Comparison of Acid phosphatase and β D-Glucuronidase Enzyme Levels in Type 2 Diabetes Mellitus with and without Periodontitis

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Abstract— The aim of the present study was to evaluate the serum acid phosphatase, and β D-glucuronidase lysosomal enzyme levels in type 2 diabetes mellitus with and without periodontitis. We studied a total of 450 subjects categorized into 3 groups: Group I (healthy subjects, n=150), Group II (type 2 diabetes mellitus with periodontitis, n=150), and Group III (type 2 diabetes mellitus without periodontitis, n=150). Serum concentration of acid phosphatase, and β D-glucuronidase were measured by means of UV absorption spectrophotometer. The mean serum lysosomal enzyme activity of acid phosphatase was elevated nearly 10 times more in type 2 diabetes mellitus with periodontitis (group II). Similarly we observed nearly a 9 fold times increased β D-glucuronidase activity among the type 2 diabetes mellitus with periodontitis (group II) subjects when compared to control. In conclusion, the elevated activities of the lysosomal enzymes- acid phosphatase, and β D-glucuronidase may play a biomarker role in showing the periodontal disease status of type 2 diabetes mellitus.

Index Terms— Acid phosphatase, β D-glucuronidase, Periodontitis, Type 2 diabetes mellitus.

1 INTRODUCTION

Periodontal disease is an inflammatory response to bacteria that exist in the gum tissue adjoining the teeth that, if left untreated, may result in recession of the gums, resorption of bone, tooth loosening and eventual loss of teeth [1]. This disease is postulated to place individuals at increased risk of type 2 diabetes mellitus and is globally underestimated. Diabetes mellitus is a systemic disease with several major complications affecting both the quality and length of life. Type 2 diabetes mellitus is a metabolic syndrome associated with hyperinsulinemia, insulin resistance, hyperglycemia, dyslipidemia and obesity. Uncontrolled or poorly controlled diabetes is associated with an increased susceptibility and severity of infections, including periodontitis [2, 3]. As with other systemic conditions associated with periodontitis, diabetes mellitus does not cause gingivitis or periodontitis. But evidence indicates that it alters the response of the periodontal tissues to local factors, hastening bone loss and delaying postsurgical healing of the periodontal tissues [4].

Polymorphonuclear leukocyte (PMNL), altered collagen metabolism, advanced glycation end products (AGE's) and bacterial pathogens in diabetes mellitus are the factors contributing to the development of periodontal disease. The primary etiologic factor of periodontal diseases are bacteria [5] and their by products, including lipopolysaccharides (LPS). The periodontal tissues are affected due to hyperglycemia, and periodontitis has been described as the sixth complication of diabetes mellitus [6]. Bacterial endotoxins, toxins and LPS can directly damage connective tissue, which cause certain cells of the periodontium to secrete enzymes. The bacteria in dental plaque when allowed to accumulate produce inflammatory mediators such as cytokines, prostanoids, and lysosomal enzymes, [7] including matrix metalloproteinases, collagenase, acid phosphatase, and β D-glucuronidase.

Lysosomes are membrane-bounded intracellular compartment responsible for the cell's waste disposal system, filled with hydrolytic acid enzymes that digest macromolecules. Mature lysosomes fuse and divide, allowing their content to be propagated throughout the compartment. Lysosomal enzymes are produced in the reticular network, enclosed in tiny vesicles that fuse with late endosomes or autophagosomes. These enzymes have been found to be up regulated in many acute and chronic pathological conditions such as trauma, sepsis and diabetes mellitus [8] Defects in the lysosomal enzymes provoke an accumulation of un-degraded molecules in the lysosomal system.

Acid phosphatase (EC 3.1.3.2) is one of the hydrolytic enzymes in lysosomes of cells from a variety of tissues. It forms a group of four isoenzymes as prostatic, lysosomal, erythrocytic and macrophagic acid phosphatases with differences originating at the structural level of the gene. The death of cells can cause a discharge of lysosomal enzymes, with a destruction of the nearby tissue. The extracellular acid phosphatase found in gingival fluid could represent an accumulation of lysosomal enzyme [9] from the rapidly desquamating epithelial cells of the crevicular epithelium or from connective tissue cells. It could also have a bacterial origin and so play a role in the formation of a pathological pocket [10].

Beta D-glucuronidase (EC 3.2.1.31) is known to be a membrane bound lysosomal enzyme, necessary in the hydrolysis of glucuronides, localized in the endoplasmic reticulum and in lysosomes. The determination of serum β D-glucuronidase is of clinical interest because it can be used in the diagnosis of several pathological conditions. Serum levels of β D- glucuronidase are increased in patients with such conditions as neoplasms, diabetes mellitus [11], in pregnant women and in gestational diabetes, atherosclerotic disease, coronary artery disease, and Gaucher's disease. Release of β D- glucuronidase is useful to detect phagocytotic cell activity, already before the actual lysis of the cell, because of increased lysosomal membrane permeability [12].

