ORIGINAL RESEARCH

Messenger RNA Expression and Quantification of L-plastin in Subgingival Tissue Samples of Generalized Severe Chronic Periodontitis Patients with and without Type 2 Diabetes Mellitus

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Abstract

Aim: The aim of this study was to assess the demographic variables and clinical parameters and to quantify the levels of L-plastin in subgingival tissue samples of generalized severe chronic periodontitis subjects with and without type 2 diabetes mellitus.

Materials and methods: The participants in this study were chosen from the Outpatient Department of Periodontology, Meenakshi Ammal Dental College (MADC), Meenakshi Academy of Higher Education and Research (MAHER), Chennai, Tamil Nadu, India. Two groups of 70 participants, each with generalized severe chronic periodontitis, were chosen. A total of 35 people in the group I had generalized, severe chronic periodontitis without any other systemic causes. Group II included people with type 2 diabetes who had generalized severe chronic periodontitis. Age, height, weight, body mass index (BMI), and income were all recorded, as well as periodontal metrics like plaque index, bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL). Both groups had subgingival tissue samples taken at the site of the periodontal surgery. The obtained samples were then subjected to molecular analysis, including real-time polymerase chain reaction (RT-PCR) for measuring L-plastin messenger RNA (mRNA) expression and enzyme-linked immunosorbent assay (ELISA) for measuring protein concentration.

Results: The mean income, plaque index, CAL, and the mRNA expression and protein quantification of L-plastin were found to be statistically significant between the groups. On correlating the periodontal parameters with the mRNA expression and protein quantification, plaque index and CAL had a significant correlation with L-plastin in both groups. However, group II showed higher periodontal destruction with the presence of this protein.

Conclusion: The subgingival tissue samples of subjects with generalized severe chronic periodontitis and type 2 diabetes mellitus showed elevated levels of mRNA expression and L-plastin protein quantification, indicating that these molecules were locally involved in the pathogenesis of both periodontitis and type 2 diabetes mellitus. It can be utilized as a biomarker for the early detection of individuals at risk for periodontitis and diabetes mellitus.

Keywords: Inflammation, L-plastin, Periodontitis, Proteomes, Type 2 diabetes mellitus.

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Introduction

The oral cavity is a perpetual source of infectious agents, and its condition often reflects the evolvement of systemic pathologies. Microorganisms have grown in importance as a major contributing component to the pathogenesis of periodontal disease over time.[1] The host's response to bacterial products, such as lipopolysaccharides, enzymes, and toxins, is usually inflammatory in nature.[2] Various systemic disorders can be at risk due to excessive inflammatory response seen in chronic periodontitis with an increase in oxidative stress.[3]

Gram-negative bacteria found in subgingival biofilms are the main etiological factor for periodontitis leading to a persistent immunoinflammatory bacterial infection of the tooth's supporting tissues.[4] Another chronic condition linked to abnormally high glucose levels is diabetes mellitus, which may result from insufficient pancreatic insulin synthesis or insufficient cell sensitivity to insulin action. This is due to the intricate interplay of genetic, environmental, and lifestyle-related factors. Diabetes patients are three times more likely to develop periodontitis. Advanced glycation end product, which is essentially nonenzymatic in nature, is another contributing factor.[5]

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One of the great challenges in periodontology is an assessment of the biomarkers for diagnostic and prognostic evaluation of diseases. Research work in the field of proteomics has promoted proteins as a pertinent biomarker for screening and predicting the onset of disease and disease activity and has paved the way for the effective diagnosis and treatment of periodontal disease. Proteins have been most commonly used as a diagnostic tool and have shown to play a pivotal role as an effective biomarker in the early detection, diagnosis, and management of any systemic diseases.[6] L-plastin is an actin-binding protein produced by leukocytes which have a crucial role in immune-mediated events. Recent studies using proteomic analyzes have shown that L-plastin is one of the few molecules consistently present in the gingival inflammatory exudate (gingival crevicular fluid) in periodontal disease.[7] Since L-plastin is proinflammatory in nature, its activation mediates leukocyte adhesion, migration and osteoclast adhesion, and bone resorption.[8]

Limited literature exists that states the determination and the quantification of proteomes in subgingival tissue samples of patients with generalized severe chronic periodontitis and type 2 diabetes mellitus and comparing it with systemically healthy generalized severe chronic periodontitis. It is suggested that protomes are seen to be elevated in the immunoinflammatory response in periodontitis as well as type 2 diabetes mellitus and hence can be used as a significant marker for the detection and prognosis of these inflammatory diseases.[9] We aimed to identify the mRNA expression and quantification of L-plastin in subgingival tissue samples of generalized severe chronic periodontitis patients with type 2 diabetes mellitus and compare them with systemically healthy generalized severe chronic periodontitis patients. We also correlated L-plastin with the demographic and periodontal variables in order to know the influence of this protein on the amount of periodontal destruction.

