

## EVALUATION OF COLLAGEN DEGRADATION IN GINGIVAL EPITHELIUM AND CONNECTIVE TISSUE OF CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT DIABETES MELLITUS.



### Dental Science

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| <b>NAVITA.S</b>            | Post Graduate Student, Department of Periodontology, RajaRajeswari Dental College & Hospital, Bangalore. |
| <b>SAVITA.S</b>            | Professor & HOD, Department of Periodontology, RajaRajeswari Dental College & Hospital, Bangalore.       |
| <b>RITHESH.K</b>           | Reader, Department of Periodontology, RajaRajeswari Dental College & Hospital, Bangalore.                |
| <b>SHIVAPRASAD.B<br/>M</b> | Reader, Department of Periodontology, RajaRajeswari Dental College & Hospital, Bangalore.                |

### ABSTRACT

**Aim-** Aim of the present study was to evaluate evidence of the histopathological changes occurring in the gingival epithelium and connective tissue in patients with chronic generalized periodontitis with and without diabetes.

**Material and methods-** A total of 30 patients diagnosed with chronic generalized periodontitis (CGP) were divided into group I with controlled diabetes mellitus and group II without diabetes mellitus. Gingival tissues were obtained from patients undergoing periodontal flap surgery or after tooth extractions. The gingival tissue samples were fixed in 10% formalin, then processed by the usual technique of paraffin inclusion followed by staining with hematoxylin-eosin and masson's trichromic stain. The histopathologic study was done to focus on the analysis of changes in the collagen fibres in two groups.

**Results-** Biopsies from gingival mucosa of controls showed a squamous epithelium of normal histological aspect, with a thin layer of keratin, with rare and small sites of parakeratosis. Degradation of connective tissue was present in diabetic subjects, due to a rich inflammatory infiltrate, destruction of reticular fibers and accumulation of dense collagen fibers (fibrosis).

**Conclusion-** Thus, the biopsies obtained from our study group shows that diabetes mellitus induces obvious histological changes, in both epithelium and gingival connective tissue.

### KEYWORDS:

Controlled diabetes mellitus, Periodontal disease, collagen degradation.

### INTRODUCTION

Periodontal disease is a mixed endogenous infection caused by microorganisms that colonize the sub-gingival dental-bacterial plaque, in a structure known as a biofilm.<sup>1</sup>

Diabetes mellitus includes a group of metabolic diseases characterized by hyperglycemia that results from a deficiency in insulin secretion and/or its reduced action. Severe hyperglycemia can cause many symptoms, including polyuria, polydipsia, polyphagia, weight loss and impaired vision.<sup>2</sup> Peripheral vascular changes occur, leading to a reduced immunological capacity of the patient and increasing susceptibility for infection.

Although diabetes mellitus has often been associated with periodontal breakdown, the exact role of this disease in the pathogenesis of periodontal disease is not completely understood.<sup>3</sup> Persons with diabetes mellitus (DM) are at greater risk of developing periodontal disease. Periodontal disease is now considered the sixth complication of DM.<sup>4</sup> Diabetes has impaired defense mechanisms involving micro- and macro-vasculatures. The increased susceptibility to infection and reduced healing capacity with altered collagen metabolism may explain the increased level of periodontal destruction.<sup>9</sup>

Several epidemiological studies have shown that there is no difference in the periodontal status between diabetic and non-diabetic patients. Herring and Shah<sup>6</sup> suggested that patients with controlled diabetes have no more periodontal destruction than healthy controls. On the contrary, some investigators have found increased periodontal destruction in diabetic patients.<sup>7</sup> So, the aim of our study was to evaluate the evidence of the histopathological changes occurring in the gingival epithelium in patients with chronic generalized periodontitis with and without diabetes.

### MATERIAL AND METHODS

Patients visiting the Department of Periodontology in Rajarajeswari Dental College and hospital, who required periodontal flap surgery or extraction of either one or more teeth were selected for the study, in the age group 20- 60 years. All subjects had at least 20 teeth with probing depth of  $\geq 5$ mm, clinical attachment level  $\geq 3$ mm. Patients with

history of systemic disease other than diabetes mellitus, who had taken medications affecting collagen metabolism, who had undergone flap surgery in the last 6 months, pregnant or lactating mothers and smokers were excluded from the study. Total of 30 patients diagnosed with chronic generalized periodontitis (CGP) were examined. Patients were divided in two groups. Group I comprised of 15 chronic generalized periodontitis patients with controlled type 2 diabetes mellitus having RBS  $\leq 180$ . Group II consisted of 15 chronic generalized periodontitis patients without diabetes mellitus.

