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Arginase: An Emerging Key Player in Periodontal Disease and Diabetes Mellitus

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Abstract

Introduction: Arginase is a semi-essential amino acid, an enzyme found in saliva which is involved in inflammation.Arginase is found mainly in the human liver, also found in salivary glands. Saliva is an important biological material containing local and systemic derived markers. Analysis of arginase may be used as a diagnostic aid to evaluate the periodontal status and to clearly understand the pathogenic process of periodontal disease.

Aims And Objectives: To evaluate arginase levels in healthy, gingivitis, chronic periodontitis, and chronic periodontitis subjects with diabetes mellitus and to compare it with clinical parameters.

Methodology: Total of 100 patients, divided into 25 healthy subjects, 25 gingivitis subjects, 25 chronic periodontitis subjects(CP), 25 chronic periodontitis (CP) subjects with Type2 Diabetes mellitus (Ty2DM). Duly signed informed consent was obtained from all the subjects and saliva samples were collected. Arginase activity was measured by UV spectrophotometry at 515nm.

Result: Arginase level in CP subjects and CP subjects with Ty2DM are significantly higher than subjects with healthy and gingivitis.

Conclusion: Early evaluation of periodontal status is essential to prevent the progression of the disease. Salivary arginase levels can be used as a reliable marker in periodontal disease also can be regarded as a novel marker for the vascular complications of diabetes.

Keywords: Arginase, Diabetes Mellitus, Pathogenesis, Periodontitis, Saliva

Introduction

Chronic periodontitis is the most commonly occurring form of periodontal disease which affects the supporting structures of the tooth [1]. Bacterial biofilm which accumulates on the surfaces of the teeth causes periodontal disease to progress [2]. TY2DM also called non–insulin dependent diabetes in which there is an excess or inappropriate secretion of insulin [3]. Diabetes is been quoted as one of the risk factor for periodontitis [4]. The diabetes and chronic periodontitis relationship gives an example for the systemic disease that influences to oral infection by vascular changes in periodontal tissues [3]. Saliva contains local and systemic derived markers of periodontal disease [5], also contains host or microbial derived enzymes including arginase, nitric oxide synthase and collagenase which are indicated to be the cause of destruction of periodontal tissues [6].

Arginase is an enzyme of the urea cycle which catalyzes the hydrolysis of L-arginine to urea and ornithine. L-Arginine, a semi-essential amino acid synthesized in the human liver and kidneys, is the common substrate for nitric oxide synthase (NOS) and arginase 7, 8. It is hypothesized that arginase may reduce NO production by depleting the common substrate L arginine [6].Modulation of host factors, in diabetes, is important for the progression or worsening of periodontal disease.The pathogenesis of periodontal disease in diabetes, result from prolonged exposure to hyperglycemia and with poor glycemic control. Diabetic persons with severe periodontal disease are susceptible to micro vascular and macro vascular complications [9]. Patients with, type 2 DM frequently exhibit vascular endothelium dysfunction and NO deficiency is a major factor contributing to endothelial dysfunction. In view of these data, our objective was to measure arginase activity in saliva of subjects in CP patients with TY2DM and to clarify the mechanism of its contribution to the disease process. We hypothesize that arginase could be a novel biomarker for the development and progression of diabetic complications.

Materials And Methods

The present study included 100 individuals visiting the Department of periodontics of PMNM Dental College and Hospital, Bagalkot, Karnataka, India. This study was approved by the ethical committee of the institution. Informed consent was obtained from all the subjects who are participating in the study after a detailed explanation of the procedures and objectives of the study. Data regarding the personal history, medical, dental, habit history was recorded. After screening, the patients were selected for the study. Based on the selection criteria mentioned below, they were divided into 4 groups. Periodontal disease status was determined by clinical periodontal assessments including plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL). Patients included in the study were aged between 18 to 60 years. Criteria for test group and control group were as follows.

Group A (Control): 25 systemically and periodontal healthy Individuals.

Group B: 25 Subjects with gingivitis

Group C: 25 Subjects with Chronic Periodontitis (According to AAP 1999 classification) [10].

Group D: 25 Chronic Periodontitis subjects with Type 2 Diabetes Mellitus.

Patients who are on insulin therapy, with systemic diseases that could influence periodontal conditions, who have undergone periodontal therapy in the past 6 months, who are on hormonal therapy, having other infections or pathology in oral cavity other than periodontitis. Subjects with any chronic illness, on any medication from last 6months, were excluded from the study [11].

Collection of Saliva Sample

Prior to recording the clinical parameters, the subjects were instructed to rinse their mouth thoroughly with water and to chew a standardized paraffin wax for stimulating the saliva, which was collected every 60 seconds to yield a total of 1.5 ml of each sample. The time of collection was approximately 9 a.m. Samples were centrifuged for 10 min at 15 000´g at 4ºC to remove any particulate matter and clear supernatant obtained was stored at -20°C until analysed[6].

