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Original Article

Dentistry Section

Gamma Glutamyl Transpeptidase, Smokeless Tobacco, Chronic Periodontitis: Exploring the Link

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ABSTRACT

Introduction: Oxidative Stress (OS) is implicated in the pathogenesis of many systemic and oral diseases such as periodontal disease. Smokeless tobacco extract produces apoptosis and causes an imbalance between reactive oxygen species and antioxidants, such as Gamma Glutamyl Transpeptidase (GGT). Epidemiological research implied serum GGT within its normal range to be an early sensitive enzyme related to OS.

Aim: To assess how GGT fares as a biomarker in periodontitis subjects with or without the usage of smokeless tobacco and correlate it with clinical parameters.

Materials and Methods: Ninety subjects were divided into three groups of healthy, chronic periodontitis, and smokeless tobacco users with chronic periodontitis from the dental outpatient department of P.M. Nadagouda Memorial Dental College and Hospital. Serum samples of patients were collected after obtaining consent and analyzed for GGT. Statistical Analysis was performed using ANOVA, and Pearson's correlation coefficient.

Results: GGT levels were found to be significantly higher in chronic periodontitis patients compared to smokeless tobacco users with chronic periodontitis and healthy subjects.

Conclusion: GGT may be used as a quick, easy and precise marker for measuring OS in patients with chronic periodontitis and smokeless tobacco users.

Keywords: Biomarker, Oxidative stress, Reactive oxygen species

INTRODUCTION

Health is achieved by being able to balance various things in life. In normal physiology, a balance is maintained between activity of Reactive Oxygen Species (ROS) and antioxidant defense capacity. An imbalance in the pro-oxidant–antioxidant levels leads to potential damage causing OS [1]. OS has been implicated in the pathogenesis of many chronic inflammatory conditions such as diabetes, aging and parkinson's disease [2,3]. Periodontitis being a multifactorial disease, its onset, course, and severity are decided by an interplay of microbiologic, genetic, immunologic, and environmental/behavioural risk factors. Various studies in recent past have emphasized the association of periodontal disease and OS [4,5].

The sites in oral cavity where smokeless tobacco is placed are at risk for developing localized mucosal lesions, gingival recession, and attachment loss [6,7]. The amount of bone loss was found to be more prevalent in smokers than nonsmokers [8]. According to National Family Health Survey (2005-2006), 36.3% males and 8.4% females were found to consume smokeless tobacco products, prevalence being more in rural compared to urban area [9]. Smokeless tobacco users were found to comprise 54.76% of the Indian population [10].

Smokeless tobacco products being a cheaper alternative to smoking have gained popularity, its use being as addictive as cigarette smoking. Recent cell culture research suggests long term smokeless tobacco use being able to generate highly reactive DNA damaging free radicals that alter the cellular antioxidant defense system [11,12]. Oral cells were reported to produce ROS after in vitro incubation in aqueous extract of smokeless tobacco [13].

Maintenance of glutathione homeostasis is one of the major functions of serum GGT, an ectoplasmic enzyme [14]. The breakdown of extracellular glutathione by cellular GGT results in provision of constituent amino acids to be reutilized in intracellular glutathione synthesis [15].

Cellular GGT plays an important role in antioxidant defense systems. GGT induction increases intracellular glutathione, which is protective against OS. Iron and copper catalyse the reaction between GGT and glutathione and cause lipid peroxidation and mutagenesis, thus leading GGT to be potentially harmful. Intracellularly it is protective whereas pro-oxidant effect is initially extracellular and then intracellular [14,16]. Serum GGT within its normal range (0-51 IU/L) can be considered as an early and sensitive enzyme related to OS [17,18].

The enzyme demonstrated its presence in the outer cell envelope of *Treponema denticola*; the anaerobic pathogen playing an important role in the progression of periodontal disease. Thus, predicting a probable role of GGT in propagation of the organism within inflamed periodontal tissues [19].

Inspite of showing promise, the benefits of this marker have somehow been underutilized. With this in mind the present study was designed to establish the reliability of GGT as OS marker in smokeless tobacco users with chronic periodontitis. GGT levels were estimated in healthy, chronic periodontitis and smokeless tobacco users with chronic periodontitis and compared and correlated with the clinical parameters. To the best of our knowledge this is one among the footmark studies to qualify GGT as a biomarker in chronic periodontitis subjects with or without the usage of smokeless tobacco.

