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Qunatification of Plaque Creatinine Levels in Healthy Gingivitis and Chronic Periodontitis Subjects

Dr. Priya Sinha[1], Dr Vikash Ranjan[2], Dr. Sakshi Priya[3]

[1]MDS, Department of Periodontics, Mumbai, Maharashtra, India

[2]MD, Department of Microbiology, Mumbai, Maharashtra, India

[3]MBBS, Bangalore, Karnataka, India

Running Title: Creatinine and Chronic Periodontitis

Abstract: ***Introduction: Periodontitis is a chronic inflammatory state characterized by persistent inflammation, annihilation of connective tissue and alveolar bone. Creatinine, 2-Amino-1-methyl-1H-imidazol-4-ol an organic compound of low molecular weight 113 dalton is a nitrogenous end product of musclecreatine catabolism. It is distributed throughout total body water. Creatinine is present not only in serum and erythrocytes but also in all bodily fluid. Creatinine in plaque seems to capture the host response to periodontopathogens. Study regarding Creatinine in periodontal disease will provide new opportunities in diagnosis and treatment. Aims and Objectives: To quantify and compare levels of plaque Creatinine level in healthy, gingivitis and chronic periodontitis subjects and compare it with bleeding on probing, gingival index, plaque index, pocket probing depth and clinical attachment level. Methodology: 120 subjects were divided into 3 groups: 40 healthy 40 gingivitis and 40 chronic periodontitis. Plaque samples of patients were collected after obtaining duly signed consent and analyzed by using liquixx Creatinine kit. Statistical analysis was done by Mann whitney U test and Spearmanscorelation test. Results: A statistically significant increase in plaque Creatinine levels was found in chronic periodontitis subjects compared to healthy and gingivitis subjects. Clinical parameters were positively correlated with increasing Creatinine levels. Conclusion: Intracellular enzymes are released from damaged cells of periodontal tissue. Creatinine can be used as reliable biochemical markers for functional condition of periodontal tissues and therapeutic intervention.***

Keywords: Creatinine, periodontitis, biochemical marker

1. Introduction

Early detection of an infection plays an utmost role for correct diagnosis and successful treatment, thereby reducing the severity and probable complication of the diseases. To rise above this challenge, medicinal researchers are committed for finding molecular disease biomarkers and their products that gives information about the unseen lethal threat before the condition becomes problematical[1].

Periodontal disease, a bacterial infection is a chronic inflammatory condition, characterised by complex host-parasite interactions leading to extermination of both hard and soft tissue [2]. It is cause primarily by specific periodontal pathogens such as gram negative anaerobic bacteria inhabiting within sub gingival plaque. Although bacteria are the major etiological agents, the host immune response to these bacteria is of fundamental importance. Hence, it is evident that periodontal disease is a multifactorial disease, affiliates with specific microorganism, social and behavioral factors, genetic or epigenetic trait, all of which are modulated and controlled by underlying immune and inflammatory response of the host.

Plaque is most important and site specific etiological factor effecting the prevalence and severity of the periodontal destruction as pathogenic microorganism which causing periodontal destruction inhabit in biofilm of dental plaque [3]. Periodontitis mostly prevails in the middle age group[4]. Quality of life is disturbed because of tooth mobility or tooth loss resulting due to long drawn out or severe inflammation. Severity and progression of disease complex requires interactions between risk factors such as microbial, immunological, environmental, age, sex, race and genetic factors[5]. To aptly diagnose and evaluate, the periodontal disease has been acknowledged lots of attention in the last decennary to avoid unnecessary treatment to the patients. In periodontology the conventional diagnostic method is determined through clinical parameters like probing pocket depth, bleeding on probing, clinical attachment loss, plaque index, gingival index and radiographs. These are unapt to discriminate the disease activity with precision as it gives data of the previous destruction and not about the current condition of the disease. Therefore, advanced methods (eg biomarkers and end products) have been projected to facilitate the diagnosis of active periodontal disease in a more objective way with providing information about the severity of periodontits and help to verify the risk of an inactive site from becoming active during maintenance and disease-monitoring phases[6]. Many biological reactions with high specificity and objectivity are mainly controlled by various biological catalyst, an enzyme [7]. Cascades of host bacterial reaction leads to production of several enzymes and end products, which are released from stromal, inflammatory or bacterial cells. From the damaged cells of periodontal tissue these products are increasingly released in gcf or saliva, Creatinine, 2-Amino-1-methyl-1*H*-imidazol-4-ol an organic compound of low molecular weight 113 dalton is a nitrogenous end product of musclecreatine catabolism. It is distributed throughout total body water. It is present not only

