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ICTP ,TIMP-1 and MMP-8 levels as salivary biomarkers in obese patients with periodontitis or without diabetes

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ABSTRACT
INTRODUCTION
One of the risk factors for periodontal diseases is obesity. The oral microbiology of obese individuals has only been the subject of a few studies. Periodontal diseases typically range from the mild, reversible infection known as gingivitis to the more severe, irreversible infection known as periodontitis, which causes the alveolar bone that supports the tooth to be destroyed and ultimately results in tooth loss. The aim of this study is to assess the levels of ICTP, TIMP-1 and MMP-8 as salivary biomarkers in obese patients with periodontitis or without diabetes.
Materials and methods
Saliva samples were collected from 40 patients aged 30-60 years visiting the Department of Periodontics Saveetha dental college and hospitals, Chennai from December 2021 to march 2022 were categorized into 4 groups (10 patients in each group) Group a- Non obese and clinically healthy gingiva, Group b- Obese patients without periodontitis, Group c- Obese + periodontitis, Group d- Obese + Periodontitis + type 2 Diabetes mellitus. Salivary biomarkers ICTP, TIMP-1, MMP-8 levels were evaluated using human ELISA KIT and statistically analyzed using one way ANOVA Test.
Results

Obese patients with diabetes mellitus are more prone for periodontal tissue destruction and low levels of salivary biomarkers ICTP, TIMP-1, MMP-8 levels when compared to non obese patients. There was a significant difference in the salivary biomarkers ICTP, TIMP-1, MMP-8 levels among different groups (P value <0.05).
Conclusion
It can be concluded that salivary ICTP, TIMP-1, MMP-8 levels is an effective non-invasive biomarker to assess early diagnosis treatment of periodontitis among obese and diabetic patients.

Keywords : Periodontitis, obesity, diabetes, proinflammatory cytokines, ICTP, TIMP-1

1. Introduction

Periodontal disease is a chronic inflammatory condition which affects approximately 10-15% of the global population and is responsible for most loss of teeth because it damages essential tooth-supporting tissues such as periodontal ligament, root cementum, alveolar bone and gingiva. Periodontal disease clinical classification depends on indicators like inflammation, pocket depth, gingival attachment loss and bone resorption (1).

MMP family is composed of six clusters of proteases that include collagenase (MMP-1, MMP-8, MMP-13), gelatinase (MMP-2, MMP-9), stromelysin (MMP-3, MMP-10, MMP-11), matrilysin (MMP-7, MMP-26), member-type matrix metalloproteinases (MMP-14,MMP-15,MMP-16,MMP-17,MMP-12) and other unclassified MMPs each having different functions. Notably collagenases are involved in periodontal disease since they have the ability to degrade native collagens fibers type I, II and III. Among them interstitial collagenase form by MMP-8 plays a crucial role (2,3). The activities of these enzymes are regulated by endogenous inhibitors such as tissue inhibitors of metalloproteinases or TIMPs and α2-macroglobulin; an imbalance usually leads to non-reversible degradation of periodontal and peri implant tissues (3).

Periodontitis and peri-implantitis, prevalent infection-induced oral inflammations, manifest as active periodontal and peri-implant degradation (APD), encompassing soft and hard tissue destruction. MMP-8, or collagenase-2, chiefly instigates this degradation process, targeting type I collagen, the primary constituent of periodontal/peri-implant tissues(4–6). The elevation of MMP-8, particularly in its active form (aMMP-8) in oral fluids, correlates with periods of heightened periodontal and peri-implant inflammation. Contrary to bacterial enzymes, MMP-8, released from neutrophils upon stimulation by periodontopathogenic bacteria and proinflammatory mediators, drives APD. Additionally, gingival fibroblasts, under pro inflammatory stimuli, contribute to MMP-8 production. Levels of active MMP-8, indicative of disease progression, show associations with clinical parameters like probing pocket depth , bleeding on probing, and clinical attachment loss (7). Following successful periodontal and peri-implant treatments, a decline in aMMP-8 levels is observed in oral fluids, indicating therapeutic efficacy (8).