MATERIALS AND METHODS

The power of this study was fixed at 90% and margin of error being 5%, so around 80 subjects were needed for the study as per statistical sample size.

In order to improve the power of study and to reduce bias 90 samples aged 18-60 years were selected from the outpatient department of Department of Periodontics, P.M. Nadagouda Memorial Dental College, Bagalkot, Karnataka, India. The study was conducted for a period of five months. The protocol for this cross-sectional study was approved by the Institutional Ethical Committee. Prior to enrolment in the study, a written consent was obtained from the candidates who fulfilled the inclusion criteria.

After clinical and radiographic examination, the subjects were divided in three groups. Group I consisted of 30 healthy subjects showing absence of clinical and radiographic manifestations of periodontal disease, at least 20 teeth present. Group II comprised of 30 subjects diagnosed as chronic periodontitis with the presence of bleeding on probing and clinical attachment level of 3 mm or more at more than 30% of all sites in the mouth [20]. Group III consisted of 30 subjects fulfilling the criteria of Group II in addition to being smokeless tobacco users. Various forms of smokeless tobacco such as mishri, gutka, mawa, khaini users were included in the study.

Subjects suffering from systemic conditions (rheumatic fever, heart diseases, hypertension, diabetes, liver and kidney disease), any infection requiring prophylactic antibiotic therapy, pregnant female, lactating women, subjects on hormonal contraceptives or on hormone replacement therapy, on steroids and NSAIDs (for previous three months) or on vitamin supplements, alcoholics and having undergone scaling and root planing in past six months were excluded from the study as they proved to affect the levels of GGT.

After proper grouping of the subjects, a full mouth periodontal examination was performed by a single examiner. The periodontal parameters pocket probing depth; clinical attachment level and gingival index (Loe and Silness 1963) were assessed using a Williams periodontal probe by a single examiner. Plaque index and OHI-S gave information about the amount of debris or calculus present but no information about the amount of inflammation present. Since in this study GGT was hypothesised to increase in inflammatory conditions, gingival index which provides an insight in the amount of inflammation present was assessed.

Biochemical Analysis

Using aseptic precautions 2 ml of venous blood was drawn from median cubital vein into a serum separating tube. Serum obtained was immediately centrifuged at 2500 rpm for 15 minutes then transferred to laboratory for analysis of GGT by VITROS 250 fully automated dry chemistry analyzer.

Principle

The VITROS GGT slide method is performed using the VITROS GGT slides and the VITROS chemistry products calibrator kit 3 on VITROS 250 /350/950/5, 1 FS and 4600 chemistry systems and the VITROS 5600 integrated system. A drop of the sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. GGT catalyses the transfer of the gamma glutamyl portion of a L-gamma glutamyl-p-nitroanilide to glycylglycine leading to the production of L-gamma glutamyl-glycylglycine (glu-gly-gly) and 3 carboxy-4-nitroaniline. p-nitroaniline at 37°C at 400 nm. The rate of change in reflection density is measured and is used to calculate the enzyme activity of GGT [21].

STATISTICAL ANALYSIS

The data collected was analyzed using computer software, IBM Statistical Package for Social Sciences Version 20.0. Analysis was done using unpaired t-test and Pearson's correlation coefficient. Data were expressed as mean and standard deviation. A $p<0.001$ were considered to be statistically significant.

RESULTS

As subjects were recruited in the study, when they came to the college outpatient department for a dental examination, age and sex matching was not possible for the three groups. The mean age of chronic periodontitis subjects is higher than the healthy group [Table/Fig-1].

GGT level was found to be highest in the chronic periodontitis group followed by smokeless tobacco users with chronic periodontitis and then the healthy group [Table/Fig-2].

The mean age of males was higher than the females in the study groups [Table/Fig-1]. Non-significant difference was found in healthy group. The mean gingival index and pocket probing depth was found to be highest in the chronic periodontitis group [Table/Fig-3,4].

These parameters showed a positive correlation with GGT levels in the study groups. Chronic periodontitis group showed a statistically significant difference in the loss of clinical attachment level than the smokeless tobacco group. [Table/Fig-5]. All the three parameters show positive correlation with the GGT levels in the study groups.