Biochemical Procedures

Arginase activity was measured spectrophotometric ally according to Chinard's method [12]. The enzyme activity was determined by measuring the amount of ornithine produced from the hydrolysis of arginine by arginase and was expressed as IU/ml. The specific enzyme activity was referred to the protein amount and calculated by dividing

the mean of enzyme activity by the mean of the protein amount. The specific enzyme activity was expressed as IU/mg protein. Briefly, saliva was diluted five-fold. The reaction mixture (0.5 ml) contained 0.5 mmol of MnCl2, 10 mmol of arginine and 30 mmol of carbonate buffer, all adjusted to pH 9.8. Then 0.05 ml of supernatant was added and after incubation at 37°C for 15 min, the reaction was stopped with 1.5 ml of concentrated acetic acid. Afterwards, 0.5 ml of ninhydrin solution was added (2.5 g of ninhydrin in a mixture of 40 ml of 6 mol/l H3PO4 and 60 ml of glacial acetic acid) and heated in a boiling water bath for 1 h. After the mixture was cooled, the absorbance was measured at 515 nm. One unit of arginase is defined as the amount of enzyme that produces 1 mmol of ornithine per minute at 37°C. Each sample was analyzed in duplicate [6].

Statistical Analysis

Data collected will be entered into a computer and analyzed using the SPSS software. Descriptive and inferential statistical analyses will be carried out in the present study. Results on continuous measurements will be presented on Mean ± SD and results on categorical measurements will be presented in Number (%). Level of significance will be fixed at p=0.05 and any value less than or equal to 0.05 will be considered to be statistically significant. Analysis of variance (ANOVA) will be used to find the significance of study parameters between the groups (Inter group analysis). Further post hoc analysis will be carried out if the values of ANOVA test will be significant.

Results

Significant differences were observed in the statistical mean value of PI, GI, PPD and CAL between the experimental and control groups. The chronic periodontitis with diabetes mellitus group had higher PI, GI, CAL and deeper PPD than the control group. Both the mean arginase activity and arginase specific activity in the experimental group were found higher than in the control group.The correlation between the amount of arginase and clinical variables in the experimental and control group was evaluated. Significant correlation was found for the amount of arginase, and the mean of clinical indices in both groups. Furthermore, highly significant correlation existed between the amount of arginase in whole saliva of the experimental group and controls (P>0.05)

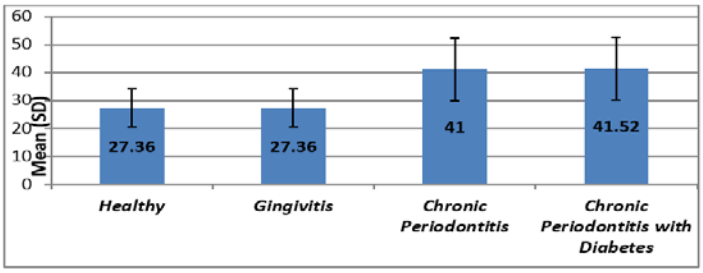

Figure 1: Comparison of age in terms of {Mean (SD)} among all the groups using ANOVA test

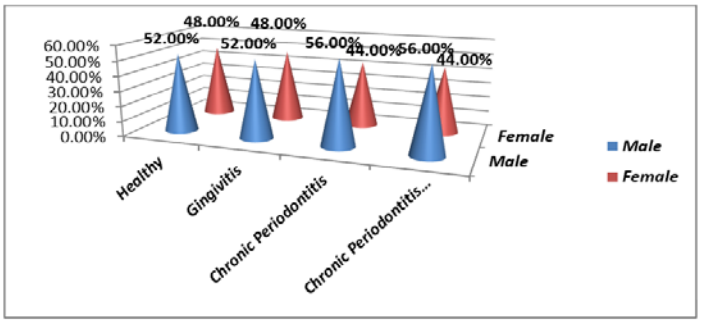

Figure 2: Gender wise distribution of the study participants among all the groups

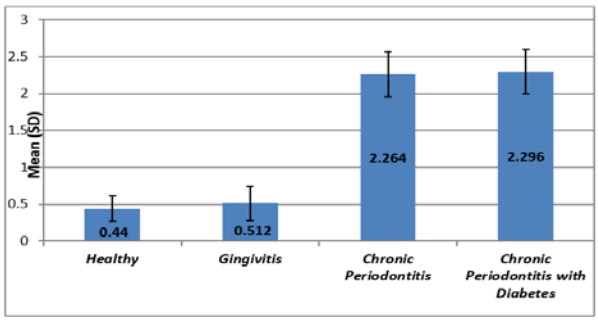

Figure 3: Comparison of gingival index values in terms of {Mean (SD)} among all the groups using ANOVA test

