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ORIGINAL RESEARCH

Comparison of neutrophil functions in diabetic and healthy subjects with chronic generalized periodontitis

Amanpreet Kaur Saini[1], Gopikrishnan Vijayakumar[2], Pooja Sharma[3]

[1]Consultant Periodontist and general dental practitioner, Chandigarh
[2]Consultant Oral Pathology and general dental practitioner, Kerala
[3]Consultant Oral Pathology and general dental practitioner, Delhi

ABSTRACT:
Background: Diabetes mellitus (DM) is a chronic condition characterized by chronic hyperglycemia that results from unregulated endocrine and metabolic pathways in glucose utilization. Periodontitis is a slowly progressing disease but the tissue destruction that occurs is largely irreversible. In the early stages, the condition is typically asymptomatic; it is not usually painful, and many patients are unaware until the condition has progressed enough to result in tooth mobility. Diabetic patients with chronic periodontitis have depressed chemotaxis compared with nondiabetic patients with chronic periodontitis. **Aim of the study:** To compare neutrophil functions in diabetic and healthy subjects with chronic generalized periodontitis. **Materials and methods:** The present study was conducted in the Department Periodontics of the dental institution. A total of 40 subjects were selected for the study. 20 were diabetics and 20 were non diabetics with chronic periodontitis. Patient included in the study had 20 teeth present and had >2 mm of attachment loss at periodontitis sites. The patients having any systemic disease other than diabetes were excluded from the study group and with history of any systemic disease were excluded from control group. The brief history of each patient including name, age, sex, past medical and dental history, plaque inde and gingival inde were recorded. **Results:** A total of 40 patients participated in this study. We observed that mean age in study group was 51.65 years and in control group was 53.24 years. Number of male participants in study group was 18 and female participants were 12. Similarly, number of male participants in control group was 16 and female participants were 14. We observed that mean neutrophil chemotaxis for study group was 20.35 and for control group was 33.65. The results on comparison were seen to be statistically significant ($p<0.05$). **Conclusion**: Within the limitations of the present study, it can be concluded that neutrophil activity is significantly reduced in diabetic patients.
Keywords: Neutrophil, periodontitis, diabetes mellitus

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Corresponding author: Dr. Amanpreet Kaur Saini, Consultant Periodontist and general dental practitioner, Chandigarh

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INTRODUCTION

Diabetes mellitus (DM) is a chronic condition characterized by chronic hyperglycemia that results from unregulated endocrine and metabolic pathways in glucose utilization. Diabetes-associated microvascular disease is a leading cause of blindness, renal failure and nerve damage, and significantly contributes to the increasing incidence of myocardial infarction and stroke in the developing world. [1] Periodontitis is a slowly progressing disease but the tissue destruction that occurs is largely irreversible. In the early stages, the condition is typically asymptomatic; it is not usually painful, and many patients are unaware until the condition has progressed enough to result in tooth mobility. The pockets deepen as a result of the further destruction of fibres of the periodontal ligament (referred to as attachment loss; Fig. 1) and the resorption of the alveolar bone that occurs in parallel with the progressing attachment loss. [2, 3] Advanced periodontitis is characterised by gingival erythema

and oedema, gingival bleeding, gingival recession, tooth mobility, drifting of teeth, suppuration from periodontal pockets, and tooth loss. Reduced PMN function has been found in patients with diabetes. PMN dysfunction studies in diabetics have exhibited defects in chemotaxis, phagocytosis and killing, and increased release of super oxide. [4] Diabetic patients with chronic periodontitis have depressed chemotaxis compared with nondiabetic patients with chronic periodontitis. Neutrophils from periodontitis patients generate abnormally high levels of oxygen radicals in response to stimuli. [5, 6] Hence, the present study was conducted to compare neutrophil functions in diabetic and healthy subjects with chronic generalized periodontitis.

MATERIALS AND METHODS

The present study was conducted in the Department Periodontics of the dental institution. The ethical clearance for the study was approved from the ethical committee of the hospital. The subjects were selected from the Outpatient department of periodontics and from diabetic clinic. A total of 40 subjects were selected for the study. 20 were diabetics and 20 were non diabetics with chronic periodontitis. Patient included in the study had 20 teeth present and had >2 mm of attachment loss at periodontitis sites. The patients having any systemic disease other than diabetes were excluded from the study group and with history of any systemic disease were excluded from control group. The brief history of each patient including name, age, sex, past medical and dental history, plaque inde and gingival inde were recorded.

