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"QUANTIFICATION OF CREATINE PHOSPHOKINASE LEVELS IN CHRONIC PERIODONTITIS"

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ABSTRACT

Introduction: Periodontitis is a chronic inflammatory state characterized by persistent inflammation, annihilation of connective tissue and alveolar bone. Creatine phosphokinase an intracellular enzyme, is a steadfast indicator of cellular damage and their increased activity is a reflection of metabolic changes in inflamed gingiva. Creatine phosphokinase in plaque seems to capture the host response to periodontopathogens. Study regarding creatine phosphokinase in periodontal disease will provide new opportunities in diagnosis and treatment. **Obejectives:** To quantify the level of plaque creatine phosphokinase level in healthy and chronic periodontitis subjects and to compare and correlate it with bleeding on probing, gingival index, plaque index, pocket probing depth and clinical attachment level. **Methodology:** 120 subjects were divided into 2 groups: 60 healthy and 60 chronic periodontitis. Plaque samples of patients were collected after obtaining duly signed consent and analyzed by using liquixx Creatine Kinase kit. Statistical analysis was done by Mann whitney U test and Spearmans corelation test. **Results:** A statistically significant increase in plaque creatine phosphokinase levels was found in chronic periodontitis subjects (1.4 ± 4.4) compared to healthy (0.2 ± 1.4).Clinical parameters were positively correlated with increasing creatine phosphokinase levels. **Conclusion:** Intracellular enzymes are released from damaged cells of periodontal tissue. Creatine

phosphokinase can be used as reliable biochemical markers for functional condition of periodontal tissues and therapeutic intervention.

KEYWORDS: Creatine phosphokinase, Chronic periodontitis, enzyme.

INTRODUCTION

For the successful treatment early detection of a disease plays a utmost role and thereby reducing the severity and probable complication of the diseases. To rise above this challenge, medicinal researchers are committed for finding molecular disease biomarker that gives information about the unseen lethal threat before the condition becomes problematical.[1] Periodontal disease, a bacterial infection is a chronic multifactorial inflammatory condition, characterised by complex host- parasite interactions leading to annihilation of both hard and soft tissue.[2] Plaque is a most important etiological factor effecting the prevalence and severity of the periodontal destruction. Pathogenic microorganism capable of causing periodontal disease inhabitant in biofilm of dental plaque.[3] Periodontitis mostly prevails in the middle age group.[4] Long drawn out and severe inflammation of periodontium results in tooth mobility or loss of tooth, influencing the quality of life .For succession and severity of periodontal disease complex interactions between risk factors such as microbial, immunological, environmental, age, sex, race and genetic factors as well interplay a foremost role.[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 incompetent to differentiate the disease activity with accuracy as it gives information of the past damage not about the present condition of the disease. Therefore, advanced methods (eg biomarkers) 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, which are released from stromal, inflammatory or bacterial cells. From the damaged cells of periodontal tissue intracellular enzymes are increasingly released in gingival crevicular fluid or saliva, such as Aspartate aminotransferase, Alanine

Aminotransferase, Gamma Glutamyl Transferase, Alkaline Phosphatase, creatine kinase etc.[8-10] Creatine phosphokinase (CPK) also known as creatine kinase (CK) or phospho-creatine kinase is 82 kDa intracellular enzyme which is proteinaceous in nature. It is normally present in tissue with high energy demands especially skeletal muscle, brain and myocardium.[11] CPK is increased in procedures associated with muscle disruption, cell damage and necrosis thus eliciting chemical changes occurring in the body.[12] It is considered as a marker of cardiovascular disease[13] and also have been used to detect periodontal diseases and determine the success of periodontal treatment[14] Clinically, CPK is assayed in blood tests as a marker of damage of CK-rich tissue such as in myocardial infarction, rhabdomyolysis, muscular dystrophy, the autoimmune myositides and in acute renal failure.[15] Creatine phosphokinase is present in bacteria within dental plaque.[16] Plaque is a structurally and functionally organised biofilm which forms a diverse microbial composition, it is relatively stable over time. Collection of plaque causes less discomfort to the patient, not technique sensitive and can be collected easily with minimum equipment. CPK in plaque seems to capture the host response to periodontopathogens. This study aims to quantify the level of plaque creatine phosphokinase in healthy and chronic periodontitis subjects and to compare and correlate plaque creatine phosphokinase 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 the creatine phosphokinase level in plaque. The use of plaque CPK levels for translational and clinical application has emerged at the forefront. The analysis of this enzyme in plaque can contribute to explanation of the pathogenesis and may provide an insight for the perfection 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 creatine phosphokinase level in healthy and chronic periodontitis. This will be the foot mark study designed to detect the estimation of creatine phosphokinase levels in plaque samples of chronic periodontitis subjects.