Gingival tissues were obtained from patients undergoing periodontal flap surgery or after extraction of periodontally compromised tooth. The gingival tissue samples were fixed in 10% formalin, then processed by the usual technique of paraffin inclusion followed by staining with hematoxylin-eosin and masson's trichromic stain. The histopathological analysis was carried out to evaluate the changes in the collagen fibres and inflammatory cell infiltrate in two groups.

### STATISTICAL ANALYSIS

The clinical parameters were analysed using chi square test, Mann-whitney U test was done to compare the inflammatory cells and collagen degradation between the two groups.  $p < 0.05$  was considered significant. Figure 5 and table 1 shows comparison of CGP with diabetic and CGP non-diabetic with respect to status of Masson trichrome stain for inflammatory cell infiltration. Figure 6 and table 2 shows comparison of CGP with diabetic and CGP non-diabetic with respect to status of Masson trichrome stain for collagen degradation.

### RESULT

The histological study of the extra cellular matrix mainly focused on the analysis of changes in the collagen fibers. In group I, collagen fibers appeared dissociated by the inflammatory cells that were inserted among them. The interstitial oedema and the hemorrhagic infiltrates were present in most of the cases and they also contributed to disorganization of the chorion architecture. Collagen bands had a discontinuous aspect and they were fragmented (Figures 1,2,3,4).

When comparing microscopic aspects of fibromucosa of the control group with those of test group, we noticed that the latter group

presented an epithelium of variable thickness, occasionally thin, with rare sites of superficial ulceration and acanthosis. The underlying connective tissue presents ectatic blood vessels surrounded by a rich inflammatory infiltrate, mainly lymphoplasmocytic. Biopsies from gingival mucosa of controls showed a squamous epithelium of normal histological aspect, with a thin layer of keratin, with rare and small sites of parakeratosis (Figures 3,4). The underlying connective tissue formed small papillae that enter through epithelium (dermal papillae). Mitotic activity in the basal layer of the epithelium that accompanies the inflammatory process, cellular intraepithelial vacuolization and acantholysis in stratum spinosum. Parakeratosis and remains of nucleus of epithelial cells were present in the superficial area of the epithelium. The capillaries and venules were dilated with perivascular lymphoplasmocytic infiltrate and thrombosis. Degradation of connective tissue was present in diabetic subjects, due to a rich inflammatory infiltrate, destruction of reticular fibers and accumulation of dense collagen fibers (fibrosis).

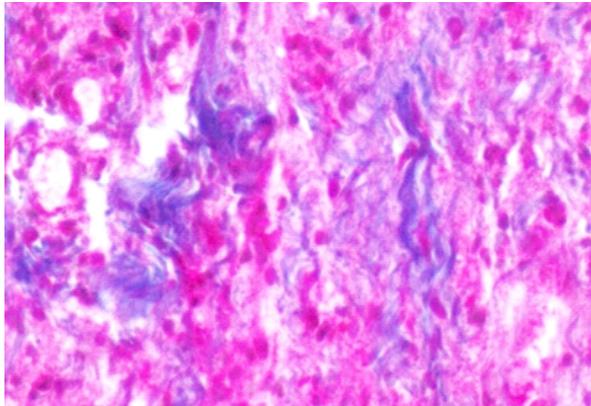


Figure 1- Distorted collagen bundle by infiltration of inflammatory cell infiltrate in group I using masson's trichromic stains

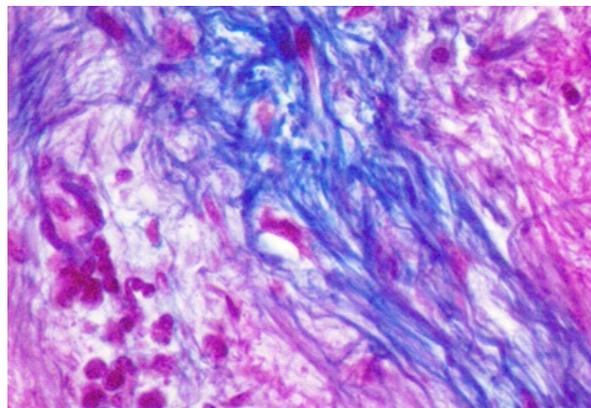


Figure 2- Arranged collagen bundle with less amount of inflammatory cell surrounding them in group II using masson's trichromic stains

