Quantification of Caspase 3 levels in patients with periodontitis with or without Diabetes Mellitus

ABSTRACT

Background

Diabetes mellitus is a debilitating systemic disease with several major complications affecting the quality and length of life. Periodontal disease has been considered another diabetic complication in addition to cardiovascular disease, nephropathy, neuropathy, peripheral vascular disease. Caspase-3 plays an important role in intracellular signaling pathways that regulate apoptosis. High levels of glucose could induce human periodontal ligament fibroblast apoptosis.

Aim: The aim of the study is to compare caspase 3 levels in periodontitis patients with or without diabetes mellitus.

Materials and methods: 30 patients were included in the study and they are divided into 3 groups: Group a- Periodontal health; Group b- Periodontitis with diabetes mellitus and Group c- Periodontitis patients without diabetes mellitus. Whole unstimulated saliva was collected from 30 patients using expectoration into sterile bulbs. Caspase 3 levels in saliva samples were measured in duplicate using a commercially available Human Caspase-3 (CASP3) enzyme linked immunosorbent assay (ELISA) Kit. Results were analyzed statistically by a one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Newman-Keuls multiple comparison test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant.

Results: From this study, it was observed that there was a significant increase of caspase 3 levels in periodontitis patients with diabetes mellitus (86.29±24.25 pmol/L) when compared to periodontitis patients without diabetes (55.06±12.90 pmol/L). The results showed a positive correlation and high level of significance when compared between periodontally healthy patients and periodontitis patients along with diabetes mellitus (86.29±24.25 pmol/L). Also, relatively significant results were observed in comparison between periodontally healthy patients (43.37±15.35 pmol/L) and patients with periodontitis without diabetes mellitus (55.06±12.90 pmol/L).

Conclusion: The present study showed that caspase-3 concentrations in saliva increases in patients with periodontitis complicated along with diabetes mellitus. Moreover, saliva concentrations of caspase-3 increase with periodontal disease and caspase-3 plays a role as a biomarker of periodontal disease and its progression.

Keywords: Periodontitis, caspase- 3, diabetes mellitus, innovative technology

1. INTRODUCTION

Diabetes mellitus is a debilitating systemic disease with several major complications affecting the quality and length of life. Periodontal disease has been considered another diabetic complication in addition to cardiovascular disease, nephropathy, neuropathy, peripheral vascular disease [1–3]. High glucose or hyperglycemia can trigger apoptosis in many tissues and cells [4–6].

Periodontitis, a common infectious disease characterized by inflammation and destruction of periodontal tissue and the major cause of tooth loss in adults, is considered one of the main complications of diabetes mellitus [7]. Apoptosis is an important biological process which is involved in regulating many physiological and pathologic pathways [8,9]. It is a highly regulated form of programmed cell death, defined by distinct morphological and biochemical features and plays a pivotal role in tissue homeostasis in multicellular organisms. Its perturbation has been associated with several disorders, which include cancer, rheumatoid arthritis, and periodontal diseases.[10–13] Various stimuli like hormones, cytokines,

and growth factors can modulate the apoptotic process. Hyperglycemia and the accompanying production of excess amounts of advanced glycation end products (AGEs), contributes to reactive oxygen species (ROS) generation leading to oxidative stress and eventually cell death or apoptosis.

Recent literature demonstrates that apoptosis is essentially mediated by a family of cysteine proteases, called caspases, which can be divided into initiator and effector caspases. [14] Initiator caspases, such as caspase-8 or -9, activate downstream effector caspases, such as caspase 3, 6, or 7, which cleave various cellular substrates [15].Caspases play an important role in modulating apoptosis, necrosis, and inflammation.[16–18] Caspase activation can lead to initiation of irreversible protein degradation.[18] Caspase-3 plays an important role in intracellular signaling pathways that regulate apoptosis. [13,19] Caspase-3, a member of the *CED-3* subfamily of caspases, initially exists as a 32 kDa inactive proenzyme known as procaspase-3.Caspase-3 modulates either partial or total proteolytic cleavage of many important key proteins, such as nuclear enzyme poly ADP ribose polymerase, which are cleaved during apoptosis. Increased expression of Caspase-3 in cell lines of lymphocytic origin suggests that it is an important mediator of apoptosis in the immune system [20].