Materials and Methods

Study Design

The study was conducted from January 2022 to December 2022 in Chennai, Tamil Nadu, India. The present study was a descriptive case-control study with the control group having a total of 90 patients within the age group of 35–60 years, diagnosed with generalized chronic periodontitis, recruited from the Outpatient Department of Periodontology, Meenakshi Ammal Dental College and Hospital, Chennai out of which 10 patients refused to participate and 10 were excluded due to other systemic complications. Finally, 70 patients were selected and were divided into two groups (group I—control and group II—test) based on the inclusion and exclusion criteria. The study was approved by "Institutional Review Board" (MADC/IRB-IX/2021/151/Sec A) at Meenakshi Ammal Dental College (MADC), Meenakshi Academy of Higher Education & Research (MAHER), Chennai, Tamil Nadu, India. The study was carried out in conformity with the 2013 revision of the 1975 Helsinki Declaration. After informing the patients in writing about the trial, they gave their signed consent.

Male patients between the ages of 35 and 60 years, with >10 natural teeth, no recent long-term antibiotic usage, no history of diabetes mellitus and willing to participate in the study were included. Patients with generalized severe chronic periodontitis subjects [30% or more sites with clinical attachment loss (CAL) >5 mm] without type 2 diabetes mellitus met the inclusion criteria for group I. Group II consisted of generalized severe chronic periodontitis patients (30% or more sites with CAL >5 mm) with known type 2 diabetes mellitus (HbA1C levels 7%) for >5 years and was under oral medication. Smokers and other behavioral factors, which may influence the study, patients who had undergone periodontal therapy within the previous 6 months, female patients (due to hormonal changes and pregnancy, which may alter the oral flora), individuals with systemic illnesses, such as those suffering from cancer, cardiovascular and respiratory disorders, human immunodeficiency virus infection, and those who were immunosuppressed were excluded from the investigation. With a sample size of 35 subjects in each group, the power was estimated to be greater than 95%.

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Demographic Data and Periodontal Evaluation

The demographic characteristics such as age, initial height, weight, BMI, occupation, and income of the selected subjects were recorded at the initial visit. Periodontal parameters such as plaque index, BOP, PPD, and CAL were also recorded at baseline. Williams periodontal probe was used for the examination and the readings were recorded to the nearest millimeter.

Subgingival Tissue Sample Collection

In both groups, subgingival tissue samples were taken during periodontal surgery from the diseased areas of the deepest periodontal pockets. For further examination, the test samples were added to the transporting medium and kept at −80°C. The acquired tissue samples underwent molecular analysis for the detection of mRNA expression of L-plastin using RT-PCR, and its protein levels were measured using ELISA, an enzyme-linked immunosorbent test.

Estimation of mRNA Expression of L-plastin Using RT-PCR

The gene expression of L-plastin was quantified by RT-PCR. This included the synthesis of complementary DNA (cDNA) from the RNA isolates by reverse transcription. The primers for L-plastin were procured from Synergy Scientific Services Private Ltd Chennai, India, and were used for the gene amplification and quantification—forward primer—5′-TGAAAGAACAATCAACAAA-3′ and reverse primer—5′-TTAATGGAACCTGGTTGG-3′. The reaction mix (20 µL) was prepared by adding 10 µL of 2 × reaction buffer, 1 µL of sense and anti-sense primer, 2 µL of cDNA, and 6 µL of sterile water. The thermal cycler protocol was as follows—initial denaturation at 95°C for 30 seconds, followed by 40 cycles of PCR, denaturation at 95°C for 5 seconds, annealing at 55–61°C for 30 seconds, and extension at 72°C for 30 seconds. All reactions were performed in triplicate, along with no template control. Melt curve analysis was performed using the thermal cycling programmed at 50–95°C for each sample to determine the presence of multiple amplicons, nonspecific products, and contaminants. The results were analyzed using RT-PCR. The present study used human glyceraldehyde 3-phosphate dehydrogenase as an invariant control.