Consuming foods with a high glycemic index has been linked to diabetes. On the other hand, foods rich in fiber are considered beneficial for health, but it's important to chew them thoroughly. These foods can promote oral and gum health while also aiding in the prevention of diabetes and obesity (9)

Obesity is a complex condition influenced by various factors such as genetics, biology, social environment, and behaviors, all contributing to a chronic imbalance between energy intake and expenditure(10). This imbalance may result in the accumulation of excessive fat, which poses risks to health (14). Globally, obesity has reached epidemic proportions, largely due to sedentary lifestyles and the consumption of high-calorie diets. By 2015, it is estimated that approximately 2.3 billion adults will be overweight and over 700 million will be obese, with developed nations being particularly affected (4,11–13). Health Canada guidelines classify a body mass index (BMI, kg/m2) of 25–30 as overweight and over 30 as obese. Obesity is further categorized into class I (BMI 30–34.9), class II (BMI 35–39.9), and class III (BMI 40 or higher) based on associated health risks (14,15).

Diabetes and obesity are interconnected conditions often triggered by unhealthy diets high in fat. High blood sugar, inflammation, and insulin resistance exacerbate inflammation and periodontitis. The link between obesity and periodontitis was first discovered by Perlstein and Bissada in animals in 1977 and later confirmed in humans by Saito et al. in 1998. Subsequent epidemiological studies have supported the notion that obesity serves as a risk factor for periodontitis(16,17) (20–23).

MATERIALS AND METHOD :

A total of 40 individuals with the age group of 30 to 60 years visiting the department of periodontics, saveetha dental college and hospitals, Chennai from December 2021 to march 2022 were categorized into 4 groups (10 patients in each group)

Group a - Non obese and clinically healthy gingiva,
Group b - Obese patients without periodontitis,
Group c - Obese + periodontitis,
Group d - Obese + Periodontitis + type 2 Diabetes mellitus.

Salivary Antioxidant levels were evaluated using human ELISA KIT and statistically analyzed using a one way ANOVA test.

Saliva collection

Saliva samples were collected randomly from each group of individuals . Whole unstimulated saliva was collected from all patients and the collected samples were immediately transported to the laboratory, where it was centrifuged at 5,000 rpm for 10 minutes and the clear supernatants were stored in aliquots at-70°C. The assay was performed within 3 months of collection. Salivary biomarkers ICTP, TIMP-1, MMP-8 levels were analyzed by using a commercially available ELISA kit.

Figure 1, saliva sample collection with ELIZA kit.