A study by Liu et al, hypothesized that high levels of glucose could induce human periodontal ligament fibroblast apoptosis by quantitatively detecting the extent of apoptosis by flow cytometry to determine how the duration of high glucose levels affected human periodontal ligament fibroblasts apoptosis and investigating the role of the caspase-3/PARP apoptotic signaling pathway on human periodontal ligament fibroblasts apoptosis. They concluded that increased glucose levels and human periodontal ligament fibroblasts apoptosis were directly proportional to time and that caspase-3/PARP apoptotic signaling pathway played an important role in this process [21].

Despite recent progress in scientific research, a great deal is still unknown about apoptosis [22,23]. Our team has extensive knowledge and research experience that has translated into high quality publications [24–36],[37–41] [42] [43]. Therefore, elucidating the mechanism of apoptosis in response to high glucose is essential in order to better understand the etiopathogenesis and pathophysiology of high glucose induced periodontitis and to develop novel medical treatments against this debilitating condition. to establish a significant relationship between increased glucose levels and periodontal fibroblast apoptosis. Hence the aim of the study is to compare the caspase 3 levels in periodontitis patients with or without diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Patient population and study design

Patients aged 30 to 60 years, visiting the Department of Periodontics, Saveetha dental college and hospitals, Chennai, India from December 2020 to February 2021 were examined. 30 patients were included in the study and divided into three groups, 10 in each group: Periodontally healthy patients (Group a), patients with periodontitis and diabetes mellitus (Group b) and patients with periodontitis only (Group c).

The enrollment criteria for the periodontitis cases are as follows: Not more than two teeth missing in each quadrant; more than or equal to 30% periodontal sites with probing depth more than or equal to 4 mm; More or equal to 20% of periodontology sites periodontal sites with interproximal clinical attachment loss more than equal to 2mm; More than or equal to 30% of sites showing bleeding on probing and radiographic evidence of bone loss visible in posterior vertical bitewing films. 24 individuals with clinically healthy periodontium of similar age, race, ethnicity and sex, who had less than 10% sites with bleeding on probing, no sites with probing depth more than or equal to 4mm, no clinical attachment loss of more than 2 mm and no radiographic evidence of bone loss visible in posterior bite wing. Exclusion criteria included individuals who had undergone periodontal treatment in the last 6 months, smoking or use of any form of tobacco, history of alcoholism and any acute periodontal conditions.

2.2 Saliva collection

Participants were instructed to refrain from eating, drinking and practicing oral hygiene procedures 12 hours before saliva collection, Whole unstimulated saliva was collected from all patients using

expectoration into sterile containers and the samples were immediately transported to the laboratory, where they were centrifuged at 5,000 rpm for 10 minutes and the clear supernatants were stored in aliquots at -70°C. The samples were thawed and the assay was performed.

2.3 Caspase 3 analysis in saliva

Caspase 3 levels in saliva samples were measured in duplicate using a commercially available Human Caspase-3 (CASP3) enzyme linked immunosorbent assay (ELISA) Kit procured from Abbkine Scientific Co., Ltd, China as per the manufacturer protocols. This assay is used to quantitatively analyse using sandwich enzyme immunoassay technique. The samples were diluted with calibrator diluent provided with a ratio of 1:4 and the assay was performed according to the instructions. Standards were included and all results were read as the value of optical density set to 450 nm. The intra and inter assay coefficient variance (CV) was found to be < 11% and <9%.

