general diseases (hypertension, ischaemic heart disease, rheumatoid arthritis), on long-term medication, antibacterial or anti-inflammatory medication within the last one month, were excluded from the study to avoid affecting blood flow results. Prior to the blood flow measurements, plaque index [PI] (17), GI (18), probing depth (PD) and attachment loss (AL) scores were recorded from the six anterior upper teeth by one of the authors using a Williams periodontal probe.

All patients were called at the same time (9:00 am) in the morning and each patient was seated in an upright position to avoid any error in the GBF measurements. Blood flow was recorded prior to the clinical indices. An acrylic splint (Ortocryl 2000, Dentaurum, Germany) for the maxillary anterior region covered the six anterior teeth and gingiva and was fabricated to position the probe. The measurement point was from the vestibular area; six holes were created from the mid-facial marginal point, five from the interdental papilla region and five from the attached gingival region. In short, all 16 holes were created on the stent to accurately place the probe (Figure).

Blood flow measurements were taken using a Periflux 4001 Master laser Doppler flowmeter device (Perimed,
Stockholm, Sweden). The LDF emitted light with a wavelength of 780 nm. The probe diameter was 1 mm. Calibration was performed with the “PF 1000 Calibration Device” (Perimed, Sweden) before the measurements (19). The measurement period, determined for each measuring site, was 30 seconds to ensure a sufficient time. During the procedure, the laser Doppler continuously measured the GBF of each person. Blood flow, volume and velocity were monitored. Blood flow was expressed as perfusion unit (PU). All signals were transferred to the computer and a mean value was calculated for each person. Sixteen average values were obtained for each group for statistical analysis.

A power analysis was performed for the study and the result was \( p = 0.95 \). A statistical analysis was performed using SPSS, version 14.0 (IBM, New York, USA). Kolmogorov-Smirnov test was firstly used for determining the distribution of data. Parametric values were analysed using one-way analysis of variance (ANOVA), followed by Tukey’s test and Pearson’s correlation analysis, used for statistical analysis of the data; \( p < 0.05 \) was considered to be statistically significant.

## RESULTS

The minimal and maximal values, averages and standard deviations are given in Table 1. A statistically significant difference (\( p < 0.05 \)) was found in PI among the groups, except for the values between groups 1 and 2 (\( p > 0.05 \)). The same result was also found in PD and AL. In comparison, among the three groups for GBF, significant results were observed between groups 1 and 2, groups 1 and 3, and also between groups 2 and 3 (\( p < 0.05 \)) [Table 2]. Moreover, the correlation among the parameters in each group showed a positive correlation between GI and AL (\( r = 0.54, p = 0.033 \)) in Group 1, and was statistically significant (\( p < 0.05 \)). Besides, there was a positive correlation between PD and AL (\( r = 0.87, p = 0.001 \)), with a statistical significance (\( p < 0.05 \)). In Group 2, there was a positive equivalence between PI and AL (\( r = 0.69, p = 0.003 \)), which was statistically significant (\( p < 0.05 \)). Likewise, a positive correlation was observed between GI and GBF (\( r = 0.65, p = 0.007 \)), which was statistically significant (\( p < 0.05 \)) [Table 2]. The remaining results were not statistically significant.

## DISCUSSION

Laser Doppler flowmetry, a non-invasive technique, was designed as an alternative to former invasive and non-invasive methods to evaluate blood flow and can be used to investigate oral tissue blood flow (20). The present study aims to detect the blood flow records from diabetic and non-
diabetic patients with chronic periodontitis. This could be helpful for a better understanding of the changes in blood flow in different forms of diseases.

The literature contains a limited number of studies comparatively investigating the blood flow between different cases. Our study differs from them with its unique objectives and patient groups. Therefore, we anticipate that it can greatly contribute to the scientific literature despite many limitations.

The present study had several limitations. First, the adaptation of the probe was very important. To fix the probe, an acrylic stent with holes to stabilize it was fabricated. Doing this, advantage was taken of the chance of a better measurement. The second limitation was the small number of patients with chronic periodontitis and Type 2 DM. A third limitation was the duration of measurements. A sufficient amount of time was needed to produce the artifacts. For this critical point, we set the measurement time to be 30 seconds for each measurement point and additional measurement points were assigned for an ultimate measurement.

There are literature reports where LDF has been applied to animal (21) and human models (19, 22). All previous research has revealed valuable results. In the present study, all clinical parameters were found to be higher in the diseased groups than in the controls. In Group 2, the GBF and GI scores were higher than in Group 1. The clinical situation in diabetics was previously reported as worse than in non-diabetics (23–25). Other factors in Type 2 DM may have an impact on the periodontal status. Moreover, in Group 2, the significant correlation between GI and GBF was very noticeable, because GI was presented as the inflammation degree in tissues and reported in direct relation with GBF in advance (21, 22).

Develioglu et al noted in previous studies (19, 22) the blood flow values with LDF from marginal gingivitis and interdental papillae. The results were in accordance with the clinical parameters and particularly with the gingival index. They help us better understand the microcirculatory events in different locations of the gingiva. Although the reliability of blood flow measurements was not high, the use of LDF on the periodontium in different areas and diseases was very practical by supporting the clinical parameters when used together.

Gleissner et al (5) investigated the local GBF at healthy and inflamed sites with LDF. The results positively correlated with the degree of inflammation. That study also observed the separate GBF results in control groups and groups with disease. The findings confirmed the results in our study.

Similarly, Kerdvongbundit et al (26) studied microcirculation in subjects with moderate gingivitis, periodontitis and healthy gingiva. Results from both groups contrasted with each other. The present study came up with similar findings. They found that the inflammation degree directly affected blood flow rates, which was parallel to our findings.

Similar to the present study, Sugiyama et al (27) evaluated the gingival vascular functions that altered in the Type 2 DM model and/or periodontitis rat models. This experimental study revealed that vascular endothelial function decreased in animal models with diabetes mellitus and/or periodontal disease due to the enhanced oxidative stress in the gingival circulation. These results can also help better understand our results. In the present study, the chronic periodontitis group with Type 2 DM had higher blood flow values than the chronic periodontitis group. This might be interpreted as the negative effect of diabetes on clinical condition and a higher severity of periodontitis in patients with chronic periodontitis, which can be attributed to the naturally higher oxidative stress in Type 2 DM. The diabetic patients had poorly controlled Type 2 DM. They were carefully selected, because we assumed that changes in blood flow values were easily noticeable and more representative in such cases compared with other groups.

Using an animal model (rats) with periodontitis, Nishikawa et al (21) have also investigated how periodontitis progressed in diabetics. Gingival blood flow changes have also been detected in animals. The authors have also expressed that nitrosative stress might be due to periodontitis in diabetic rats. In short, nitrosative stress might play a crucial role in the exacerbation of periodontitis in diabetic patients. Scardina et al (28), in their study, concluded that the increase in capillary density could suggest the presence of active inflammatory phenomena or a tendency for a greater susceptibility to inflammatory phenomena. Finally, it was shown that there was some peripheral damage to the microcirculation at the masticatory mucous level in diabetics and such changes could be shown and evaluated via the videocapillaroscopic method.

CONCLUSION

Within the limits of our study, it has been demonstrated that the blood flow changes in different disease groups can be monitored non-invasively by using LDF. It seems to be a practical and easy way to make clinical evaluations. However, further studies with higher number of subjects are required to better understand its role and importance in clinical practice.

REFERENCES