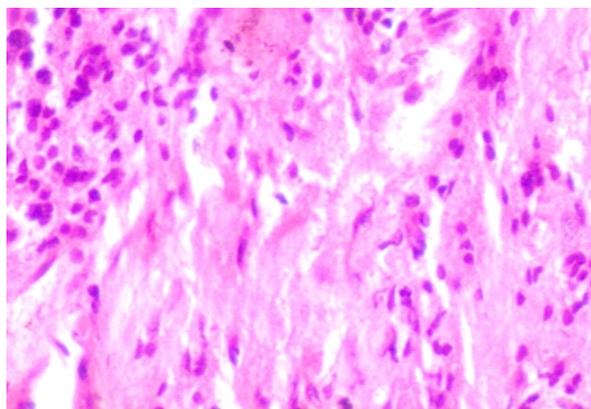


Figure 3- Loss of chorion architecture of collagen fibre due to infiltration of dense inflammatory cell infiltrate in group I using hematoxylin-eosin stains

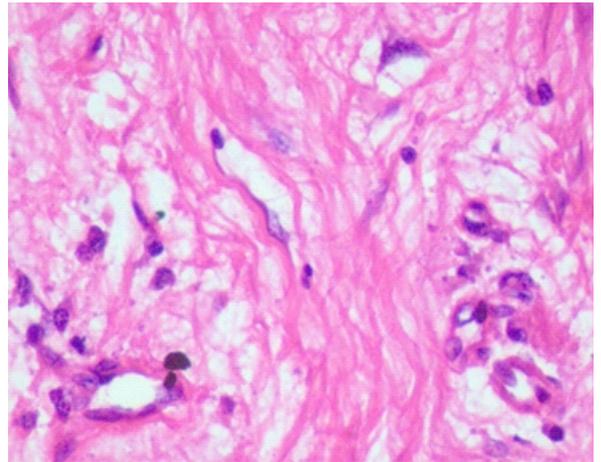


Figure 4- Arranged collagen bundle surrounded by sparse inflammatory cells in group II using hematoxylin-eosin stains

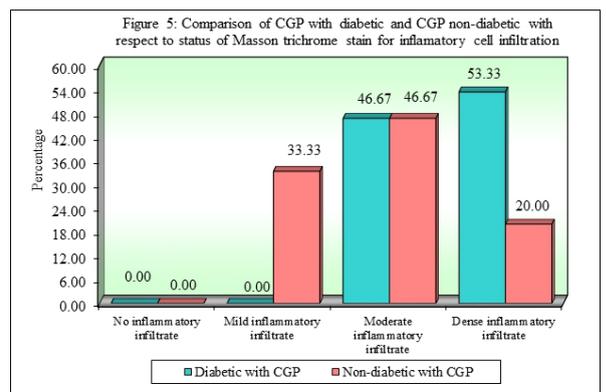
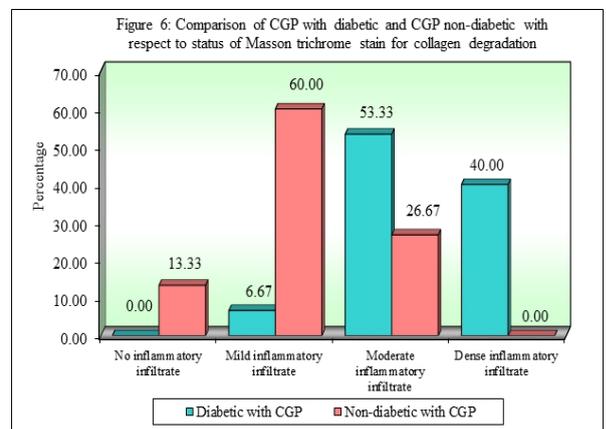


Table 1. Comparison of diabetic with CGP and non-diabetic with CGP with respect to status of Masson trichrome stain for inflammatory cell infiltration

| Masson trichrome stain for inflammatory cell infiltration | Diabetic with CGP | %      | Non-diabetic with CGP | %      | Total |
|---|-------------------|--------|-----------------------|--------|-------|
| No inflammatory infiltrate                                | 0                 | 0.00   | 0                     | 0.00   | 0     |
| Mild inflammatory infiltrate                              | 0                 | 0.00   | 5                     | 33.33  | 5     |
| Moderate inflammatory infiltrate                          | 7                 | 46.67  | 7                     | 46.67  | 14    |
| Dense inflammatory infiltrate                             | 8                 | 53.33  | 3                     | 20.00  | 11    |
| Total   | 15                | 100.00 | 15                    | 100.00 | 30    |