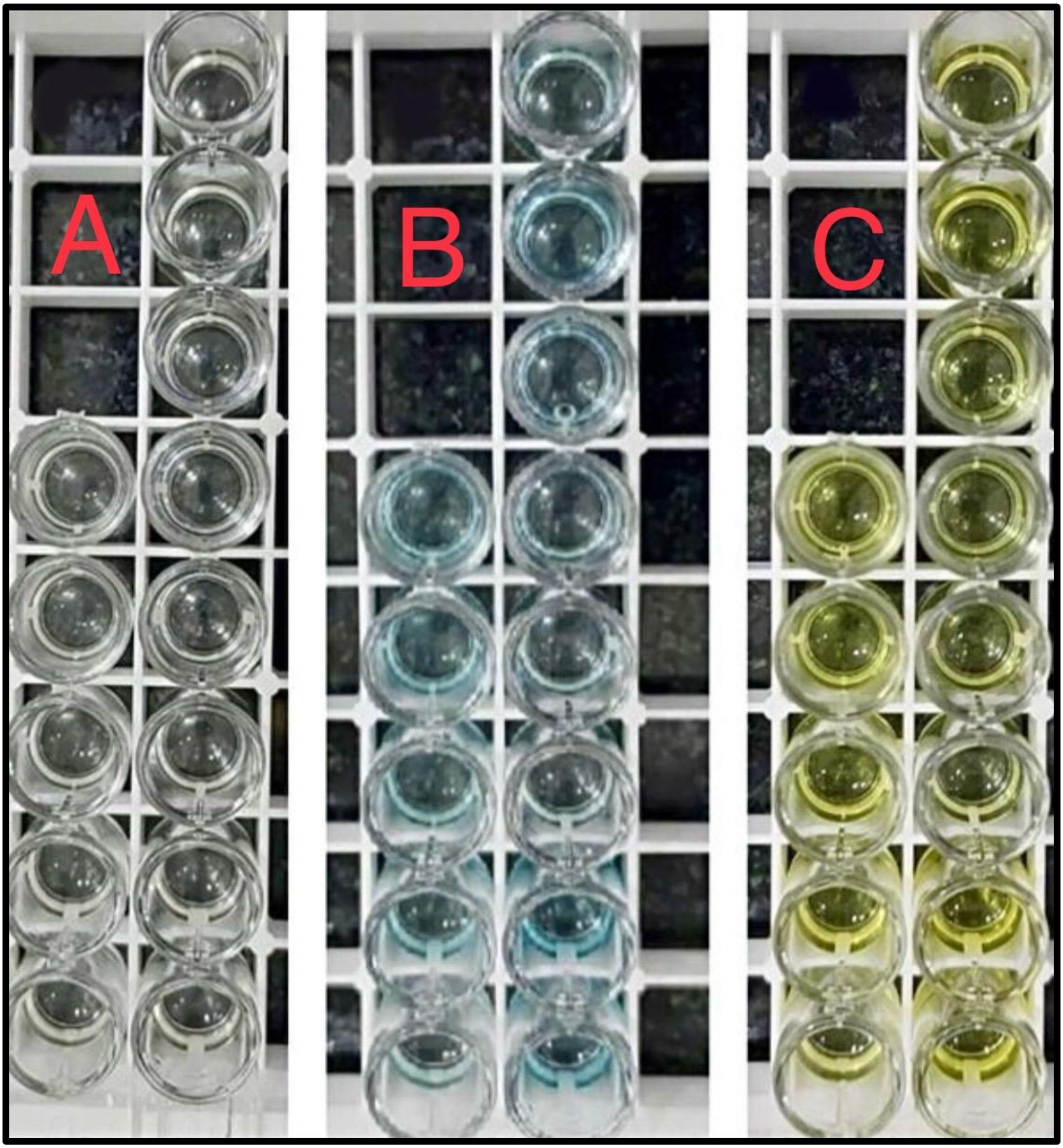

Figure 2,
A) Testing sample wells with 10 microliter of saliva sample of each group.

B)Testing sample wells with HRP - conjugated detection antibody 50 microliter to each well, covered with plate cover Incubated for 30 minutes at 37 degree Celsius. Add chromogen solution A 50 microliter to each well and incubate for 15 minutes at 37 degree Celsius.

C)Testing sample wells with 50 microliter of stop solution to each well.where the color of Well changes from blue to yellow.

Statistical analysis :

The triplicate analysis results of the experiments were expressed as mean ± standard deviation and the data were analyzed statistically by one-way analysis of variance (ANOVA). The results with the $p < 0.05$ level were considered to be statistically significant.

RESULTS AND DISCUSSION :

Obese patients with diabetes mellitus are more prone for periodontal tissue destruction and low levels of salivary biomarkers ICTP, TIMP-1, MMP-8 levels when compared to non obese patients. There was a significant difference in the salivary biomarkers level among different groups (P value <0.05)

MEAN :

	Group (A) (pg/ml)	**Group (B) (pg/ml)**	**Group (C) (pg/ml)**	**Group (D) (pg/ml)**
ICTP	0.2225	0.3485	0.3915	0.497
TIMP-1	0.333	0.511	0.5935	0.673
MMP-8	0.247	0.3485	0.406	0.4905