in serum and erythrocytes but also in all bodily secretions[8]. It is normally present in tissue with high energy demands especially skeletal muscle[9].

The normal serum creatinine (^{s}Cr) for the adult male, the normal range is 0.6 to 1.2 mg/dl, or 53 to 106 µmol/L by the kinetic or enzymatic method, and 0.8 to 1.5 mg/dl, or 70 to 133 µmol/L by the older manual Jaffé reaction. For the adult female, with her generally lower muscle mass, the normal range is 0.5 to 1.1 mg/dl, or 44 to 97 µmol/L by the enzymatic method .[9] Creatinine formation begins in the kidney with the transamidination from arginine to glycine to form glycocyamine or guanidoacetic acid (GAA).The GAA is transported to the liver where it is methylated by S-adenosyl methionine (SAM) to form creatine. Creatine enters the circulation, and 90% of it is taken up and stored by muscle tissue. In muscle creatine and creatine phosphate are irreversibly converted to creatinine by non-enzymatic degradation, there is a continuous need for their replacement[10]. Level of creatine phosphokinase is raised during muscle damage, cell destruction and necrosis thus eliciting chemical fluctuations happening in the body[11]. It is deliberated as anindicator of cardiovascular disease[13]and also have been used to detect periodontal diseases and determine the success of periodontal treatment [14]. There is a presence of creatine phosphokinase in bacteria with dental plaque[16] ·Plaqueis an architecturally and functionally organised biofilm forming a diverse microbial composition, is fairly stable over time with site specificity . Plaque collection causes less uneasiness to the patient, is less technique sensitive and can be collected easily with minimum equipment.

Thus this study aims to quantify the level of plaque creatinine in healthy, gingivitis and chronic periodontitis and to compare and correlate plaque creatinine level with the bleeding on probing, gingival index, plaque index, pocket probing depth and clinical attachment level. Since there is no relevance study based on creatinine levels in plaque, thus the analysis of this enzyme in plaque can contribute to clarification of the pathogenesis and may provide an insight for the improvement in making a prompt diagnosis of the periodontal disease and different systemic conditions. This study highlights recent advances in the use of biomarker disease diagnostics that focus on the identification of active periodontal disease from plaque sample. To the best of our knowledge there is yet no scientific data available on plaque creatinine level in healthy, gingivitis and chronic periodontitis.

2. Materials and Methods

In the present cross sectional, single blinded study a total of 120 subjects of both the sexes with age ranging between 18-60 years were selected from the outpatient department of Department of Periodontic, P.M. Nadagouda Memorial Dental College, Bagalkot, Karnataka, India for a period of two months. The study protocol was approved by the Institutional Ethical Committee. Aforementioned signed inform consent form was obtained from the subjects prior to enrolment in the study. After clinical and radiographic examination, the subjects were divided in three groups. Group I consisted of 40 healthy subjects showing absence of clinical and radiographic manifestations of periodontal disease, at least 20 teeth present. Group II 40 gingivitis subjects and Group III comprised of 40subjectsdiagnosed as chronic periodontitis with the presence of bleeding on probing and clinical attachment level of 3 mm or more at more than30% of all sites in the mouth[17].