L-plastin Analysis by ELISA

Protein concentration of L-plastin was analyzed by ELISA methods from the kit procured from Bioassay Technologies Laboratory, Shanghai, China (Human L-plastin—Cat. No E4410Hu standard curve range—10 ng/L–2000 ng/L Sensitivity—5.24 ng/L). The assay was performed at room temperature. A blank well was set without any solution. Around 50 μL negative controls were added to the negative control wells. And 40 μL of L-plastin samples were added, followed by 10 μL anti-L-plastin antibody. The samples were diluted with sample diluents, covered with a plate sealer, and incubated at 37°C for 30 and 60 minutes. The plate was then washed five times with 350 μL wash buffer. Nearly 50 μL streptavidin-horseradish peroxidase was added to sample wells and standard wells and mixed well. The plate was covered with a sealer and incubated for 60 minutes at 37°C. The sealer was then removed, and the plate was washed five times with wash buffer. The wells were again washed with 0.35 mL wash buffer for 30 seconds to 1 minute for each wash. 50 μL substrate solution of A and B was added to each well. The plate was covered in the dark with a new sealer for 10 minutes at 37°C. When 50 μL stopped, the solution was added to each well, and the blue color changed into yellow immediately. The optical density (OD value) of each well was determined using a microplate reader set at 450 nm within 30 minutes after adding the stop solution.

Statistical Analysis

Statistical analysis was done using Statistical Package for the Social Sciences version 20.1 (Chicago, United States of America Inc). For both groups, the parameters' means and standard deviations were estimated. The link between variables was assessed using Pearson's correlation test. Mann–Whitney test was done to analyze data that did not follow a normal distribution. In the present study, $p < 0.05$ was considered as the level of significance.

Results

When demographic variables were compared between the groups, it was found that age (group I—45.49 ± 6.42; group II—46.89 ± 5.66) was insignificant with p-value = 0.337. BMI (group I—25.91 ± 3.45; group II—26.60 ± 3.16) also did not show any significant differences. They were found to be more or less similar. On comparing periodontal parameters, the mean plaque index (group I—1.50 ± 0.47; group II—2.07 ± 0.24), and CAL (group I—6.51 ± 0.82; group II—7.2 ± 0.67) were found to be significant between group I and group II with a p-value of <0.001 whereas BOP and PPD were insignificant. Similarly, the mean mRNA expression of L-plastin in groups I and II was 1.00 ± 0 and 2.79 ± 0.24, respectively, which also showed a statistically significant difference with a p-value of <0.001. The mean L-plastin protein levels in group I was 290.78 ± 52.04 ng/L and in the group, II was 447 ± 41.92 ng/L, which again showed a significant difference with a p-value of <0.001 (Table 1).

The mean income per month in groups I and II was Rs. 21,342.9 ± 12,288.1 and it was Rs. 16,485.7 ± 11,508 with a mean rank of 40.44 and 30.56, respectively, which was found to be statistically significant (p-value of 0.042) (Table 2).

On correlating the study variables, plaque index (p = 0.000) and CAL (p = 0.001) showed a positive correlation with the mRNA expression and protein levels of L-plastin, showing a significant p-value. The other parameters, such as height, weight, BMI, income, BOP, and PPD, did not show any statistically significant correlation with the tested protein (Table 3). mRNA expression of L-plastin showed a positive correlation with its protein level, which was found to be highly significant with a p-value = 0.000 (Table 4).

Discussion

The analysis of proteomes has brought great change among various biomedical disciplines such as medicine, genetics, molecular biology, and dentistry. The identification of the proteins associated with periodontal disease has gained importance in the recent era of diagnosis as potent biomarkers.[10] Protein analysis has been performed using various sources such as saliva and blood however has been identified to vary scarcely in pathologic tissue from the diseased periodontium, which is mainly considered the primary lesion of the periodontal disease.[11] According to Tonetti and Claffey, the periodontal pocket is the anatomopathological lesion exhibiting a site of an active periodontal disease that may be considered one of the main biological areas for proteomic analysis.[12] We analyzed the mRNA expression of L-plastin and its protein levels in the subgingival tissues of the surgical sites of generalized severe chronic periodontitis patients with and without type 2 diabetes mellitus.