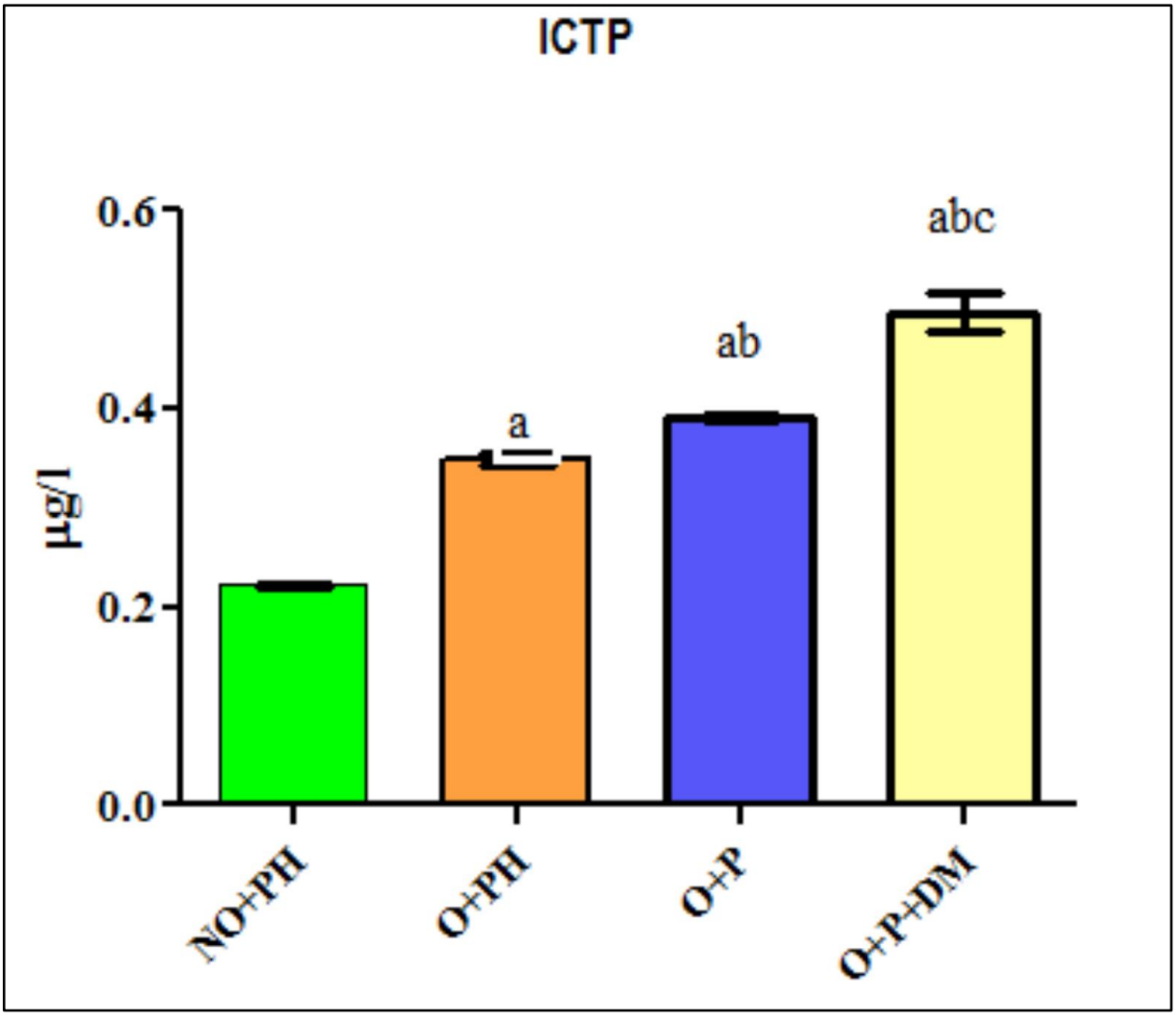

Figure 3, The above graph shows that, there is significant difference in the level of salivary biomarker ICTP among different groups. (P value <0.05).
Group a - Non obese and clinically healthy gingiva with mean value of 0.2225,
Group b - Obese patients without periodontitis with mean value of 0.3485,
Group c - Obese + periodontitis with mean value of 0.3915,
Group d - Obese + Periodontitis + type 2 Diabetes mellitus with mean value of 0.497.

