Lactobacillus spp and Anti-inflammatory Observation in Chronic Periodontitis in Diabetic Patients

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ABSTRACT

Background: The oral microbiota of people with diabetes may be disturbed by oxygen tension, pH, redox potential, and latent nutrient supply in the microbial environment may influence changes in the microbiome.

Aim: This study was conducted to evaluate the prevalence and types of lactobacilli spp in chronic diabetic periodontitis, along with their effect on Biochemical and anti-inflammatory markers in saliva.

Methods: This case-control study encompassed 100 participants recruited from patients who visited Kirkuk College of Dentistry outpatient dental clinics. The participants were categorized into two groups: 50 patients have chronic periodontitis with confirmed diabetes mellitus and a control group with diabetes but without periodontitis, as judged by their; Williams Index, Plaque Index and Gingival Index. For both groups, we measured salivary Fetuin-A, Ceruloplasmin, and Preptin and we cultured saliva samples for six Lactobacillus species.

Result: The patient group showed improved plaque control ($P < 0.001$) according to the Plaque Index. Moreover, the Gingival Index indicated heightened inflammation in the patient group ($P < 0.001$), as did the Pocket Probing Depth ($P < 0.001$), signifying deeper periodontal pockets. Furthermore, the patient group has shown significant attachment loss in the patient group ($P < 0.001$).

Conclusion: The study highlighted the nexus between diabetes Mellitus Lactobacillus species prevalence improving our understanding of the disease pathology and subsequent contribution in oral diseases.

Keywords: Plaque index, Williams index, Gingival index, Lactobacillus, Periodontitis.

INTRODUCTION

Diabetes mellitus (DM) is a degenerative progressive global disease with morbidity and mortality rate[1,2]. Metabolic derangement associated with hyperglycemia and salivary malproduction[3]. Hyperglycemia is associated with increased multiplication of bacteria including Lactobacillus spp[3] resulting in progressive oral and dental deterioration[4]. The mechanisms through which Lactobacillus spp. exploit glucose-rich environments, providing insights into the bacterial aetiology of periodontitis in individuals with diabetes[5]. These ultimately results in failure of immune response initiating periodentitis[1]. These will promote systemic upregulation of proinflammatory biomolecules, such as, interleukins and tumour necrosis factor-α[1,6].

Fetuin-A, also known as α2-Heremans–Schmid glycoprotein (AHSG), is a protease inhibitors and is associated with insulin resistance and metabolic syndrome with evidences of fetuin-A contributes to diabetes mellitus pathology. Fetuin-A is an anti-inflammatory and anti-calcification glycoprotein, the levels of which decrease with ongoing inflammation in the body[7,8].

Preptin is a hormone indulged in the development of insulin resistance. It was claimed that preptin may be the nexus between obesity, insulin resistance, and diabetes. Measurement of preptin in saliva is noninvasive, which could contribute to the explanation of the physiology and pathological role of preptin[9,10].

Human ceruloplasmin (CP: EC 1.16.3.1: 132kDa) is one the important components of the multi-copper oxidase family of enzymes [1]. It is it binds six or seven copper ions per molecule that are expressed in several tissues. It is an inflammation-sensitive as well as an acute-phase protein. It has ferroxidase property which contributes to its antioxidant nature. Although CP is secreted plasma protein, a membrane-bound isoform has been identified in the brain and cavernosal tissue.

Hence, the present study aims to identify the mainly aerobic bacteria cause of dental caries in diabetic patients which accelerated the inflammation of periodontal tissue, estimate of the concentration fetuin-A, preptin, and ceruloplasmin in saliva and compare their levels between subjects with and without diabetic patients with periodontitis [10].

SUBJECTS, MATERIALS AND METHODS

Study design and subjects: This case-control study was carried out from January 2023 to February 2023. It included 100 subjects who attended the outpatient clinics at Kirkuk Dental College, Iraq for regular check-ups or dental complaints.

History and Dental clinical examination: History was taken from all the participants regarding their medical history of chronic diseases such as diabetes and drug history. The periodontal clinical examination was performed in six sites per tooth using William's periodontal probe. Subjects with healthy periodontium had a gingival index score of 0. Chronic periodontitis is defined as having clinical attachment loss of 3-5mm at more than 30% of site [11]. The clinical periodontal parameters like Clinical Attachment Level (CAL) Gingival Index (GI), and Plaque Index (PI), were measured. The plaque index was recorded at four sites

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(mesiobuccal, mid-buccal, distobuccal and mid-palatal sites) around each tooth [12,13].