Subjects with 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 creatinine.

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) plaque index (silness and Loe 1964), bleeding on probing were assessed using a Williams periodontal probe by a single examiner. Plaque index and gingival index gave information about the amount of debris or calculus present and about the amount of inflammation present.

Biochemical Analysis

After proper isolation subgingival plaque sample was collected from all smooth dental surfaces of incisors and molar regions using sterile periodontal curettes[19] The collected samples was transferred to sterile ,chilled plain tubes containing 10 ml phosphate buffered saline (Ph 8.0) (0.12 M Nacl, 5 Mm NaH2PO4, 0.01 M Na2HPO4, Ph 7.4) .Sample was immediately centrifuged at 3000 rpm for 2 mins and then was transferred for further analysis of creatine level using liquixx creatinine kit in automated dry chemistry analyzer. Most of those in current use are automated and give clinically reliable and reproducible results.

3. Statistical analysis

The data collected was analyzed using computer software, IBM statistical package for social science version 20. Analysis was done using Spearmans correlation testand Mann Whitney U –test. Data were expressed as mean and standard deviation. A $p<0.001$ were considered to be statistically significant.

4. Results

The mean plaque creatinine level 24.0, 30.66, and 43.74 among periodontally healthy, gingivitis and chronic periodontitis subjects respectively. Plaque creatinine levels were found to be higher in chronic periodontitis compared to gingivitis and healthy subjects. All the clinical parameters i.e bleeding on probing, pocket probing depth, clinical attachment loss , gingival index , and plaque index showed positive correlation with the study group . According to the increase in the clinical parameters there was significant increase in the creatinine levels among all the groups.

Comparison of mean plaque creatinine levels in the all the group showed statistically significant difference p<0.001 [Table/Fig-1].

The mean age of chronic periodontitis subjects is higher than the healthy group [Table/Fig-2]. Creatinine level was found to be higher in the chronic periodontitis group compared to gingivitis and healthy group [Table/Fig-1].The mean gingival index, plaque index, bleeding index and pocket probing depth was found to be higher in the chronic periodontitis group [Table/Fig-2]. These parameters showed a positive correlation with creatinine levels in the study groups. Chronic periodontitis group showed a statistically significant difference in the loss of clinical attachment level compaered with healthy group . [Table/Fig-2]. All the parameters show positive correlation with the creatinine levels in the study groups [Table/Fig-2].

Table 1: comparison of healthy, gingivits and chronic periodontitis subjects

Group		N	Mean (SD)	Range	Median (Q1-Q3)	Kruskal Wallis test		Mann Whitney U test (p-value)		
						Chi square value	p-value	1 vs 2	1 vs 3	2 vs 3
Gingival index	Normal	50	0.58 (0.21)	0.20 - 0.90	0.65 (0.40 - 0.73)	100.25	<0.001*	0.87(NS)	<0.001*	<0.001*
	Gingivitis	50	0.58 (0.22)	0.20 - 0.90	0.65 (0.40 - 0.80)					
	CP	50	2.25 (0.26)	1.50 - 2.70	2.30 (2.08 - 2.43)					
Plaque index	Normal	50	0.75 (0.27)	0.10 - 1.40	0.80 (0.50 - 0.90)	100.43	<0.001*	0.57(NS)	<0.001*	<0.001*
	Gingivitis	50	0.78 (0.26)	0.10 - 1.40	0.80 (0.60 - 0.90)					
	CP	50	2.24 (0.31)	1.40 - 2.70	2.40 (2.08 - 2.50)					
Bleeding index	Normal	50	1.25 (0.51)	0.20 - 2.00	1.20 (0.80 - 1.70)	97.62	<0.001*	0.84(NS)	<0.001*	<0.001*
	Gingivitis	50	1.23 (0.52)	0.20 - 2.00	1.20 (0.80 - 1.70)					
	CP	50	2.95 (0.41)	1.80 - 3.60	3.00 (2.70 - 3.23)					
Creatinine	Normal	50	0.15 (0.05)	0.10 - 0.20	0.10 (0.10 - 0.20)	120.62	<0.001*	<0.001*	<0.001*	<0.001*
	Gingivitis	50	0.31 (0.15)	0.10 - 0.90	0.30 (0.20 - 0.33)					
	CP	50	0.92 (0.24)	0.50 - 1.60	0.90 (0.80 - 1.10)					