Out of the demographic variables examined, mean income was found to be significant. (p = 0.000) This was in accordance with the study done by Agardh et al., who reviewed the association of socioeconomic factors and diabetes mellitus and found that lower-income increased the risk of diabetes by 40% even after statistically controlling for clinical factors and risk behaviors.[13] He included the level of education, income, and other contributing

Table 1: Comparison of all the variables between group I and group II

	Mean ± standard deviation			
Variables	*Group I (control group)*	*Group II (test group)*	*t–value*	*p-value*
Age (years)	45.49 ± 6.42	46.89 ± 5.66	0.96	0.33^NS
Height (cm)	164.68 ± 12.55	164.77 ± 10.12	0.031	0.97^NS
Weight (kg)	70 ± 7.88	72.31 ± 8.05	1.21	0.22^NS
BMI	25.91 ± 3.45	26.60 ± 3.16	0.86	0.39^NS
PI	1.50 ± 0.47	2.07 ± 0.24	6.28	<0.00*
BOP (%)	79 ± 8	81 ± 6	1.29	0.199^NS
PPD (mm)	5.87 ± 0.64	6.02 ± 0.58	1.01	0.31^NS
CAL (mm)	6.51 ± 0.82	7.2 ± 0.67	3.81	<0.00*
mRNA expression L-plastin	1.00 ± 0	2.79 ± 0.24	43.34	<0.00*
Protein quantification L-plastin (ng/L)	290.78 ± 52.04	447.00 ± 41.92	13.83	<0.00*

Level of significance; $p < 0.05$, p-value; * significant; p-value; NS, not significant

Table 2: Descriptive analysis of income by Mann–Whitney test

Variable	*Mean ± standard deviation*		*Mean rank*		*p-value*
Income (Rs) per month	Group I (control group)	Group II (test group)	Group I	Group II	0.042*
	21,342.9 ± 12,288.1	16,485.7 ± 11,508	40.44	30.56	

Level of significance; $p < 0.05$, *p*-value; * significant

Table 3: Correlation of mRNA expression and protein quantification of L-plastin with the demographic variables and periodontal parameters in both the groups

		mRNA expression	*Protein quantification*
Correlation		*L-plastin*	*L-plastin*
Height (cm)	Correlation	−0.022	0.106
	p-value	0.858^NS	0.384^NS
Weight (kg)	Correlation	0.125	0.215
	p-value	0.301^NS	0.075^NS
BMI	Correlation	0.112	0.047
	p-value	0.357 ^NS	0.697^NS
Income (Rs) per month	Correlation	−0.177	−0.111
	p-value	0.144^NS	0.362^NS
Plaque index	Correlation	0.583	0.588
	p-value	0.000*	0.000*
Bleeding on probing (%)	Correlation	0.106	0.112
	p-value	0.382^NS	0.357^NS
Probing pocket depth (mm)	Correlation	0.113	0.14
	p-value	0.352^NS	0.247^NS
Clinical attachment level (mm)	Correlation	0.383	0.374
	p-value	0.001*	0.001*

Level of significance; $p < 0.05$, *p*-value; * significant; *p*-value; NS, not significant

Table 4: Overall correlation between mRNA expression of L-plastin and its protein quantification

		Protein quantification ELISA
Pearsons correlation		*L-plastin*
Group I (35) + group II (35)		70 (No.)
mRNA expression-PCR: L-plastin	Correlation	0.854
	p-value	0.000*

Level of significance; $p < 0.05$, *p*-value; * significant

factors that would influence socioeconomic status. It can be stated that low income may influence the nutritional status of individuals. Moreover, it may also restrict the good medical facilities, which may further aggravate the disease. Age and BMI did not show any significant differences and were found to be more or less similar.