Neutrophil function tests

The tests include chemotaxis and super oxide estimation. Five milliliters of venous blood was drawn from the antecubital vein with a needle and a disposable syringe; 2.5 ml of this blood was transferred into a plain vial (for phagocytosis), and the remaining 2.5ml into the vial containing EDTA and transported to the laboratory. The cells were prepared in RPMI 1640 solution and the neutrophils adjusted at 5 × 10[6] /ml, making sure that the cell concentration was same in the test and control samples. The lower compartment of the chemotactic chamber was filled with FMLP as the chemotactic factor. The upper compartment was filled with cell suspension and placed inside the lower compartment, and the chamber was incubated at 37°C in air for 3 hours. At the end of 3 hours, the test was taken out of the incubator and the upper compartment was removed and emptied. The upper compartment was then immersed in 70% ethanol or methanol for a few minutes so that the glue melted and the filter became loose from the bottom of the syringe. This was picked up with a tweezer, taking care not to touch the rim and stain it. Once fixed, it was mounted under a cover slip with the lower side of the filter facing up and examined for the presence of neutrophils that have crossed to the lower surface of the filter.

The statistical analysis of the data was done using SPSS version 11.0 for windows. Chi-square and Student's t-test were used for checking the significance of the data. A p-value of 0.05 and lesser was defined to be statistically significant.

RESULTS

A total of 40 patients participated in this study. 20 patients were diabetics and formed study group. 20 patients were non diabetics and consisted control group. Table 1 shows demographics of study group and control group. We observed that mean age in study group was 51.65 years and in control group was 53.24 years. Number of male participants in study group was 18 and female participants were 12. Similarly, number of male participants in control group was 16 and female participants were 14. Table 2 shows mean neutrophil chemotaxis for study group and control group. We observed that mean neutrophil chemotaxis for study group was 20.35 and for control group was 33.65. The results on comparison were seen to be statistically significant ($p<0.05$) [Fig 1].

Table 1: Demographics of study group and control group

Variables	Study Group	Control Group
Total number of patients	30	30
Mean age (years)	51.65	53.24
No. of male participants	18	16
No. of female participants	12	14

Table 2: Mean neutrophil chemotaxis for study group and control group

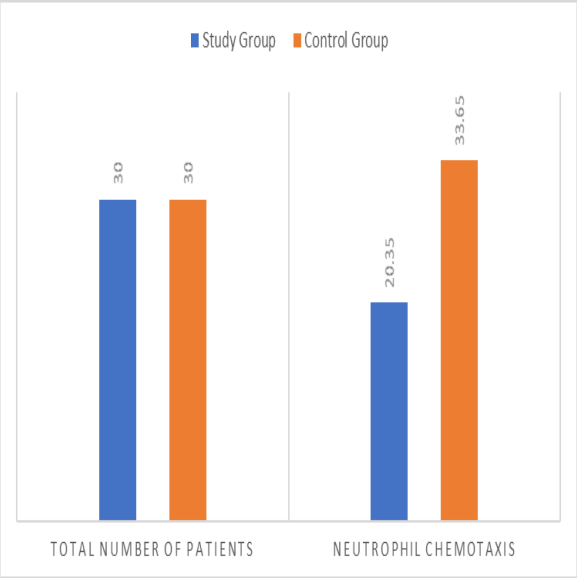
Variables	Study Group	Control Group
Total number of patients	30	30
Neutrophil chemotaxis	20.35	33.65
SD	4.34	5.65
p-value	0.002	

DISCUSSION

In the present study, we observed that neutrophil chemotaxis was significantly reduced in diabetic patients as compared to non diabetics. The results were compared to previous studies from literature and was found to be consistent with the results. Shetty N et al evaluated PMN functions in 15 diabetic patients with chronic generalized periodontitis. Chemotaxis, superoxide production, phagocytosis and killing of Porphyromonas gingivalis by diabetic PMNs were evaluated relative to healthy and matched controls. These analyses revealed a significant depression in the number of diabetic PMNs migrating along an fMLP gradient. In addition, a significant enhancement of diabetic PMN superoxide production was observed.

Phagocytosis and killing by diabetic PMN of P. gingivalis was also impaired significantly. Bhansali RS et al evaluated neutrophils chemotaxis, phagocytosis, microbicidal activity and superoxide generation in LAP patients of Indian origin. Eleven LAP patients and nine healthy subjects were included in the study. Neutrophil chemotaxis was evaluated against an alkali-soluble casein solution using Wilkinson's method. Phagocytosis and microbicidal activity assay were performed using Candida albicans as an indicator organism.