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The aim of this work was to evaluate activities of acid phosphatase, and β D- glucuronidase lysosomal enzymes in the serum of type 2 diabetes mellitus with and without periodontitis in order to see whether these enzymes show any comparable changes.

2 MATERIALS AND METHODS

2.1 Study Subjects and Ethical Approval

The study group consisted of a total of 450 subjects between the age group 25 to 55 years and were catagorized into three groups as control (Group I, n=150), type 2 diabetes mellitus with periodontitis (Group II, n=150), and type 2 diabetes mellitus without periodontitis (group III, n=150). Group II and group III subjects were selected from the outpatients attending Department of Periodontology & Oral Implantology, SRM Dental College, India. The study protocol was approved by the Institutional Ethical Committee of Medical and Health Sciences, SRM University, India and an informed consent was obtained from all the subjects..

2.2 Clinical Assessment of Study Subjects

Information about the age, gender, blood pressure, body mass index (BMI), duration of diabetes mellitus, current medications (insulin supplementation, oral hypoglycemic agents), diet and diabetes mellitus complications were obtained by a standardized questionnaire. For all subjects, the basic clinical history and demographic data were recorded. The clinical assessment for periodontitis subjects included examination of gingiva, intra oral examination- number of teeth present and missing, pathological migration, and probing depth. Mean pocket probing depth, and clinical attachment loss (CAL) were measured using mouth mirror and William's periodontal probe to assess the periodontal status.

Pocket probing depth was measured as the distance from the gingival margin to the bottom of the probed pocket. Probing depths were recorded at six sites per tooth, rounded up to the nearest millimetre. Periodontitis was confirmed by bone loss evident on radiographic examination. The periodontal status was examined by a trained Periodontist of SRM Dental College, Department of Periodontology, Chennai -600 089. The healthy controls were not on any kind of prescribed medication or dietary restrictions

2.3 Inclusion and Exclusion Criteria

Inclusion criteria include known diabetic type 2 patients of both sexes, for at least the past 5 years diagnosed by a physician by means of the oral glucose tolerance test. All periodontitis individuals included under the category of periodontitis should have more than 30% of the sites with Clinical attachment level (CAL) ≥ 3mm, pocket depth (PD) ≥ 4 mm, and at least 2 teeth in each quadrant with the condition of 20 teeth in all the subjects.

Smokers, alcoholics, drug abused, patients who had periodontal therapy six months prior to the study, patients under antibiotics and having systemic disease other than diabetics, taking hormone drugs, lipid lowering drugs, oral contraceptives, and pregnant women were excluded from the study.

2.4 Measurement of Clinical Parameters and Enzyme Activities

Blood samples were collected after an overnight fast for each subject. The fasting blood glucose (FBG), measured using the glucose oxidase - peroxidase method, was expressed in milligrammes per deciliter (mg/dl) and blood glycated hemoglobin (HbA1c), analyzed by high-performance liquid chromatography method (Biosystems S.A, Costa Brava, Spain) was expressed in percentage. Serum was obtained by centrifuging the blood at 1500 rpm for 10 minutes. It was used for the estimation of acid phosphatase, and β D-glucuronidase activity. Acid phosphatase (ACP) was analysed by the technique described by Gutman and Gutman [13], using di sodium phenyl phosphate as substrate. The incubations were performed at 37°C for 1 hour, and the reaction was stopped by adding 10% trichloroacetic acid. After removal of the precipitate, the concentration of acid phosphatase was determined by the differences in extinction at 620 nm against the reagent blank in a spectrophotometer. This difference in extinction was used as a measure of enzyme activity. The enzyme activity was expressed as micromoles of substrate hydrolysed/ min/ 1000 ml serum.

β D-glucuronidase was measured using p-nitrophenyl β D glucuronide as the substrate [14]. 0.05 ml substrate and 0.05 ml acetate buffer were incubated with 0.1 ml enzyme at 37°C for 1 hour. The reaction was arrested by adding 3 ml of glycine buffer. Standard were also made. Colour developed was read at 400 nm against a reagent blank. The enzyme activity was expressed as micromoles of p-nitrophenol liberated per minute per litre of serum. One unit of enzyme is defined as the amount of enzyme that releases 1 micromol of p-nitrophenol in 1 min at 37°C.

2.5 Statistical Analysis

The data are presented as mean ± SD (standard deviation). An unpaired Student's t test was used to evaluate the significance of differences, accepting $P < 0.05$ as the level of significance. Data were analyzed using the Graphpad Prism 6 for windows statistical software package (San Diego, California).