Group	No of participants	Age Mean (SD)
Healthy subjects	Males:13	16.46 (1.8)
	Females:17	16.53 (4.5)
Chronic periodontitis	Males:19	53.37 (49.8)
	Females:11	50.0 (32.9)
Smokeless tobacco users with chronic periodontitis	Males:20	30 (9.9)
	Females:10	27.80 (9.5)

[Table/Fig-1]: Age wise distribution mean (SD) of the study participants among the three groups using unpaired t-test.
* p-value<0.001

Group	GGT levels Mean (SD)
Healthy subjects	16.50±3.5
Chronic periodontitis	52.13±4.7
Smokeless tobacco users with chronic periodontitis	29.27±9.7

[Table/Fig-2]: Comparison of values between cases and controls by one way ANOVA test.
* p-value<0.001

Gingival Index		GGT Levels
Chronic periodontitis	Pearson correlation	0.075
	Sig. (2-tailed)	0.696
	N	30
Smokeless tobacco users with chronic periodontitis	Pearson Correlation	0.237
	Sig. (2-tailed)	0.208
	N	30

[Table/Fig-3]: Comparison of three groups (healthy, chronic periodontitis and Smokeless tobacco users with chronic periodontitis) with respect to pocket probing depth scores by Pearson's correlation coefficient.

Pocket Probing Depth		GGT Levels
Chronic periodontitis	Pearson correlation	0.399*
	Sig. (2-tailed)	0.029
	N	30
Smokeless tobacco users with chronic periodontitis	Pearson correlation	0.818**
	Sig. (2-tailed)	<0.001
	N	30

[Table/Fig-4]: Comparison of three groups (healthy, chronic periodontitis and smokeless tobacco users with chronic periodontitis) with respect to pocket probing depth scores by Pearson's correlation coefficient.
*Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Clinical Attachment Level		GGT Levels
Chronic periodontitis	Pearson Correlation	0.294
	Sig. (2-tailed)	0.115
	N	30
Smokeless tobacco users with chronic periodontitis	Pearson Correlation	0.772**
	Sig. (2-tailed)	<0.001
	N	30

[Table/Fig-5]: Comparison of three groups (healthy, chronic periodontitis and smokeless tobacco users with chronic periodontitis) with respect to clinical attachment level scores by Pearson's correlation coefficient.
**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Chronic periodontitis is usually seen affecting the higher age group. This fact is very well reflected in the present study [Table/Fig-1]. Neutrophils are at the forefront of diseased periodontium. They face the bacterial challenge by producing proteolytic enzymes and oxygen by inducing oxidative burst. Lipopolysaccharides and DNA from plaque bacteria cause activation of Activator protein-1 (AP-1) and NF-kappa β pathways in gingival fibroblasts via CD14 and Toll like receptor-4 which causes activation of osteoclasts and increases concentration of Matrix-Metallo Proteinase (MMPs) which ultimately causes tissue damage. Recruitment and activation of hyper responsive Polymorpho-Nuclear (PMNs) cells by inflammatory cytokines produced by bacteria hastens the ROS production [22]. Enzymatic antioxidant glutathione plays a major role in maintaining the intracellular redox balance and thus regulating signaling pathways which are affected by OS.

GGT being an intracellular enzyme is a good indicator to assess cellular damage. It reflects the pathological changes occurring in the periodontal tissues [23]. Inflamed gingival tissues undergo various metabolic changes that cause it to be released in high amounts in various body fluids such as blood, saliva, gingival crevicular fluid etc. In the present research chronic periodontitis subjects showed higher levels of GGT as compared to healthy subjects [Table/Fig-2], which is in accordance to a study done by Sreeram M [17]. Other studies showing similar results in saliva and gingival crevicular fluid include those by Todorovic T et al., and Agnihotram G et al., [23,24].

Smokeless tobacco is a known risk factor for periodontitis, affecting the prevalence, extent and severity of periodontal disease. Tobacco in any form is known to cause potential alteration in the systemic as well as oral health [6]. Monocyte and oral keratinocyte production of inflammatory mediators is affected thus eventually leading to development of localized tissue alterations [7]. It's use also induces dose dependent increase in hepatic mitochondrial and microsomal lipid peroxidation as well as hepatic DNA damage thus producing oxidative tissue damage and apoptosis. This strongly suggests that smokeless tobacco induces an OS [7]. The smokeless tobacco users in the present study also exhibited OS. The results obtained were in accordance to a study done by Alwar V et al., [25]. A study done on experimental rats by Ugbor CI et al., also gave similar results [26].