STATISTICAL ANALYSIS:

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ± standard deviation. Results were analyzed statistically by a one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Newman-Keuls multiple comparison test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Apoptosis is a tightly regulated cellular suicidal program that plays a central role in the homeostasis of multicellular organisms by eliminating cells with defects during normal metabolism. In addition, apoptosis is also considered to be essential for the health of periodontal ligament cells and tissues [23]. Diabetes mellitus is a very common systemic disease, and periodontitis is considered to be one of the main oral complication associated with it [24, 25]. Therefore, it is important to evaluate how increased glucose levels could lead to human periodontal ligament fibroblasts apoptosis and cell death.Only a few studies have incorporated the caspase 3 using salivary samples. An inflammatory exudate derived from the periodontal tissues called gingival crevicular fluid, is composed of serum and locally generated materials such as tissue breakdown products, inflammatory mediators, and antibodies directed against dental plaque bacteria [44]. When compared to collection of saliva, collection of GCF is found to be more difficult.

The amount of caspase-3 was measured by the ELISA method. On comparing the three groups, the p value was found to be p=0.001 which was statistically significant. The significance was considered at the levels of p<0.05. The present study describes the association of caspase 3 level in periodontitis patients with and without diabetes mellitus and comparing the levels with periodontally healthy patients. The test done with ELISA showed that the caspase 3 level was increased in periodontitis patients who were also affected with diabetes mellitus. From this study, it was observed that there was a significant increase of caspase 3 levels in periodontitis patients with diabetes mellitus (86.29±24.25 pmol/L) when compared to periodontitis patients without diabetes (55.06±12.90 pmol/L). The results showed a positive correlation and high level of significance when compared between periodontally healthy patients and periodontitis patients along with diabetes mellitus (86.29±24.25 pmol/L). Also, relatively significant results were observed in comparison between periodontally healthy patients (43.37±15.35 pmol/L) and patients with periodontitis without diabetes mellitus (55.06±12.90 pmol/L). The results obtained were statistically significant with a p value of p<0.0001 (p<0.05 level of significance) (Figure 1 and Table 1).

Previous literature studies have emphasized the association of diabetes mellitus in relation to periodontitis. [45] Results obtained in this study are in concordance with previous data reported by Pradeep et al., who reported that GCF concentration of caspase-3 proportionally increases with the progression of periodontal disease. [46] A study by Malak et al, demonstrated the increase in caspase 3 level in GCF samples collected from periodontitis patients with diabetes mellitus. [47] Results from a

study by Liu et al, show that high glucose could induce human periodontal ligament fibroblasts apoptosis in a time dependent manner and caspase-3 apoptotic signaling pathway plays an important role in this process. [21]

This high level of caspase 3 in poorly controlled patient could be attributed to accumulation of advanced glycation end products (AGE) as shown by Takeda et al, who reported that increased AGE in the gingival crevicular fluid from diabetic patients compared with non-diabetic controls are significantly associated with deterioration of periodontitis.[48] Thus it can be assumed that worsening of glycemic control may lead to more accumulation of AGE and hyper responsive monocyte and this results in the increased release of cytokines, hence the increase in the level of caspase 3 concentration. This provides a plausible explanation for the increased incidence and severity of periodontal destruction in patients with diabetes mellitus. [49]

Hence, this study has ensured the strong association of caspase 3 in periodontitis patients and its impact with diabetes mellitus using salivary sample collection. Thus, caspase 3 activity can be used as a novel biomarker to predict periodontitis in diabetic patients, and can be used to diagnose and elicit a comprehensive periodontal therapy with reduction of blood glucose levels.

The present study also has certain limitations, as the study population was restricted within the geographical limit and can be established in large scale population. Due to the use of restrictive inclusion and exclusion criteria, in an attempt to minimize the occurrence of confounding factors, the small sample size is one limitation of this study. Further multicenter, longitudinal, prospective studies with larger sample sizes are required for the validation of the results of the present study.