The periodontal parameters such as Pi, bleeding index, PPD, and CAL were compared between the groups. The mean plaque index was found to be statistically significant (*p*-value of 0.001). This was in line with studies by Abaas and Kudva et al., who illustrated a higher plaque index score in patients with periodontitis suffering from diabetes mellitus as compared to the healthy group.[14,15] This states that the bacterial load in the form of dental plaque and severity of periodontitis is associated with glycemic control and the time interval of diabetes mellitus. Similarly, mean CAL was also found to be statistically significant (*p*-value of 0.001). This was inconsistent with the findings of Azawy et al., who also presented similar findings.[16] Loss of periodontal attachment and loss of alveolar bone in periodontitis is usually seen to be associated with type 2 diabetes mellitus keeping glycemic control as an important determinant, as reported by Poplawska and Szelachowska.[17] We could not find significant BOP percentages among the groups. Acute host inflammatory responses are said to be responsible for BOP. Insignificant BOP in our study could be due to the fact that both groups comprised generalized severe chronic periodontitis patients. Moreover, group II was under medication, and its diabetic levels were under control; therefore, it did not show any active signs of inflammation. This was consistent with the findings by Katagiri et al., who found an effective glycemic control by glycemic intervention therapy and reported an improvement in the BOP within 6 months without any periodontal intervention.[18]

It is stated that cytokines manifest potent proinflammatory and catabolic activities and play a key role in periodontal tissue breakdown with attachment loss. We found the mean PPD to be similar among the groups, which was statistically insignificant. This could probably be due to the fact that both were chronic periodontitis subjects of similar age. Moreover, the diabetic patients (group II) were on medication without any active signs of periodontal inflammation. This was in accordance with Campus et al., who showed a decrease in PPD in well-controlled diabetic subjects when compared to the poorly controlled diabetics with periodontitis.[19]

L-plastin mRNA expression and its protein levels were found and expressed in the subgingival tissue samples of both groups

from the disease sites. The mRNA expression of L-plastin in group II was expressed as a fold change over the group I assuming the value of group I to be one as given by Schmittgen and Livak.[20] This was found to be a 1.79–fold change denoting a significantly elevated expression. L-plastin showed elevated levels in group II, emphasizing its involvement in generalized chronic periodontitis with the type 2 diabetes mellitus group. Ozturk et al. reported elevated L-plastin mRNA expression in the gingival tissue and gingival crevicular fluid among chronic periodontitis subjects.[7] Shinomiya et al., and Arpin et al., reported the role of L-plastin in immune cell-mediated events.[21,22] However, in contrast, Chan et al. observed the presence of L-plastin in the saliva of diabetic patients, which did not reach significant levels.[23] This may be because of the small sample size. L-pastin also showed a significant positive correlation with the plaque index (p-value of <0.001) and CAL (p-value of <0.001), indicating an association with the severity of the disease. Ma et al. reported that the activation of L-plastin mediates leukocyte adhesion, migration, osteoclast adhesion, and bone resorption.[8] The proteomic profile of L-plastin in our study has brought a possible role of this molecule in periodontal disease and diabetes mellitus. This is the first study to analyze this protein from the tissue of the periodontally active sites from periodontitis patients with type 2 diabetes. Analyzes of earlier studies have been done in serum, gingival crevicular fluid, and saliva; however, so far, attempts have not been made from the diseased sites. This was also the first attempt to find out the expression and protein levels of L-plastin in periodontitis patients with type 2 diabetes mellitus and compare them with systemically healthy generalized severe chronic periodontitis individuals.

One of the limitations of the study was that only the diabetic group was not included. In the future, a larger sample size with the inclusion of this group would further explain the role of L-plastin in diabetic patients without periodontitis. Within the limitation of this study, the investigation of this protein has revealed that it has a specific role in the etiology of periodontitis, which has shown the impact of diabetes mellitus, thus making it a more promising marker for both of these inflammatory diseases. This protein may specifically involve in the etiology and progression of both diabetes mellitus and periodontitis and act as a potent novel biomarker for predicting the onset, diagnosis, and management of disease activity. Future research may reveal new signaling pathways that connect diabetes mellitus and periodontal inflammation by using L-plastin as one of the major proteins in periodontal destruction. Both of these disorders may have this as a multifaceted therapy target. It may be highly promising to look at the complex biochemical connections between the proteomes in chronic generalized periodontitis and type 2 diabetes mellitus in order to develop specialized therapeutic approaches.

Conclusion

In conclusion, the higher mRNA expression of L-plastin and its protein levels were observed in chronic periodontitis patients with type 2 diabetes mellitus. Further longitudinal and interventional studies are required to explore the mechanisms that are more reliable in influencing the pathogenesis of both periodontal disease and diabetes mellitus.

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