Assessment of Gingival Microvasculature Changes in Young Diabetic Patients and Its Correlation with Systemic Complications

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ABSTRACT

Background: Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. The microvascular complications of diabetes include retinopathy, nephropathy and neuropathy. Gingival microvasculature represents vascular changes elsewhere in the body and hence gingival biopsies may be subjected for microscopic analysis to evaluate the microvasculature changes in diabetic patients.

Aim: The present study aims at demonstrating microvascular changes in gingiva of diabetics and analyze its correlation with systemic complications of diabetes.

Materials and methods: A total of 46 outpatients with known diabetes between the age group of 10 and 35 years, visiting SDM College of Dental Sciences, Dharwad and Day Care Centre, Hubli were included in the study. A total of 40 control subjects (20 males and 20 females) were also selected. Gingival biopsies were performed; specimens were stained and visualized under the microscope to assess the microvasculature changes in diabetic patients.

Results: Signs of microangiopathy were prominent in diabetics. A total of 16 specimens (34.8%) showed widening and reduplication of basement membrane, 33 specimens (71.7%) had increased staining in basement membrane, nine (19.6%) showed splitting of basement membrane, 28 (60.9%) had swelling of endothelial cells and 5 (10.9%) showed proliferation of endothelial cells with or without lumen obliteration. Out of the 46 diabetic subjects, grade I microangiopathy was seen in 33 subjects, grade II in seven subjects and grade III in six subjects. Retinopathy was present in five subjects, all with uncontrolled diabetes, nephropathy was present in 22 subjects out of which 90.9% were uncontrolled diabetics and neuropathy was seen in 23 subjects out of which 86.9% were uncontrolled diabetics.

Keywords: Diabetes mellitus, Gingival microvasculature, Diabetic complications.

INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute (impaired production) or relative (impaired utilization) deficiency of insulin.1 It is the most common endocrine disease which is also characterized by metabolic abnormalities and by long-term complications involving the eyes, kidneys, nerve and blood vessels.2 Lack of insulin affects the metabolism of carbohydrates, proteins, fat, water and electrolytes.1 Diabetes can be divided into two main types: Type 1 (insulin-dependent diabetes mellitus) and type 2 (noninsulin-dependent diabetes mellitus).3

The vascular system is more susceptible to functional and structural changes in diabetes mellitus, leading to microvascular complications in diabetes.4 The complications of diabetes include retinopathy, nephropathy and neuropathy.5 The microvascular changes reported in diabetes are widening of basement membrane in capillaries and precapillary arterioles, thickening of the walls of small blood vessels, proliferation and accumulation of endothelial cells and occlusion of capillary lumen.5 Diffuse thickening of the basement membrane is the most consistent feature of diabetes. The thickening is most evident in the capillaries of the skin, retina, renal glomeruli and renal medullae.6

Evidence indicates that improved glycemic levels and blood pressure control can slow and perhaps even stop the development of microvascular changes that lead to systemic complications. In addition, early recognition provides opportunity for early treatment, which has been shown to preserve vision, renal function and limb integrity. Screening of early signs of diabetic microvascular changes ensures that patients with diabetes live longer and lead a better life.5

Gingival microvasculature represents the microvascular changes elsewhere in the body and hence gingival biopsies may be subjected for microscopic analysis to evaluate the
microvasculature changes in diabetic patients. Hence, the present study aims at demonstrating microvasculature changes in gingiva of diabetics and analyses its correlation with diabetic microvasculature complications.

MATERIALS AND METHODS
A total of 46 outpatients with known diabetes, visiting SDM College of Dental Sciences, Dharwad and Day Care Centre in Hubli were included in the study. All subjects (28 males and 18 females) were between the age of 10 and 35 years. Also, 40 control subjects (20 males and 20 females) between the age of 10 and 35 years were selected. Diabetic patients, who were beyond 35 years of age, with family history of diabetes, those suffering from hypertension and other cardiovascular disorders, which lead to arteriosclerosis, were excluded from the study.

A thorough medical examination of all subjects was conducted and findings were recorded in standard medical proforma. Subjects were investigated for the fasting and post-prandial blood sugar levels. Qualified personnel subjected the diabetic as well as nondiabetic group for fundoscopic examination for retinal changes. One hour prior to examination, patient was given 2 drops of homatropine eye drops for dilatation of the pupil. The results were noted as simple and proliferative retinopathy or normal optic fundus. All patients were subjected to urine albumin test using a Uristix to detect nephropathic changes. Neuropathy was determined from clinical history of burning and tingling sensation of hands and feet.

Biopsy Procedure
Gingival biopsies were performed by a sharp dissection using local anesthesia. Interdental papilla between the lower right first and second premolar was the site selected for obtaining biopsy. Sections were prepared and stained with periodic acid Schiff’s reagent using McManus method.