In this study less clinical periodontal damage was exhibited by smokeless tobacco users with chronic periodontitis which showed relatively lower OS compared to chronic periodontitis indicating the presence of a positive correlation between the amount of destruction and the value of GGT obtained. The vasoconstrictive effects of nicotine interfere with the inflammatory signs and symptoms [4]. The tar products produced due to consumption of tobacco result in black or brown staining on the tooth surface. Smokeless tobacco users being more concerned about the unaesthetic appearance of teeth reported earlier to clinic thereby with less destruction of underlying periodontal tissues. This may be the probable explanation of lower GGT values in the smokeless tobacco subjects compared to the chronic periodontitis subjects.

Currently in the field of OS, biomarkers have been gaining a lot of interest lately. Emphasis is on development of functional biomarkers of OS status, i.e, biomarkers integrating effects of exposure to oxidants along with full range of antioxidant protective mechanisms in vivo [18]. Traditional methods of diagnosis of periodontal disease based on clinical and radiographic parameters are being replaced by newer methodologies wherein the periodontal disease activity can be quantified and differentiation can be done between active and quiescent site. The newer technologies being developed although highly sensitive and specific are invasive and expensive. The development of a simple, less invasive, quicker, easy to interpret, economical and yet confirmatory test is the need of the hour. Radiographs require a longer period to detect measurable changes in bone. The quest for a reliable biochemical test that detects inflammatory changes in a short period is still on. Biochemical investigations are currently preferred due to them being simple, less invasive, less time consuming, comparatively economical and may also be useful for pre and post-treatment evaluation of therapy. Moreover, biochemical alteration reflects tissue changes at cellular level.

According to a study by Lim JS et al., the activity of serum GGT was found to be unchanged in repeated tests during 40 weeks when frozen stored samples were thawed and then frozen again after each testing [18]. The value of their activity can correlate with the amount of damage and can indicate the prognosis of the course of this disease [23].

From the present study, it is evident that by estimation of GGT in circulation of chronic periodontitis patients, the degree of oxidative damage can be assessed. So we speculated that its estimation would have important clinical significance to prevent further periodontal damage and thereby maintain a healthy periodontium in the high risk cases. By correcting the underlying deficiency of antioxidants the treatment plan can be improved. This may be helpful for successful management of periodontitis, thereby arresting it in early stages and avoiding the possible consequences i.e., mobility and thus loss of teeth. The limitations of the present study includes having a smaller sample size. So, further elaborate studies on the estimation of before and after scaling and root planing levels are needed to ascertain the actual role of these parameters in the initiation and promotion of periodontitis.

CONCLUSION

There is a lack of gold standard methods for measuring OS generated during periodontal tissue destruction. There is a paradigm shift in the techniques and advanced researches for detection of free radicals. Measurement of serum GGT being an easy, reliable and inexpensive test, it can be put to use as a diagnostic chair side test for early detection of OS suggesting high scope for it to be recognized as a biomarker for OS damage. Researchers have confirmed that periodontal diseases are not just confined to the oral cavity but are also known to have an effect on systemic health. Hence, GGT can be a promising option to establish both a good oral and systemic health. Further long term studies are needed to justify its role as a biomarker.