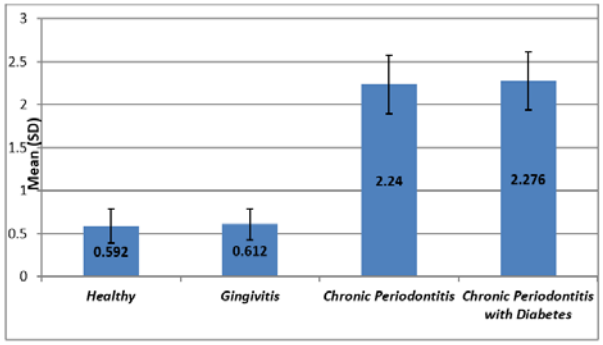

Figure 4: Comparison of plaque index values in terms of {Mean (SD)} among all the groups using ANOVA test

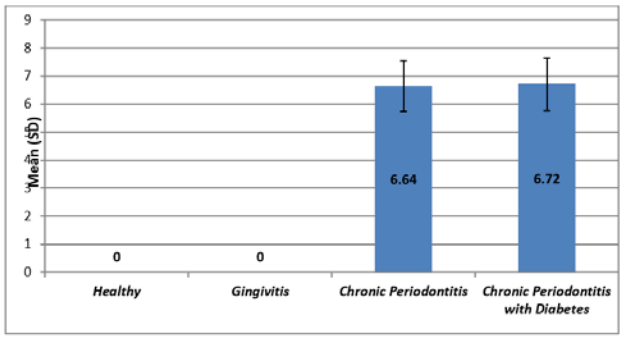

Figure 5: Comparison of pocket probing depth values in terms of {Mean (SD)} among all the groups using ANOVA test.

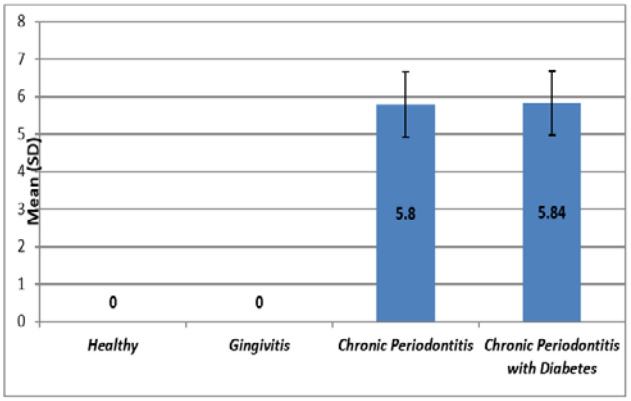

Figure 6: Comparison of clinical attachment level values in terms of {Mean (SD)} among all the groups using ANOVA test

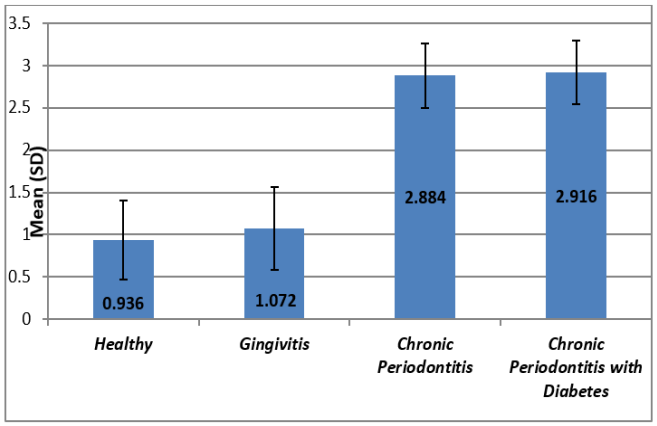

Figure 7: Comparison of bleeding index values in terms of {Mean (SD)} among all the groups using ANOVA test

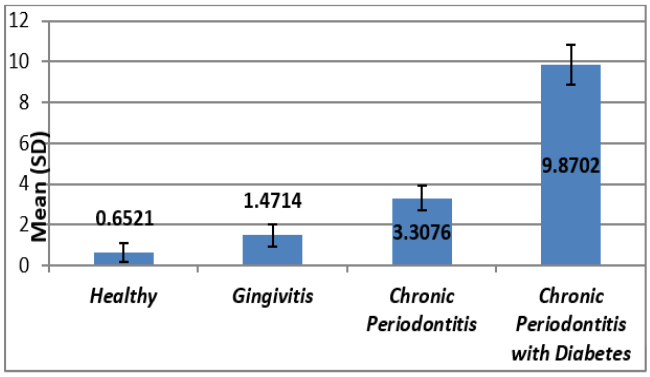

Figure 8: Comparison of arginase level values in terms of {Mean (SD)} among all the groups using ANOVA test