Setting up the Microscope
Accurate measurements of length in the microscope require a calibrated scale superimposed on the specimen so that both scale and specimen are simultaneously in focus. A stage micrometer is used in conjunction with micrometer eyepiece. The stage micrometer consists of 3 × 1 inch slide, on which a millimeter scale is graduated in hundredths of a millimeter. The micrometer eyepiece consists of a special eyepiece which has a micrometer thread. Each division of the eyepiece scale corresponds to 10 divisions of the millimeter scale. The stage micrometer was removed and the stained section was placed. The width of the vessel wall covering the number of divisions of the eyepiece micrometer was noted. In every section, 5 vessels were measured and the mean value was taken as the thickness of the vessel wall.

Calculation and Standardization of Each Division of Eyepiece Micrometer in Microns
The number of divisions on the eyepiece scale corresponding to the definite number of divisions on the millimeter scale was determined. It was found that 1 division of the table micrometer was corresponding to 10 divisions of the eyepiece scale.

Hence, the value of each division of the eyepiece micrometer was calculated as:

<table>
<thead>
<tr>
<th>Table micrometer</th>
<th>Eyepiece micrometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 division</td>
<td>10 divisions</td>
</tr>
<tr>
<td>Each division</td>
<td>10 microns</td>
</tr>
<tr>
<td>1 division of eyepiece micrometer</td>
<td>01 micron</td>
</tr>
</tbody>
</table>

Measurement of Vessel Wall Thickness
After determining the value of each division of eyepiece micrometer, the stage micrometer was removed and the stained section was placed. The width of the vessel wall covering the number of divisions of the eyepiece micrometer was noted. In every section, 5 vessels were measured and the mean value was taken as the thickness of the vessel wall.

Criteria for Grading Microangiopathy

Grade I: Vessels showing changes in the basement membrane like widening, reduplication, splitting and increased staining intensity.

Grade II: Vessels showing changes in the basement membrane and swelling of endothelial cells.

Grade III: Vessels showing changes in the basement membrane with proliferation of endothelium cells and with or without partial or complete obliteration of lumen.

All observations were recorded on tables according to criteria.

RESULTS
The statistical data collected were analyzed by employing appropriate tests viz, Chi-square test. Signs of microangiopathy were prominent in diabetics as depicted in Table 1. Sixteen specimens (34.8%) showed widening and reduplication of basement membrane (Figs 1 and 2), 33 specimens (71.7%) had increased staining in basement membrane, 9 (19.6%) showed splitting of basement membrane (Fig. 3), 28 (60.9%) had swelling of endothelial cells and 5 (10.9%) showed proliferation of endothelial cells with or without lumen obliteration.

The distribution of different grades of microangiopathy among controlled and uncontrolled diabetic and nondiabetic groups is shown in Table 2. Out of the 46 diabetic subjects, grade I microangiopathy was seen in 33 subjects, grade II in seven subjects and grade III in six subjects.

The distribution of subjects for presence or absence of retinopathy, nephropathy as well as neuropathy and its correlation with grades of microangiopathy in the study and control groups is shown in Graph 1. Out of the 46 subjects in study group, five (10.9%) showed retinal changes, 22 (47.8%) showed nephropathic changes and 23 (50.0%) showed neuropathic changes indicating that these complications are associated with diabetic microangiopathy. However, no correlation was seen between the complications and the different grades of microangiopathy.

A comparison of mean values of thickness of vessel wall was made between the study and control groups. It was found...
that the mean vessel wall thickness in study group was 5.363 (SD = 2.545) as compared to the control group showing the mean value of vessel thickness as 1.230 (SD = 0.189). Hence, the vessel wall thickness of study group was found to be statistically significant (p < 0.01).

The distribution of subjects with retinopathy, nephropathy and neuropathy and its correlation to the state of control of diabetes is shown in Graph 2. Yates corrected Chi-square test value for comparison of distribution of retinopathy ($\chi^2 = 5.9691$), nephropathy ($\chi^2 = 14.1576$) and neuropathy ($\chi^2 = 11.3834$) among controlled and uncontrolled diabetics was found to be significant at 5% level of significance (p < 0.05). Retinopathy was present in five subjects, all with uncontrolled diabetes, nephropathy was present in 22 subjects out of which 90.9% were uncontrolled diabetics and neuropathy was seen in 23 subjects out of which 86.9% were uncontrolled diabetics.

**DISCUSSION**

Diabetes mellitus (DM) is a complex multisystemic disorder characterized by a relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues. Its prevalence is increasing in the present scenario of a sedentary lifestyle in the general population. The World Health Organization (WHO) estimates the global burden of diabetes to be 299 million cases by the year 2025.

Although according to the definition, the pathophysiological basis and much of management of diabetes mellitus is glucocentric, it is a true metabolic disorder, and a number of metabolic disturbances have been characterized. While good glycemic control can prevent or reduce the likelihood of the possible complications of DM, approximately 50% of patients with diabetes mellitus develop vascular chronic complications following years of disease.