Fig 1: Pictorial representation of neutrophil chemotaxis comparison on diabetics and controls



Nitrobluetetrazolium (NBT) test was used to assess superoxide generation by neutrophils using E. coli endotoxin. The chemotactic activity and phagocytic and microbicidal activity were observed to be significantly reduced in LAP neutrophils. On the contrary, superoxide generation was observed to be significantly increased in LAP neutrophils compared with healthy individuals. They that neutrophil functions, namely chemotaxis, phagocytosis and microbicidal activity, are deficient LAP patients. However, superoxide generation was significantly increased when stimulated by endotoxins, which may explain the tissue damage seen in LAP. These abnormal neutrophil functions may predispose to increased susceptibility for LAP. [7, 8]

Herrmann JM et al investigated gingival cellular inflammatory responses in individuals with previously undiagnosed T2DM with CP or CP alone and in systemically and periodontally healthy controls (H) in vivo and established an ex vivo technique permitting quantitative and qualitative assessments of gingival crevicular immune cells. T2DM + CP, CP, and H individuals (n = 10, each) received a 2-week oral hygiene regimen (OHR). Afterwards, a noninvasive sampling technique was performed to evaluate gingival inflammation induced under standardized conditions in vivo, that is, in the absence of severe periodontal destruction and inflammation at clinically healthy sites. Stimuli (casein/test or phosphate-buffered saline w/o. Ca2+ or Mg2+, PBS(−/−)/control) were randomly applied contralaterally in the gingival sulci of participants' upper dentes canini. One day after completion of the OHR, gingival crevicular fluid (GCF) was kinetically assayed between the time of the baseline (BL) measurement and 55 minutes. Polymorphonuclear leukocyte (PMN) content (PMNGCF) was quantitated at an optimum time of 35 minutes. PMNGCF counts reflect local inflammation. Ex vivo samples were fluorimetrically labeled, gated according to the donor's peripheral blood polymorphonuclear neutrophils (PMNPB), and then counted, employing flow cytometry. PMNGCF counts in unstimulated gingival crevices (at BL) in the T2DM + CP group were higher than those in the CP and H groups. PMNGCF counts were elevated in casein vs PBS(−/−)-stimulated gingival crevices in all groups. Patients with T2DM + CP showed increased PMNGCF counts compared to those with CP according to scatter plots. CD45+ counts in the stimulated sites in T2DM + CP patients were higher than those in CP and H patients. Under stimulation conditions, the CD45+ counts differed from those under placebo conditions, indicating augmented, inducible inflammatory leukocyte infiltrate in T2DM + CP patients. They concluded that this noninvasive technique permits quantitative assessment of (experimental) gingival inflammation in vivo, revealing an influence of T2DM + CP on the number of primary immune cells in the gingival crevice. Patients who are challenged with (local) leukocytosis are likely at risk of collateral damage to the gingival crevice neighboring tissues, favoring the severity and progression of CP and consequently T2DM. Cutler CW et al evaluated the polymorphonuclear leukocyte (PMN) function in a poorly controlled adult insulin-dependent diabetic patient (IDDM) with severe recurrent periodontitis, while describing the microbiological and clinical findings. Chemotaxis, superoxide production, and phagocytosis and killing of Porphyromonas (Bacteroides) gingivalis by the IDDM PMN were evaluated 1 week before treatment relative to a healthy, matched control. These analyses revealed a significant depression in the number of IDDM PMNs migrating along an FMLP gradient (Boyden chamber assay). In addition, a significant enhancement of IDDM PMN superoxide production in response to opsonized zymosan (cytochrome C reduction) was observed. Phagocytosis and killing by IDDM PMN of two P. gingivalis strains was also impaired significantly. The subgingival microflora contained significant levels of P. gingivalis, Actinobacillus actinomycetemcomitans, Eikenella corrodens, and Peptostreptococcus micros. Periodontal treatment consisted of extraction of hopeless teeth, scaling and root planing and 3 weeks

of Augmentin therapy. The antibiotic therapy resulted in unrecoverable numbers of the putative pathogens and a reduction in both gingival inflammation and disease progression. The IDDM healing response to previous surgical treatment and extractions was poor, presumably due to a marked thrombocytopenia. [9, 10]

CONCLUSION

Within the limitations of the present study, it can be concluded that neutrophil activity is significantly reduced in diabetic patients.

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