REFERENCES

[1] Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol 2000. 2007;43:160-232.
[2] Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol. 2003;17:24-38.
[3] Serra J, Domınguez R, Marschoff E, Guareschi E, Famulari A, Boveris A. Systemic oxidative stress associated with the neurological diseases of aging. Neurochem Res. 2009;34:2122–32.
[4] Aziz A, Kalekar MG, Suryakar AN, Benjamin T, Prakashan MJ, Ahmed BMN, et al. Assessment of some biochemical oxidative stress markers in male smokers with chronic periodontitis. Ind J Clin Biochem. 2013;28(4):374–80.
[5] Miricescu D, Totan A, Calenic B, Mocanu B, Didilescu A, Mohora M, et al. Salivary biomarkers: Relationship between oxidative stress and alveolar bone loss in chronic periodontitis. Acta Odontol Scand. 2014;72:42-47.
[6] Robertson PB, Walsh M, Greene J, Einster V, Grady D, Hanck W. Periodontal effects associated with the use of smokeless tobacco. J Periodontol. 1990;61:438-43.
[7] Malhotra R, Kapoor A, Grover V, Kaushal S. Nicotine and periodontal tissues. J Indian Soc Periodontol. 2010;14(1):72-79.
[8] Baharin B, Palmer RM, Coward P, Wilson RF. Investigation of periodontal destruction patterns in smokers and non-smokers. J Clin Periodontol. 2006;33:485–90.
[9] International Institute for Population Sciences (IIPS) and Macro International. 2007. National Family Health Survey (NFHS-3), 2005-06: India: Volume I, Mumbai: IIPS.
[10] Parmar G, Sangwan P, Vashi P, Kulkarni P, Kumar S. Effect of chewing a mixture of arecanut and tobacco on periodontal tissues and oral hygiene status. J Oral Sci. 2008;50:57-62.
[11] Lam EWN, Kelley EE, Martin SM, Buettner GR. Tobacco xenobiotics release nitric oxide. Tobacco Induced Dis. 2003;1:207–11.
[12] Kilinc M, Okur E, Kurutas EB, Guler FI, Yildirim I. The effects of Maras powder

(smokeless tobacco) on oxidative stress in users. Cell Biochem Funct. 2004;22:233–36.
[13] Bagchi D, Hassoun EA, Bagchi M, Stohs SJ. Protective effects of free radical scavengers and antioxidants against Smokeless Tobacco Extract (STE) induced oxidative stress in macrophage J774A.1 cell cultures. Archives of Environmental Contamination and Toxicology. 1995;29(3):424-28.
[14] Whitfield JB. Gamma glutamyl transferase. Crit Rev Clin Lab Sci. 2001;38(4):263–355.
[15] Bailey SM, Patel VB, Young TA, Asayama K, Cunningham CC. Chronic ethanol consumption alters the glutathione/glutathione peroxidase-1 system and protein oxidation status in rat liver. Alcohol Clin Exp Res. 2001;25:726–33.
[16] Takahashi Y, Oakes SM, Williams MC, Takahashi S, Miura T, Joyce-Brady M. Nitrogen dioxide exposure activates gamma-glutamyl transferase gene expression in rat lung. Toxicol. Appl. Pharmacol. 1997;143:388–96.
[17] Sreeram M, Suryakar AN, Dani NH. Is gamma-glutamyl transpeptidase a biomarker for oxidative stress in periodontitis?. J Indian Soc Periodontol. 2015;19:150-54.
[18] Lim JS, Yang JH, Chun BY, Kam S, Jacobs DR Jr, Lee DH. Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress? Free Radic Biol Med. 2004;37:1018-23.
[19] Mäkinen PL, Mäkinen KK. Gamma-glutamyltransferase from the outer cell envelope of *Treponema denticola* ATCC 35405. Infect Immun. 1997;65:685-91.
[20] American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal Diseases and Conditions. J Periodontol. 2015;86(7):835-38.
[21] Shaw LM, Strømme JH, London JL, Theodorsen L. IFCC methods for measurement of catalytic concentration of enzymes. Part 4. IFCC methods for gamma glutamyl transferase. J Clin Chem Biochem. 1983;21:633-46.
[22] Dahiya P, Kamal R, Gupta R, Bharadwaj R, Chaudhary K, Kaur S. Reactive oxygen species in periodontitis. J Indian Soc Periodontol. 2013;17(4):411-16.
[23] Todorovic T, Dozic I, Vicente-Barrero M, Ljuskovic B, Pejovic J, Marja¬novic M, et al. Salivary enzymes and periodontal disease. Med Oral Patol Oral Cir Bucal. 2006;11:E115-19.
[24] Agnihotram G, Singh MTR, Pamidimarri G, Jacob L, Rani S, Sravanthi. Study of clinical parameters in chronic periodontitis. International Journal of Applied Biology and Pharmaceutical Technology. 2010;1(3):1202-08.
[25] Alwar V, Ramesh R, Niranjan G, Kala C. Biochemical assessment of liver damage in smokeless tobacco users. Int J Cur Res Rev. 2013;5(23):63-69.
[26] Ugbor CI, Okogun GRA. Okonkwo LO, Eze NC, Asogwa BE, Ebo JO. The effect of tobacco snuffs consumption on liver enzymes. IJHPR. 2013;2(2):20-27.

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