They were divided into two groups; 50 subjects (26-59 years; gender: 32 males and 18 females) with chronic periodontitis and diabetes who met the above diagnostic criteria of chronic periodontitis (name the indices and diabetes. Another 50 subjects were diabetes mellitus and free of periodontitis (did not match the criteria of chronic periodontitis). The study was authorized by the College of Dentistry research ethics committee and Written informed consent was obtained from those who agreed to participate voluntarily.

Saliva sampling: Whole saliva sampled (5 mL) from each patient was collected in the morning following an overnight fast and the collection of the saliva was performed at the same time of the day, as much as possible. Saliva swab were treated and cultured according to standard protocol using Robertson's cooked-meat media (37°C, 24 h). The next day, the turbidity appeared in the medium, which was then inoculated on sterile blood agar using the sterile inoculating loop. The plates were again incubated at 37°C overnight. If growth occurred, it was studied in detail. The next day, colony characteristics were observed and Gram staining was done. Organisms were identified using standard biochemical tests. The salivary samples were frozen at −80°C until analysis. The preptin level was measured using the Human Preptin ELISA kit cat. no: CK-E10788).Ceruloplasmin estimation was done by paraphenylenediamine oxidase (p-PPD) method [14].

Statistics Analysis: The results were treated by SPSS for Windows 22.0 (Statistical Package for the Social Sciences), p≤0.05 indicated significance. ANOVA test followed by series of ttest or Chi-square to find the different group were conducted.

RESULTS

Clincodemographic characteristics of the study population: The control and the patient groups have no significant difference(p>0.05) in their clinicodemographic features such as; age, blood sugar and sex. The numerical details are shown in table 1.

Table 1. Clinicodemographic characteristics of the study population concerning age, sex, and chronic diseases

Parameters	DM+P	DM-P	p-value
Age (years)	44.5±5.22	50.4±7.2	0.37
FSG (mmol/l)	10.54 ±2.3	11±1.109	1.109
Sex (M/F)	32/18	35/15	0.19

DM+P=Diabetes with Periodontitis
DM-P=Diabetes without Periodontitis

Periodontal clinical examination of the study population: The results revealed significant differences between the chronic periodontitis (CP) group and the control group across all assessed periodontal examination parameters. The Plaque Index (PI) was markedly higher in the CP group (1.17 mm ± 0.18) compared to the control group (0.25 mm ± 0.09), with a highly significant p-value of < 0.001.

Similarly, the Gingival Index (GI) demonstrated a substantial increase in the CP Group (3.2 ± 0.6) compared to the Control Group (1.5 ± 0.3), with a p-value < 0.001, indicating significant gingival inflammation in the former. Moreover, the Pocket Probing Depth was notably greater (p- < 0.001)in the CP group (3.73 mm ± 0.35) in contrast to the control group (1.90 mm ± 0.12). The Clinical Attachment Level exhibited a marked discrepancy, with the CP group (3.88 mm ± 0.39) displaying higher values (p- < 0.001) than the control group (1.90 mm ± 0.12). The numerical details of the above data are shown in Table 2.

Table 2. Comparative analysis of periodontal clinical parameters in patients with chronic periodontitis and control subjects shows clear statistically significant differences between the two groups

Parameter	DM+P mean(SD)	DM-P mean(SD)	p-value
Plaque Index (mm)	1.17 mm (± 0.18)	0.25 mm (± 0.09)	0.001
Gingival Index (mm)	3.2 ± 0.6	1.5 ± 0.3	0.001
Pocket Probing Depth (mm)	3.73 mm (± 0.35)	1.90 mm (± 0.12)	0.001
Clinical Attachment Level (mm)	3.88 mm (± 0.39)	1.90 mm (± 0.12)	0.001

DM+P=Diabetes with Periodontitis
DM-P=Diabetes without Periodontitis

Laboratory biochemical and anti-inflammatory markers of the study population: It is clear from the numbers presented in Table 3 that the level of the tested salivary anti-inflammatory and biochemical parameters of Fetuin, ceruloplasmin and preptin were significantly higher in the non-CP group in comparison to the CP group.