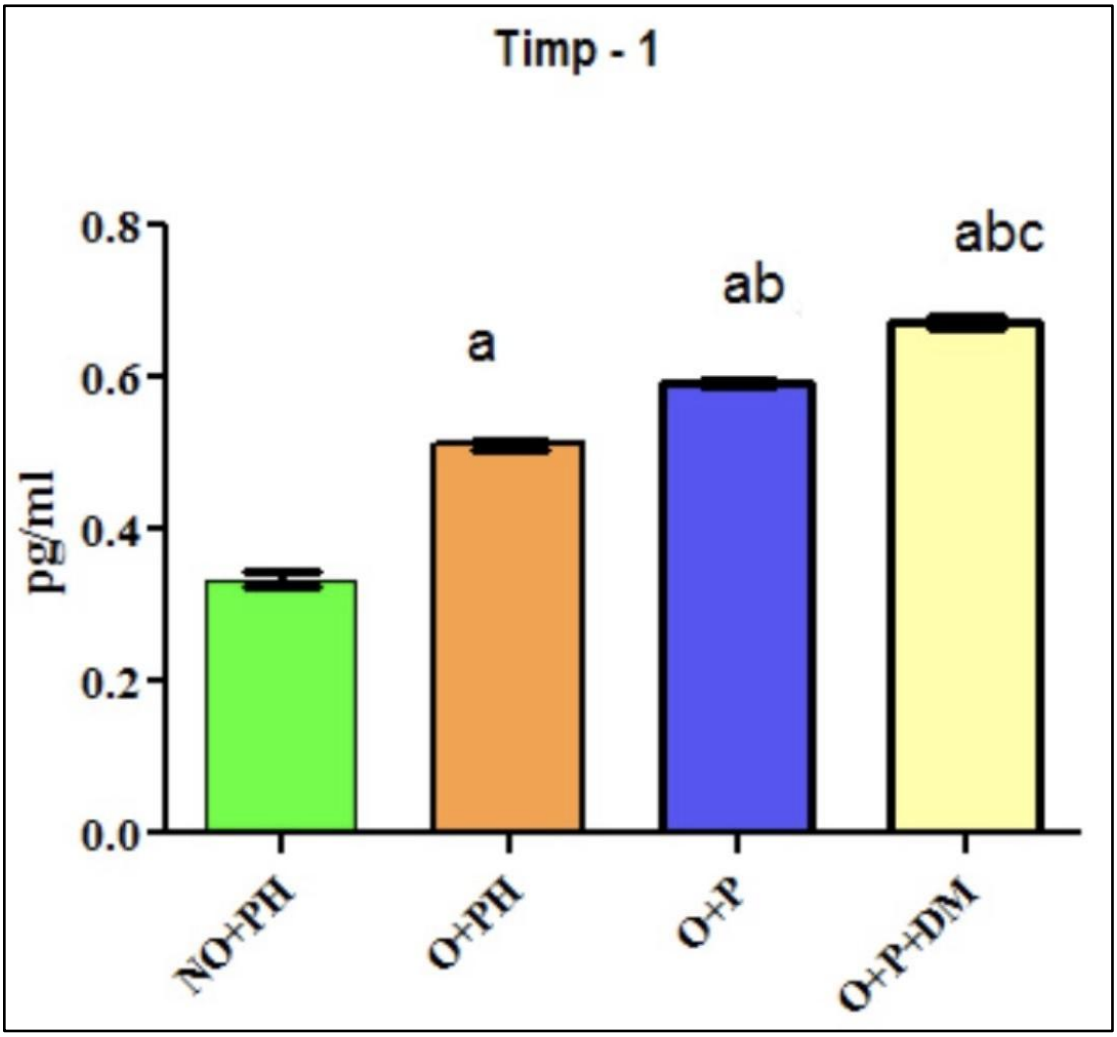

Figure 3, The above graph shows that, there is significant difference in the level of salivary biomarker TIMP-1 among different groups. (P value <0.05).
Group a - Non obese and clinically healthy gingiva with mean value of 0.333,
Group b - Obese patients without periodontitis with mean value of 0.511,
Group c - Obese + periodontitis with mean value of 0.5935,
Group d - Obese + Periodontitis + type 2 Diabetes mellitus with mean value of 0.673.