The vascular system is more susceptible to functional and structural changes in diabetes mellitus, leading to the

**Table 1:** Distribution of subjects according to the observed signs of microangiopathy

<table>
<thead>
<tr>
<th>Signs of angiopathy</th>
<th>Diabetic</th>
<th>Non diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>No. 16</td>
<td>% 34.8</td>
</tr>
<tr>
<td>Absent</td>
<td>No. 30</td>
<td>% 65.2</td>
</tr>
<tr>
<td>Increase intensity of staining of basement membrane Present</td>
<td>No. 33</td>
<td>% 71.7</td>
</tr>
<tr>
<td>Absent</td>
<td>No. 13</td>
<td>% 28.3</td>
</tr>
<tr>
<td>Splitting of basement membrane Present</td>
<td>No. 9</td>
<td>% 19.6</td>
</tr>
<tr>
<td>Absent</td>
<td>No. 37</td>
<td>% 80.4</td>
</tr>
<tr>
<td>Swelling of endothelial cells Present</td>
<td>No. 28</td>
<td>% 60.9</td>
</tr>
<tr>
<td>Absent</td>
<td>No. 18</td>
<td>% 39.1</td>
</tr>
<tr>
<td>Proliferation of endothelial cells Present</td>
<td>No. 5</td>
<td>% 10.9</td>
</tr>
<tr>
<td>Absent</td>
<td>No. 41</td>
<td>% 89.1</td>
</tr>
</tbody>
</table>

**Table 2:** Distribution of different grades of microangiopathy among controlled and uncontrolled diabetic and nondiabetic group

<table>
<thead>
<tr>
<th>Grades of angiopathy</th>
<th>Diabetic</th>
<th>Non diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controlled</td>
<td>Uncontrolled</td>
</tr>
<tr>
<td>0</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1</td>
<td>19 (79.2%)</td>
<td>14 (63.6%)</td>
</tr>
<tr>
<td>2</td>
<td>3 (12.5%)</td>
<td>4 (18.2%)</td>
</tr>
<tr>
<td>3</td>
<td>2 (8.3%)</td>
<td>4 (18.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>22</td>
</tr>
</tbody>
</table>

Graph 1: Distribution of subjects for presence or absence of retinopathy, nephropathy and neuropathy and its correlation with grades of angiopathy in the study and control group

Graph 2: Distribution of different grades of microangiopathy among controlled and uncontrolled diabetic and nondiabetic group
microvasculature complications in diabetes. The complications of diabetes include retinopathy, nephropathy and neuropathy. The present study was designed to assess the gingival microvasculature changes in diabetic patients and to correlate these changes with complications of diabetes, such as diabetic retinopathy, nephropathy and neuropathy.

The investigation conducted revealed that the basement membrane of the vessel wall showed widening and reduplication (Figs 1 and 2) in 34.8% of diabetic patients. There was also increased staining of basement membrane in 71.7% of diabetics, and only 19.6% showed splitting of basement membrane (Fig. 3), 28 subjects showed swelling of the endothelial cells as well. Nondiabetics, on the other hand did not present with any of these findings.

Campbell MJA (1971) in the light and electron microscopic examination of blood vessels from the gingival tissues observed that there was thickening of the basement membrane in diabetic subjects, which was not evident in non-diabetics.

Another finding observed in the present study was that thickening of vessel wall was a constant finding in all biopsy sections. Frantzis TG et al (1971) observed that the thickening of vessel wall was pronounced in the gingival tissues of diabetics, which led them to suggest its diagnostic importance in identifying the disease process.

The mean thickness of vessel wall greatly increased in diabetics (5.363) as compared to non-diabetics (1.230). Sepp Ala B et al (1971) in their study of morphometric analysis of cellular and vascular changes in gingival connective tissue in long-term insulin-dependent diabetic subjects seemed to have greater mean areas for the cross sectional area of their gingival blood vessels than controlled insulin dependent diabetics and control group (nondiabetics).

In the present study, it was found that retinopathy was present in five diabetics, all suffering from uncontrolled diabetes. Becerra A et al (1998) in their study stated that glycemic control is related with diabetic retinopathy in both insulin-dependent diabetes mellitus and noninsulin-dependent diabetes mellitus.

Microalbuminuria was present in 22 diabetics in the study, out of which 90.9% subjects suffered from uncontrolled diabetes while only 9.1% had diabetes under control. This indicates that the state of control plays an important role in development of these microvascular complications.

Chase M et al (1989) measured glycohemoglobin value of insulin-dependent diabetes mellitus to relate glucose control with renal and retinal complications. They suggested that poor control had 3.6 times greater presence of microalbuminuria and 2.5 times the prevalence of retinopathy than found in subjects with long-term good control.

SUMMARY AND CONCLUSION

From the present study it was concluded that the microvasculature changes are seen in diabetics as compared to nondiabetes. The diabetic microvasculature complication (retinopathy, nephropathy and neuropathy) seem to be related to the state of control of diabetes. However, no statistical significance was established with grades of microangiopathy which needs to be further evaluated.

REFERENCES