MATERIALS AND METHODS

In the present cross sectional study a total of 120 subjects of both the sexes age 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. Prior to enrolment in the study, a written consent was obtained from the subjects.

After clinical and radiographic examination, the subjects were divided in two groups. Group I consisted of 60 healthy subjects showing absence of clinical and radiographic manifestations of periodontal disease, at least 20 teeth present. Group II comprised of 60 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.[17]

Subjects with systemic conditions (rheumatic fever, heart diseases, hypertension, diabetes, liver and kidney diseases) , 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 CPK.[18]

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 sub gingival 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 2mins and then was transferred for further analysis of creatine phosphokinase level using liquixx creatine kinase kit in automated dry chemistry analyzer.

Principle

Modification by IFCC methods is used to estimate CPK. Creatine phosphokinase reversibly catalyzes the transfer of a phosphate group from creatine phosphate to adenosine diphosphate

(ADP) to give creatine and adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6- phosphate and ADP from glucose. This reaction is catalyzed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose- 6-phosphate is oxidized by the action of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340/660 nm due to the formation of NADPH is directly proportional to the activity of Creatine phosphokinase in the sample.[20]

Creatine Phosphate + ADP+ATP + Glucose-------->G-6-P+ NADP+CPK HK,Mg2+ G6PDH+Creatine+ ATP+ADP+ Glucose-6-phosphate (G-6-P) 6-phosphogluconate +NADPH2

III. Statistical analysis

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

IV. Results

All subjects were enlisted in the study, when they visit to the college outpatient department for a dental examination, age and sex matching was not possible for the two groups. The mean age of chronic periodontitis subjects is higher than the healthy group [Table-1]. Creatine phosphokinase level was found to be higher in the chronic periodontitis group compared to healthy group 1.4± 4.4 and 0.2 ± 1.4 respectively [Table-1]. The mean gingival index and pocket probing depth was found to be higher in the chronic periodontitis group [Table-1]. These parameters showed a positive correlation with creatine phophokinase levels in the study groups [Table-2] Chronic periodontitis group showed a statistically significant difference in the loss of clinical attachment level compared with healthy group [Table/Fig-1]. All the parameters show positive correlation with the creatine phosphokinase levels in the study groups [Table-2].

i) Comparison of the study parameters between the healthy and CP subjects by using Mann whitney U test

Group		N	Mean (SD)	Range	Median (Q1-Q3)	U Statistic	p-value
Age	Healthy	60	32.35 (6.58)	19 - 50	32.5 (29- 36.75)	575.00	<0.001*
	CP	60	43.97 (8.90)	24 - 59	45 (38- 50)		
Gingival index	Healthy	60	0.65 (0.21)	0.2 - 0.9	0.7 (0.5- 0.8)	0.00	<0.001*
	CP	60	2.28 (0.25)	1.7 - 2.7	2.3 (2.1- 2.475)		
Plaque index	Healthy	60	0.69 (0.18)	0.1 - 0.9	0.7 (0.6- 0.8)	0.00	<0.001*
	CP	60	2.29 (0.26)	1.6 - 2.6	2.4 (2.1- 2.5)		
Pocket probing depth	Healthy	60	0(0.00)	0 - 0	0 (0- 0)	0.00	<0.001*
	CP	60	6.73 (0.66)	6 - 8	7 (6- 7)		
Clinical attachment level	Healthy	60	0(0.00)	0 - 0	0 (0- 0)	0.00	<0.001*
	CP	60	6.82 (0.75)	6 - 8	7 (6- 7)		
Bleeding index	Healthy	60	1.09 (0.35)	0.2 - 1.7	1.15 (0.9- 1.38)	0.00	<0.001*
	CP	60	3.02 (0.34)	2 - 3.5	3.2 (2.8- 3.3)		
Creatine kinase	Healthy	60	0.70 (0.34)	0.2 - 1.4	0.65 (0.4- 0.9)	0.50	<0.001*
	CP	60	2.79 (0.80)	1.4 - 4.4	2.75 (2.1- 3.5)		