Discussion

Clinical periodontal parameters such as PD, CAL, bleeding on probing, plaque index, and radiographic evidence of alveolar bone loss are used to assess the severity of periodontal disease. Salivary markers have been used as diagnostic markers or risk factors for periodontal disease [13]. One of the salivary components is represented by arginase. Arginase in salivary glands may function in the synthesis of polyamies which are found in saliva and are nutritionally important to man oral bacteria, which has the ability to decrease NO synthesis, causing decrease in its antimicrobial properties making periodontal tissues more susceptible to existent pathogens

[6]. Gullu et al evaluated the arginase activity present locally in the gingival tissues [1], whereas in our present study, the arginase activity was observed in the saliva of periodontitis patients. The degradation of arginine by asaccharolytic anaerobic Gram-positive rods, including Eubacterium minutum, Filifactor alocis, E. infirmum, E. sulci, and E. saphenum has been shown in periodontal pockets.A study from Sosroseno et al. emphasized the importance of bacteria in arginase activity. They showed that Actinobacillus actinomycetemcomitans-lipopolysaccharide is able to stimulate arginase activity by murine macrophages. Arginase activity in patients diagnosed with chronic periodontitis was previously studied by Gullu et al 6, 14. Özmeriç et al. showed higher salivary arginase activity in periodontitis patients compared to healthy controls. These authors justified their results based on the fact that arginase in saliva might originate from host cells, such as macrophages [15].Gheren et al. study also discovered similar results. In their study salivary arginase was increased in chronic periodontitis patients compared to the controls. This was in contrary to findings of Kolte et al. study, where no significant difference was noted [11].Konarska et al measured the arginase activity in saliva and stated that the presence of the bacterial flora had no influence on the arginase activity. Since the periodontal status of the patients had not mentioned in that study, it was not clear whether the source of the arginase activity was the pathogenic bacteria as the periodontopathogenic bacteria might also exist in the saliva [6].Hirose et al. have found that NO production by macrophages and polymorph nuclear leukocytes via the iNOS pathway was enhanced in periodontal lesions and resulted in the progress of periodontitis. Activated macrophages were shown to produce arginase and this might also be valid for our patients since macrophage infiltration and activation are known to be characteristics of chronic inflammation as in periodontitis [16].

The present study was conducted to further evaluate the role played by salivary arginase on periodontal status of patients with diabetes mellitus. Elevated, arginase can compete with nitric oxide synthase (NOS) for their common substrate, Larginine. Reduction in L-arginine availability to NOS can lead to decreased production of NO, and possible NOS uncoupling and increased superoxide formation. Arginase and NOS has been reported to be involved in disease states such as hypertension and vascular complications of diabetes [17].

Thus, in the present study increased salivary arginase levels caused a decrease in NO synthesis, leading to decrease in the antibacterial property of saliva causing periodontal tissues to become more susceptible to existing pathogens and also leads to increase production of polyamines in favor of oral bacterial growth further contributing to the disease process.

The present study demonstrated an increase in salivary arginase levels in the group of CP with TY2DM when compared to the control group, results based on the fact that arginase in saliva might originate from host cells, such as macrophages or from oral bacteria, such as periodontal pathogens. Significant differences were observed in the statistical mean value of PI, GI, PPD and CAL between the experimental and control groups (Figure 3, 4, 5, 6, and 7).This was in accordance with the study conducted by Gheren LW et al 1, Ozmeric et al [6] and Shruthi Hegde et al [11]. Probing depth reflects a cumulative history of periodontal disease as there is a continuous progressive destruction of periodontal tissues. Disease activity at each individual pocket varies with time. Clinical parameters are reflective of an altered state of the periodontium that is changed by the repeated insults of bacteria and their byproducts associated with

periodontitis [18].Statistically significant difference between control group and study groups were noted in our study. (Figure 8)

The present study demonstrates that salivary arginase activity is increased in chronic periodontitis subjects with diabetes mellitus when compared to healthy individuals. This finding coincides with findings by Kashyap et al. who conducted a study on a small number of 12 diabetic patients. Increase in arginase level can lead to development of vascular dysfunction by limiting L arginine availability [11].

Conclusion

Chronic periodontitis is one of the most commonly encountered oral diseases with significant effects on oral and systemic health. Early evaluation of the periodontal status is essential to prevent the progression of the disease. In the present study demonstrates that salivary arginase levels were increased in chronic periodontitis subjects; further increase in arginase level is seen in chronic periodontitis subjects with diabetes mellitus when compared to control healthy individuals, indicate that levels of arginase can function as a predictory marker of increased morbidity of diabetic state. We suggest that salivary arginase activity could be an additional tool to connect salivary markers with periodontal status and could be a marker of early development of vascular dysfunction of diabetes. Arginase inhibitors may have therapeutic benefits in diabetic patients by preventing vascular dysfunction and maintaining NO levels.

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