*p<0.05 Statistically significant,
p>0.05 Non Significant, NS

Table 2: Correlation of Clinical Parameters With Creatinine Level

Group			Plaque index	Pocket probing depth	Clinical attachment level	Bleeding index	Creatinine	Age
Healthy	Gingival index	Correlation Coefficient	0.93	.	.	0.92	0.76	0.62
		p-value	<0.001*	.	.	<0.001*	<0.001*	<0.001*
	Plaque index	Correlation Coefficient	1.00	.	.	0.96	0.77	0.64
		p-value	.	.	.	<0.001*	<0.001*	<0.001*
	Bleeding index	Correlation Coefficient		.	.	1.00	0.79	0.76
		p-value		.	.	.	<0.001*	<0.001*
	Creatinine	Correlation Coefficient		.	.		1.00	0.53
		p-value		.	.		.	<0.001*
Gingivitis	Gingival index	Correlation Coefficient	0.96	.	0.06	0.78	0.72	0.62
		p-value	<0.001*	.	0.70(NS)	<0.001*	<0.001*	<0.001*
	Plaque index	Correlation Coefficient	1.00	.	0.10	0.83	0.74	0.69
		p-value	.	.	0.51(NS)	<0.001*	<0.001*	<0.001*
	Bleeding index	Correlation Coefficient		.		1.00	0.87	0.81
		p-value		.		.	<0.001*	<0.001*
	Creatinine	Correlation Coefficient		.			1.00	0.61
		p-value		.			.	<0.001*
CP	Gingival index	Correlation Coefficient	0.96	0.87	0.84	0.92	0.95	0.75
		p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Plaque index	Correlation Coefficient	1.00	0.91	0.87	0.95	0.94	0.71
		p-value	.	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Pocket probing depth	Correlation Coefficient		1.00	0.88	0.85	0.88	0.55
		p-value		.	<0.001*	<0.001*	<0.001*	<0.001*
	Clinical attachment level	Correlation Coefficient			1.00	0.89	0.86	0.64
		p-value			.	<0.001*	<0.001*	<0.001*
	Bleeding index	Correlation Coefficient				1.00	0.96	0.83
		p-value				.	<0.001*	<0.001*
	Creatinine	Correlation Coefficient					1.00	0.80
		p-value					.	<0.001*

Spearman's Correlation test
*p<0.05 Statistically significant,
p>0.05 Non Significant, NS

5. Discussion

The prevalence rate of periodontitis is high occupying more than 50% of the indian community[21] mostly appreciated in population with middle age, this fact is very well reflected in table 1. Periodontitis, a chronic, multifactorial inflammatory condition affecting the attachment of connective tissue and supporting bone around the teeth.[22]Principal etiological factor accountable for commencement and advancement of periodontitis is dental plaque biofilm[3]. Of 600 different bacteria and 150 to 200 different species roughly 10% of bacteria play a causal role in the initiation of periodontal disease. Once with after initiation, there is detruction of fibroblast (collagen), apical shifting of juctionalepithelium, deepening of gingival sulcus leading to formation of periodontal pocket with alveolar bone resorption .Periodontal disease progression is episodic in nature with a period of extensive destruction followed by quiescent periods[23]. In response to these destructionmany enzymes and their products are released from stromal, epithelial, bacterial or inflammatory cells in gingival crevicular fluid, saliva or anybody fluid[20]. These enzymatic biomarkers and their end product play an innate role for diagnosis, evaluating treatment outcome, monitor cellular and chemical constituent[23]. Periodontal disease biomarker permit earlier detection of disease and may be released during defensive activity done against bacterial invasion.[20]