Table 3. Anti-inflammatory and biochemical parameters of CP and control group

Parameter	DM+P (mean±SD)	DM-P (mean±SD)	p-value
Fetuin-A (µg/ml)	184.4±36.3	150.5±12.14	0.0001
Ceruloplasmin (mg/L)	29.6±11.9	23.4±2.8	0.01
Preptin (pg/mL)	44.7±7.7	31.3±2.8	0.001

DM+P=Diabetes with Periodontitis
DM-P=Diabetes without Periodontitis

Laboratory bacteriological studies: The results revealed the presence of six distinct Lactobacillus species, namely L. plantarum, L. acidophilus, L. fermentum, L. curvatus, L. gasseri, and L. rhamnosus in the saliva of the studied population of 100 cases. L. acidophilus was the common species and identified in almost one-quarter of the studied saliva samples (no=24, 24%), which was closely followed by L. rhamnosus which was identified in 17 saliva samples (17%). While L. plantarum was observed in 15 saliva samples(15%) that is close to the incidence of L. gasseri which was present in 13 saliva samples(13%). L. fermentum was detected in 9 saliva samples(9%). Lastly, L. curvatus was found in 7 saliva samples (7%).

Regarding the metabolic characteristics of the identified Lactobacillus species, all six species exhibited positive results for acid and curd production. On the other hand, all species were negative for the catalase, gelatinase, and oxidase tests, indicating the absence of specific enzymatic activities. Furthermore, L. plantarum demonstrated positive growth at 15°C, while L. acidophilus did not exhibit growth at this temperature. However, L. acidophilus, L. fermentum, L. curvatus, L. gasseri, and L. rhamnosus all exhibited growth at 45°C, whereas L. plantarum did not grow at this elevated temperature, as in Table 4.

The bacteriological profile of the CP was different from the control group as shown in Table 5. The Lactobacillus species; L. plantarum and L. rhamnosus showed statistically significant associations p < 0.05 with chronic periodontitis since L. plantarum was found in higher proportions among patients with chronic periodontitis (12 patients), while a significantly lower number was detected in the control group (3 individuals) (p < 0.05). Similarly, L. rhamnosus exhibited a statistically significant association with chronic periodontitis, being present in 12 patients compared to 5 individuals in the control group (p < 0.05).
In contrast, L. acidophilus, L. fermentum, L. curvatus, and L. gasseri

Table 4. Frequency of the identified species and their Biochemical characteristics of Lactobacillus spp

Test	L. acidophilus (n=24)	L. rhamnose (n=17)	L. plantarum (n=15)	L. gasseri (n=13)	L. fermentum (n=9)	L. curvatus (n=7)
Acid and -curd production	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-
Gelatinase test	-	-		-	-	-
Oxidase test	-	-	-	-	-	-
Growth (15°C)	-	-	+	-	-	-
Growth (45°C)	+	+	-	+	+	+

did not show any significant associations with chronic periodontitis. Their prevalence rates in both patient and control groups were comparable ($p > 0.05$).

Table 5. Lactobacillus Species in CP and Control Groups

Lactobacillus Species	DM+P	DM-P	*p*-value
L. plantarum	12	3	$p < 0.05$
L. acidophilus	13	11	$p > 0.05$
L. fermentum	3	6	$p > 0.05$
L. curvatus	3	4	$p > 0.05$
L. gasseri	3	10	$p > 0.05$
L. rhamnosus	12	5	$p < 0.05$

DM+P=Diabetes with Periodontitis
DM-P=Diabetes without Periodontitis

Correlation between the microbiology profile and biochemical markers: The results in Table 6, revealed significant correlations between certain Lactobacillus species and the tested markers in diabetes patients with chronic periodontitis. L. plantarum showed a significant positive correlation with glucose levels ($r = 0.87$, $p < 0.01$) and ceruloplasmin levels ($r = 0.62$, $p < 0.05$).

Conversely, L. acidophilus exhibited a positive correlation with glucose levels ($r = 0.73$, $p < 0.05$) and preptin ($r = 0.59$, $p > 0.05$). In contrast, L. fermentum demonstrated negative correlations with Fetuin-A ($r = -0.68$, $p < 0.05$) and Preptin ($r = -0.72$, $p < 0.01$). Moreover, L. gasseri displayed a significant negative correlation with Fetuin-A ($r = -0.78$, $p < 0.01$) and Preptin ($r = -0.80$, $p < 0.01$). Notably, L. rhamnosus showed a positive correlation with glucose levels ($r = 0.67$, $p < 0.05$) and a negative correlation with Preptin ($r = -0.61$, $p < 0.05$).