3 RESULTS

The demographic data, clinical and periodontal parameters of the study population are summarized in Table 1. The lysosomal enzyme activities of serum acid phosphatase, and β D-glucuronidase were shown in Table 2.

As expected the mean levels of periodontal probing depth (PD) and clinical attachment level (CAL), were significantly greater than 4mm in type 2 diabetes mellitus with periodontitis (group II) compared to type 2 diabetes mellitus without periodontitis and healthy subjects. Comparing to control, type 2 diabetic subjects with periodontitis (group II) and type 2 diabetic subjects without periodontitis (group III) presented mean percentage of HbA1C levels 8.38 ± 1.17 and 7.74 ± 1.31 respectively, Table 1.

TABLE 1
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF STUDY POPULATION

Parameters	Control Group I	Type 2 diabetes mellitus with periodontitis Group II	Type 2 diabetes mellitus without periodontitis Group III
No of samples	150	150	150
Gender (M/F)	80/70	77/73	78/72
Age, years	35.46 ± 10.74	44.42 ± 10.37***	46.26 ± 10.02***
Duration of diabetes, years	-	8.70 ± 4.82	8.39 ± 5.35
HbA1c %	5.20 ± 0.51	8.38 ± 1.17***	7.74 ± 1.31***
BMI, kg/m^2	22.72 ± 1.5	24.07 ± 1.51 ^{NS}	23.32 ± 1.49 ^{NS}
Systolic blood pressure(mm Hg)	119.5 ± 4.65	128.8 ± 5.09	126.4 ± 5.70
Diastolic blood pressure(mm Hg)	72.93 ± 2.10	79.05 ± 3.03	75.14 ± 1.78
FBG, mg/dl	95.28 ± 12.51	176.7 ± 59.12***	183.7 ± 57.16***
PD (mm)	1.45 ± 0.13	4.61 ± 0.51***	1.42 ± 0.17 ^{NS}
CAL (mm)	0.70 ± 0.27	4.91 ± 0.37***	0.64 ± 0.15 ^{NS}

Values are expressed as Mean ± SD; except for gender (Male, M / Female, F).
Glycosylated hemoglobin, HbA1c; Body mass index, BMI; Fasting blood glucose, FBG; Probing depth, PD; Clinical attachment level, CAL.
*Differences were considered significant level at *** p <0.0001; ** p <0.001; *p<0.05 of parameters of group II, III, IV vs group I and NS, non-significant*

The serum acid phosphatase, and β D-glucuronidase lysosomal enzymes activities was found to be significantly increased in group II when compared to control. It is interesting to note that, the activity of acid phosphatase was elevated nearly 10 times more in type 2 diabetes mellitus with periodontitis (group II) whereas it was only about 3 times higher in type 2 diabetes mellitus without periodontitis (group III) subjects. Similarly we observed nearly a 9 fold times increased β D-glucuronidase activity among the type 2 diabetes mellitus with periodontitis (group II) and it was found to be 2 times increased among group III subjects, Table 2.

TABLE 2

ACID PHOSPHATASE, AND BETA D-GLUCURONIDASE LEVEL IN TYPE 2 DIABETES MELLITUS WITH AND WITHOUT PERIODONTITIS

Parameters	Control Group I	Type 2 diabetes mellitus with periodontitis Group II	Type 2 diabetes mellitus without periodontitis Group III
No of samples	150	150	150
Acid phosphatase µ moles /min/L	1.18 ± 0.47	10.09 ± 2.46 ***	3.50 ± 1.27 ***
β D glucuronidase µ moles/min/L	3.29 ± 2.61	27.65 ± 10.46***	6.53 ± 1.68***

*Values are expressed as mean ± standard deviation; Differences were considered significant level at *** p <0.0001 of group II, III vs group I*

4 DISCUSSION

Number Lysosomes and endosomes are outfitted with a perplexing variety of endopeptidases. Evidence from our results demonstrates that, diabetes can alter serum lysosomal enzyme levels in periodontitis subjects. Perturbations in acid phosphatase, and β D-glucuronidase are more pronounced in type 2 diabetes subjects with periodontitis. It is not known whether differences in en zymes levels are a consequence of diabetes to develop periodontitis, or alternatively whether they contribute to the expression of the disease.

Acid phosphatase or orthophosphoric monoester phosphohydrolase activity is widespread throughout nature. We found an increased level of 10 fold times higher acid phosphatase activity among the type 2 diabetes mellitus with periodontitis subjects than control. The presence of acid phosphatase in serum arises from various sources, including erythrocytes, leukocytes, platelets, kidneys, spleen and liver, each of which contribute molecular variants (isoenzymes) of acid phosphatase that are specific to the organ or cells of origin. Various studies have shown that acid phosphatase, which is an important marker of phagocytic activity in professional phagocytes [15]. The rise in the levels of acid phosphatase enzyme during type 2 diabetes mellitus with periodontitis might accompany an increase of their release between the intra- and extra-cellular environments. It plays a role in the catabolism of glycoproteins and aminoacids.