Creatinine and urea production are interrelative processes. Creatine is used for creatinine synthesis, and is produced from the amino acid arginine, an intermediate of urea production. One of the intermediates of creatine synthesis is ornitine, a substrate for the first chemical reaction of urea production (ornitine cycle). Most serum creatinine is derived from skeletal muscle as a metabolite of creatine. Thus, normal serum creatinine concentrations are proportional to muscle mass, which is an important target organ of insulin[24]The described parameters of saliva in the patients (creatinine, proline, hydroxyproline, protein) reflected the protein metabolism; the important clinico-diagnostical value for this pathology Creatine phosphate an intracellular enzyme is a good indicator to assess cellular damage or cell necrosis[11]. It reflects the pathological and metabolic changes occurring in the inflamed swollen periodontal tissues. When these gingival tissue becomes sick they released enzymes in high amounts in various body fluids such as blood, saliva, gingival crevicular fluid. In the present cross sectional single blinded study chronic periodontitis subjects showed higher levels of creatinine as compared to gingivitis and healthy subjects [Table/Fig-1], Creatine phosphate levels were significantly increased in periodontal disease as because proinflammatory cytokines (IL-6, and IL-1) are produced and accumulated in gingival crevicular fluid of patients with periodontal disease ,this accumulation of circulating cytokines results in soft tissue damage as mentioned by Tidballwhere as on doing experiment on genetically engineered mice by Tsujinaka *et al* found that over expression of IL-6 is associated with an increased degradation of muscular proteins.[25] According to huang 1990, creatinephophokinase is present in higher proportion in gingival fibroblast obtained from patient with periodontits . Connective tissue of periodontium is made up of fibroblast. Therefore, degradation of this connective tissue in periodontits leads to increase in release of creatine phosphate thereby causing increase in count of creatinine in chronic periodontitis subjects.[26] The results obtained were in accordance to a study done Todorovic T et al in saliva.[18]

In gingivitis, as mentioned in literature that there is increase in load of neutrophil count, creatine phosphate stored in specific granules and secretory vesicles of the neutrophils and are principally released during migration to the site of infection .This may be the probable explanation for showing significant positive correlation between Creatinine level and gingival index among healthy, gingivitis and chronic periodontitis as gingival index measure the severity of inflammation[16]. NADPH oxidase is present in cell membrane of phagosome. This oxidase converts NADPH2 to NADP. Stimulation of phagocytic cells leads to an increase in cellular consumption of molecular oxygen, a process termed as Respiratory burst . This is associated with the generation of various reactive oxygen species further leading to tissue damage. With increase in destruction there is increase in creatine level, since conversion of creatine and creatinine is interconnected process thereby showing positive correlation with the probing depth and clinical attachment loss[20].

From the present study, it is found that by estimation of creatinine in chronic periodontitis patients is useful in interpreting the cellular necrosis or cellular damage. So we after theorizing thoroughly, estimation of this creatinine may provide golden prospect for prognosis of disease thereby maintaining a healthy periodontium, arresting disease in early phase providing successful therapy and liming the loss of teeth thus improving the quality of life. The limitations of the present study includes having a smaller sample size. So, further studies are needed to establish the actual role of these parameters in the initiation and promotion of periodontitis.

6. Conclusion

Activity of creatinine reflects the depth of pathological process and damage of periodontal tissues, indicating the prognosis of the disease. It also reflects the metabolic changes in the inflammed gingiva thus providing new opportunities in diagnosis and treatment protocol .Researchers have confirmed that periodontal disease is not just the disease of oral cavity but also effect the systemic health. Hence creatinine levels estimation can help us to save millions oral health.

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Authore Name	Contribution
Dr Priya Sinha	60%
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DrSakshi Priya	10%

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