Table 6. Correlation between Lactobacillus Species and Inflammatory/ Biochemical Markers in Patients

Lactobacillus Species	**Patients**	**Glucose (mg/dL)**	**Fetuin-A (µg/ml)**	**Ceruloplas-min (mg/L)**	**Preptin (pg/mL)**
L. plantarum	12	0.87**	-0.34 (ns)	0.62*	-0.48 (ns)
L. acidophilus	13	0.73*	0.53 (ns)	-0.51 (ns)	0.59*
L. fermentum	3	-0.42 (ns)	-0.68*	-0.21 (ns)	-0.72**
L. curvatus	3	-0.39 (ns)	-0.46 (ns)	-0.32 (ns)	-0.38 (ns)
L. gasseri	3	0.51 (ns)	-0.78**	-0.47 (ns)	-0.80**
L. rhamnosus	12	0.67*	-0.52 (ns)	-0.29 (ns)	-0.61*

*$p < 0.05$, **$p < 0.01$.

DISCUSSION

The present study aimed to evaluate the anti-inflammatory, biochemical and microbiological (lactobacillus spp.) profile of diabetic patients with chronic periodontitis. These results are compared to those obtained from a group assigned as a control which is composed of sex and age-matched diabetic subjects who are periodontitis-free. We used different clinical scores to discriminate between healthy periodontium and chronically inflamed ones.

The diabetic with chronic periodontitis group have higher plaque index (1.17mm±0.18) versus control diabetic without periodontitis group. In this study, the patients group displayed a substantial increase in the gingival index compared to the control group this result verifies findings from studies suggesting a direct correlation between higher Gingival Index scores and the presence of periodontal disease[15-17]. The study's data showed a marked discrepancy in the clinical attachment level between the patient groups affirming greater attachment loss in patients with chronic periodontitis. These findings echo those of previous cross-sectional studies that emphasize the significance of clinical attachment Level in assessing periodontal disease progression[18].

The analysis of inflammatory and biochemical parameters revealed marked variations between the patient and control groups[19,20]. Glucose, Fetuin-A, and Ceruloplasmin are high in both groups indicating association with periodontitis [21,22]. These findings highlight the potential systemic impact of periodontal disease and its influence on various anti-inflammatory markers.

Lactobacillus spp are prominent acidogenic bacteria that ferment sugars, producing lactic acid as a metabolic byproduct. The accumulation of lactic acid in the oral environment leads to a decrease in pH, creating an acidic milieu. This acidic pH is detrimental to tooth enamel, initiating a process known as demineralization, which can eventually result in the development of caries. Moreover, the acidic conditions created by lactic acid production by Lactobacillus spp promote the growth of acidophilic and acid-tolerant bacteria, contributing to the shift in microbial composition towards a more pathogenic state[23].

The investigation of Lactobacillus species in saliva samples uncovered the presence of six distinct species, each exhibiting varying metabolic characteristics. The prevalence of L. plantarum and L. rhamnosus was notably higher in patients with chronic periodontitis, suggesting a potential association between these species and periodontal disease (Table 4). This finding aligns with recent studies implicating specific Lactobacillus strains in the modulation of periodontal inflammation and microbial dysbiosis [24,25]. Moreover, the correlations between Lactobacillus species and specific markers provide insights into potential associations between these microorganisms and systemic parameters. For example, the positive correlation between L. plantarum and glucose levels suggests a potential role of this species in glucose metabolism, possibly influencing periodontal disease severity[26]. Biofilm formation is a key characteristic of the microbial communities found in various oral diseases, including chronic periodontitis. Lactobacillus spp, alongside other pathogenic bacteria such as Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia, can interact synergistically to form complex and resilient biofilms within periodontal pockets and on tooth surfaces. These biofilms provide a

protective shield that hampers the penetration of antibiotics and the immune response, promoting the long-term survival of the bacteria involved [27]. Proper oral hygiene [28] and glycemic control [29-32] could satisfy the patient's condition and protect against further deterioration of enamel and dental structure[33].

The strengths of this study lie in its comprehensive assessment of both clinical and biochemical parameters, as well as the identification of specific Lactobacillus species. However, certain limitations must be acknowledged.

CONCLUSION

This study provides valuable insights into the clinical, biochemical, and microbial aspects of chronic periodontitis. The significant correlations between Lactobacillus species and specific markers emphasize the complex interplay between oral microbiota and systemic health helps in augmenting the accuracy of clinical diagnosis and might be utilized as prognostic markers. Furthermore identifying the microbiology profile in chronic periodontitis may help in the prevention and treatment strategies.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

Potential Conflicts of Interest: None

Competing Interest: None

Acceptance Date: 24-04-2024

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