*p<0.05 statistically significant P>0.05 Non significant, NS

ii) Correlation between study parameters in healthy and CP subjects using Spearmans corelation test

Parameters		**Creatine kinase**	
		Healthy	**CP**
Gingival index	Correlation Coefficient	0.93	0.97
	p-value	<0.001*	<0.001*
Plaque index	Correlation Coefficient	0.89	0.97
	p-value	<0.001*	<0.001*
Pocket probing depth	Correlation Coefficient	-	0.72
	p-value	-	<0.001*
Clinical attachment level	Correlation Coefficient	-	0.70
	p-value	-	<0.001*
Bleeding index	Correlation Coefficient	0.97	0.91
	p-value	<0.001*	<0.001*

*p<0.05 statistically significant , P>0.05 Non significant, NS

DISCUSSION

The prevalence rate of Periodontitis is high in India, with involvement of more than 50% of the Indian community[21] mostly appreciated in population with middle age, this fact is very well reflected with our study [table1]. Periodontitis is an chronic, multifactorial inflammatory condition affecting the attachment of connective tissue and supporting bone around the teeth[22] Primary etiological factor responsible for commencement and progression 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 the initiation progress with the presence of major responsible factors, there are further destruction of fibroblast (collagen), apical shifting of juctional epithelium, 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 destruction some enzymes are released from stromal, epithelial, bacterial or inflammatory cells in gingival crevicular fluid, saliva or any body fluid.[20] These enzymatic biomarker play an innate role for diagnosis, evaluating treatment outcome, monitor cellular and chemical constituent.[23] Periodontal disease biomarker permits earlier detection of disease and may be released during defensive activity done against parasite invasion. Enzymes those are released in response to tissue destruction are asapartate transferase, alkaline phosphatase, lactate dehydrogenase, creatine phosphokinase , etc [20]

Creatine phosphokinase an intracellular enzyme is a steadfast 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 damaged that's when they released enzymes (CPK) in higher 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 CPK as compared to healthy subjects [Table/Fig-1], which is in accordance to a study done by keerthana et al[20] Deepika v et al[16] and other study by Alshail F showing similar results in blood.[24]

CPK levels were significantly increased in periodontal disease as because pro inflammatory cytokines (IL6, IL1) 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 Tidball, whereas on doing experiment on genetically engineered mice by Tsujinaka et al, they found that over expression of IL-6 is associated with an increased degradation of muscular proteins.[24] According to huang 1990, creatine phophokinase is present in higher proportion in gingival fibroblast obtained from patient with periodontitis. Connective tissue of periodontium is made up of fibroblast. Therefore, degradation of this connective tissue in periodontitis leads to increase in release of creatine

phosphokinase, thereby showing significant increase in chronic periodontitis subjects.[25] The results obtained were in accordance to a study done Todorovic T et al in saliva.[18]

In periodontitis, as mentioned in literature that there is increase in load of neutrophil count, creatine phosphokinase are 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 creatine phosphokinase level and gingival index among healthy and chronic periodontitis as gingival index measure the severity of inflammation.[16]

Creatine Phosphate + ADP + ATP + Glucose --------> G-6-P + NADP + CPK HK, Mg2+G6PDH+Creatine+ ATP+ADP+ Glucose-6-phosphate (G-6-P) 6-phosphogluconate +NADPH + H

Creatine kinase catalyse the transfer of a phosphate group from creatine phosphate with liberation of ATP. This ATP is used by glucose in presence of G6PD, MG^{+2} and hexokinase to produce G6P and NADPH. 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 phosphokinase level, thereby showing positive correlation with the probing depth and clinical attachment loss.[20]

From the present study, it is found that estimation of creatine phosphokinase in chronic periodontitis patients is useful in interpreting the cellular necrosis or cellular damage. So we after theorizing thoroughly, estimating the level of creatine phosphokinase may provide golden perspect for prognosis of disease thereby maintaining a healthy periodontium, arresting disease in early phase, providing successful therapy and limiting 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.

V. CONCLUSION

Activity of creatine phosphokinase 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 inflamed gingiva thus providing new opportunities in diagnosis and treatment protocol. Creatine phosphokinase can serve as a useful biomarker for screening of periodontal disease.

Researchers have confirmed that periodontal disease is not just the disease of oral cavity but also effect the systemic health. Hence creatine phosphokinase along with saving millions heart it can help us to save millions oral health.

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