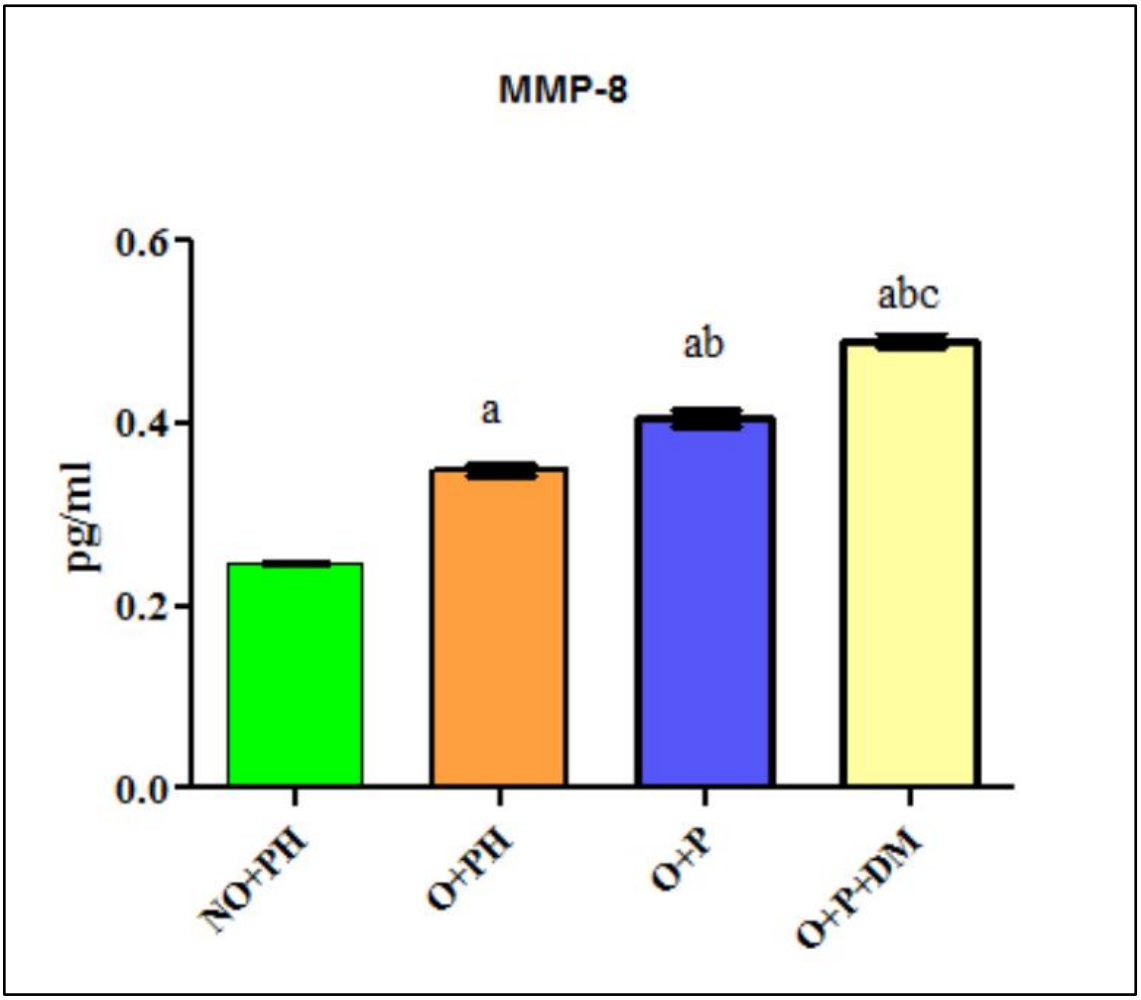

Figure 3, The above graph shows that, there is significant difference in the level of salivary biomarker MMP-8 among different groups. (P value <0.05).
Group a - Non obese and clinically healthy gingiva with mean value of 0.247,
Group b - Obese patients without periodontitis with mean value of 0.3485,
Group c - Obese + periodontitis with mean value of 0.406,
Group d - Obese + Periodontitis + type 2 Diabetes mellitus with mean value of 0.4905.

Gingivitis and periodontitis are the two main illnesses that make up periodontal disease, which is one of the most common diseases in the world (7,18). Inflammation of the gingival tissue is a feature of the disease's milder, treatable form, gingivitis. Gingivitis can develop into periodontitis, a chronic infectious illness of the tissues supporting the teeth, in people who are vulnerable to the condition. Although bacteria are the cause of periodontal illnesses, it is thought that the host's response is crucial in the degeneration of bone and connective tissue (19). Innate and adaptive immune responses are involved in the inflammatory and immune response induced by microbial antigens and virulence factors. According to potential variations in cytokine and other antimicrobial responses, environmental factors, and the subject's genetics, the response varies between individuals (20–22).

Saliva contains antioxidant enzymes such as salivary peroxidase, catalase, peroxidase, and glutathione reductase, in addition to nonenzymatic antioxidants such as uric acid, reduced glutathione, albumin, and lactoferrin, as well as polyphenols (23). The youngest subjects (ages 2-14) had considerably higher salivary biomarkers ICTP, TIMP-1 activity in NWS than the other groups (24,25). Additionally, it was

noticed that higher salivary biomarkers MMP-8 activity in elderly people's NWS compared to middle-aged persons, kids, and teenagers (26)(27).

CONCLUSION :

Within the limitations of the study it was concluded that salivary biomarkers ICTP, TIMP-1, MMP-8 is an effective non-invasive biomarker to assess early diagnosis and treatment of periodontitis among obese and diabetic patients.

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