Periodontal disease has been shown to be more severe in

diabetics as compared to non-diabetics [16]. A Significant progressive destruction of periodontal apparatus occurs in diabetics as compared to non-diabetics and significantly higher level of β -Glucuronidase levels exists in patients with poorer diabetic control [17]. We observed low levels of β -glucuronidase activity in control group I and the levels were found to be elevated in group II (type 2 diabetes mellitus with periodontitis). These results are consistent with the findings of Bang et al [18] and Lamster et al [19].These authors observed that β -glucuronidase increases with the development and severity of inflammation resulting into increased probing pocket depth and attachment loss. Also, the higher levels of β -glucuronidase can be predictive of attachment loss on a site specific and whole mouth basis with high levels of sensitivity and specificity.

The marked increase of β D-glucuronidase might reflect its major role in the catabolic proteoglycan pathway of the ground substance, generally involved in sphingolipid storage disorders [20]. The deficit of a specific enzyme and the consequent impairment of the lysosomal function quite likely set in an achievement and a sort of compensation mechanism involving the other enzymes of the lysosomal apparatus. The increase of plasma β-D glucuronidase might be due to the endothelial lesions in periodontitis, as already seen in chronic degenerative pathologies with vascular damage, like diabetes mellitus [21]. This was supported by finding a highly significant increase in β D-glucuronidase levels in type 2 diabetes subjects with periodontitis than type 2 diabetes subjects without periodontitis in our study.

The increase in β D-glucuronidase activity observed in serum may be as a result of damage in lysosomal membrane during diabetes with periodontitis, which results in the leakage of lysosomal enzymes into cytosol. Damaged lysosomal membrane leads to leakage of glycosidases into the cytosol, which could cause intracellular destruction [22]. It was observed that there is a significant correlation of hormonal influence with glycemic indices and lipid profile in the patients with type 2 diabetes mellitus [23]. The increase in the levels of these enzymes during type 2 diabetes mellitus with periodontitis might accompany an increase of their release–uptake mechanism between the intra- and extra-cellular environments. This process regards all cells, but particularly blood cells such as lymphocytes and leukocytes [24].

The present study also demonstrates that there is higher level of acid phosphatase and β -glucuronidase activity in patients of type 2 diabetes mellitus with periodontitis as compared to type 2 diabetes mellitus without periodontitis. This finding is in accordance with study by Bacic et al. [25] who showed that periodontal disease is more frequent and severe in diabetics as compared to non-diabetics. Wah Ching Tan et al [26] demonstrated that in diabetics with periodonitis, other than impaired glucose metabolism, genetic predisposition plays an important role in the progression of disease. Accumulation of glucose-mediated AGEs in diabetic patients impairs chemotactic and phagocytic function of polymorphonuclear leukocytes [27, 28]. The elevated levels of serum lysosomal enzymes observed in our study may trigger the production of reactive oxygen species (ROS). In an earlier study by Albandar [29] showed that sites with high numbers of microorganisms had the highest β glucuronidase activity. Type 2 diabetes mellitus with periodontitis could cause systemic inflammation and deterioration of insulin resistance through the production of inflammatory cytokines. Formation of advanced glycation end products (AGE) as a result of glycosylation of proteins and lipids are among the factors that can lead to periodontal disease development and increase oxidative stress in gingiva in diabetes patients [30, 31]. The level of β-glucuronidase correlates significantly with attachment loss that may subsequently occur in individuals with adult periodontitis [32].

This is the first study to show clearly that elevated levels of serum lysosomal enzymes acid phosphatase, and β D-glucuronidase was seen in subjects among type 2 diabetes mellitus with periodontitis. Periodontitis if untreated leads to tooth loss and hence a proper intervention is required from stage to stage in order to retain the teeth in the oral cavity. The elevated level of lysosomal enzymes liberated in serum can be used as a diagnostic tool for the early detection of type 2 diabetes mellitus with periodontitis.

5 Conclusion

Finally, we conclude that there was a significant increase in the enzyme activities of acid phosphatase, and β D-glucuronidase in type 2 diabetes mellitus with periodontitis subjects. The changes in lysosomal enzyme activities indicate that these enzymes stand as markers of possible efficacy. The presentation of periodontal signs in the latter stages of diabetes mellitus cause more periodontal tissue destruction, gingival bleeding, and delayed wound healing and show a higher risk of periodontitis for patients with diabetes. Therefore acid phosphatase and β D-glucuronidase can be used as a biomarker for diagnosing the type 2 diabetes mellitus with periodontitis cases.

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