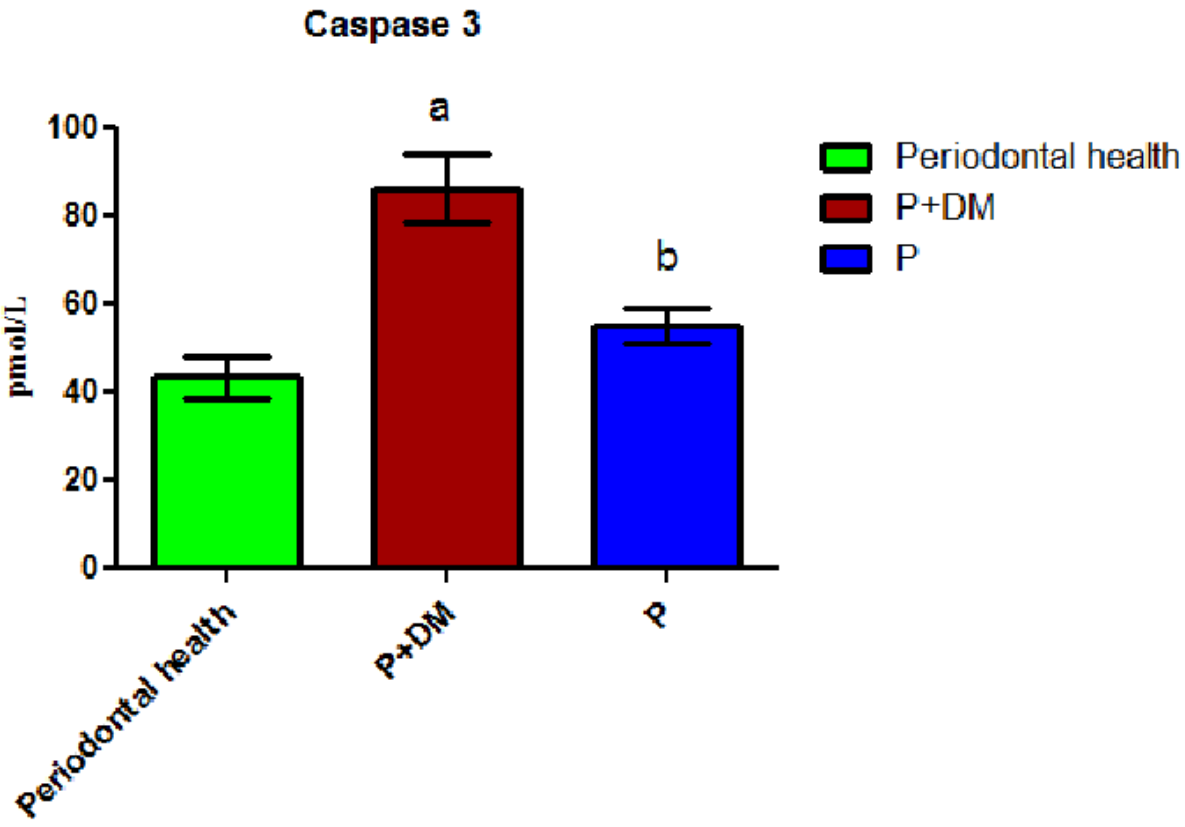

Fig. 1. Assessment of salivary caspase-3 concentration among periodontal health, periodontitis (P) and periodontitis with diabetes mellitus (P+DM). The levels of salivary caspase-3 were assessed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Significance at p <0.05, a- compared with the periodontal health group. b-compared with periodontitis with diabetes mellitus.

Table 1: Comparison of salivary caspase-3 levels among 3 groups, periodontal health, periodontitis (P) and periodontitis with diabetes mellitus (P+DM). The values are expressed in pmol/L. The levels of salivary caspase-3 were assessed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Significance at p <0.05.

Groups	Periodontal health	P+DM	P	P value
Caspase-3 (pmol/L)	43.37±15.35	86.29±24.25	55.06±12.90	P<0.0001

4. CONCLUSION

The present study showed that caspase-3 concentrations in saliva are higher in patients with periodontitis along with diabetes mellitus when compared with periodontal disease only. Thus diabetes mellitus have an impact causing increased periodontal destruction. Therefore, caspase-3 plays a role as a biomarker of periodontal disease in diabetes mellitus and its progression.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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