Chi-square= 7.2732, p=0.0263\*  
Mann-Whitney U, Z=-2.2813, p=0.0225\*



**Table 2- Comparison of diabetic with CGP and non-diabetic with CGP with respect to status of Masson trichrome stain for collagen degradation**

| Masson trichrome stain for collagen degradation | Diabetic with CGP | %      | Non-diabetic with CGP | %      | Total |
|---|-------------------|--------|-----------------------|--------|-------|
| No inflammatory infiltrate                      | 0                 | 0.00   | 2                     | 13.33  | 2     |
| Mild inflammatory infiltrate                    | 1                 | 6.67   | 9                     | 60.00  | 10    |
| Moderate inflammatory infiltrate                | 8                 | 53.33  | 4                     | 26.67  | 12    |
| Dense inflammatory infiltrate                   | 6                 | 40.00  | 0                     | 0.00   | 6     |
| Total   | 15                | 100.00 | 15                    | 100.00 | 30    |
| Chi-square= 15.7333 p=0.0012*                   |                   |        |                       |        |       |
| Mann-Whitney U, Z=-3.6501, p=0.0003*            |                   |        |                       |        |       |

**DISCUSSION**

Periodontitis is stated to be the sixth complication of diabetes.<sup>8</sup> Majority of well-controlled studies show a higher prevalence and severity of periodontal disease in diabetics than in non-diabetics with similar local irritation including greater loss of attachment, greater alveolar bone loss, increased bleeding on probing, and increased tooth mobility resulting in tooth loss.

Golub et al found in experimentally induced diabetes, impaired production of bone matrix component by osteoblasts, decreased collagen synthesis by gingiva and periodontal ligament fibroblasts, and increased collagenase activity. Collagen from diabetic patients has been reported to be more insoluble and resistant to digestion, directly impairing degradation and remodeling. Increased crevicular fluid collagenolytic activity and decreased synthesis of collagen by gingival fibroblasts was found in diabetic patients. Administration of insulin prevents the onset and corrects the defective collagen production.<sup>10</sup>

In 2008, Silva JA et al. reported atrophy and pleomorphism of the gingival epithelium, with decreased cellular organelles and increased intercellular spaces, and a thickened keratin layer with the reduction of height of dermal papilla. According to these authors, increased mitotic activity in epithelial basal layer can contribute to the compromise of epithelial cells differentiation, leading to a thickening of the keratin layer and flattening of dermal papillae. Furthermore, they observed a reduction in collagen solubility and the distortion of reticular fibers.<sup>11</sup> The primary factor responsible for the development of diabetic complications is prolonged tissue exposure to hyperglycaemia, which results in the production of advanced glycation end products (AGEs). This leads to an increase in collagen cross-linking and the generation of reactive oxygen intermediates, such as free radicals.<sup>16</sup> The modified collagen fibres accumulate in the tissues, resulting in thickening of the basement membrane. This impairs oxygen diffusion, waste elimination, leukocyte migration and the diffusion of immune factors and may thereby contribute to the pathogenesis of periodontitis.<sup>17</sup>

Altered collagen metabolism may predispose patients with diabetes not only to periodontal disease but also to other abnormalities of connective tissues, such as impaired wound healing. Elevations of collagenase activity in gingival crevicular fluid<sup>12</sup> and decreases in gingival collagen synthesis<sup>13</sup> in patients with diabetes have been observed. In diabetics, the periodontium is probably affected by increased collagenase action, functional abnormalities of neutrophil degranulation as a source of gingival crevicular fluid collagenase or other metabolic abnormalities in periodontal ligament fibroblast. Vascular changes in properly controlled long-term diabetes shows microangiopathy.<sup>14,15</sup>

**Conclusions**

According to the results of biopsies obtained from our study group, diabetes mellitus induces obvious histological changes, in both epithelium and gingival connective tissue. We noticed accelerated mitosis in basal epithelial layer, acanthosis in spinocellular layer, parakeratosis and hyperkeratosis in superficial layer even in controlled diabetes patients. Hence, more cautious approach is required to treat periodontal diseases in controlled diabetes patients.

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