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Enzymatic Profile of Alkaline and Acid Phosphatase In Gcf of Patients with Chronic Periodontitis.

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Abstract:*Objective: This Study Was Done To Evaluate The Level Of ALP And ACP As Potential Biochemical GCF Markers Among Chronic Periodontitis Patients. Materials And Methods: A Total Of 60 Subjects Were Selected For The Study. The Study Population Was Further Divided Into 3 Group (Group 1 – Clinically Healthy Periodontium, Group – 2 Gingivitis, Group – 3 Periodontitis)Based On Clinical Assessment Of Probing Depth , Bleeding On Probing And Radiographic Evaluation Of Bone Loss. GCF Samples Were Taken To Assess The Level Of Enzymes .Results: Obtained Results Were Shown Statistically Significant Increase In The Level Of ALP Activity In GCF From Patients With Gingivitis And Periodontitis In Relation To Control Group. There Was Positive Correlation Between The Activity Of Examined GCF Enzymes And Values Of The Gingival Index And Periodontal Disease Index. Conclusion: Based On These Results, It Can Be Assume That Activity Of These Enzymes In GCF May Be Used As Potent Biochemical Markers For Periodontal Destruction*.

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I. Introduction

Microbial Biofilm Adhering To Teeth, Serves As A 'Slow- Delivery System' Of Oral Pathogens Into The Systemic Circulation Leading To A Chronic Microbial Challenge And Downstream Effects, As A Consequence Of An Altered Host Immune Response. Because Of The Increasing Prevalence And Associated Co Morbidities Screening And Diagnostic Modalities For The Early Identification Of Oral Disease Initiation And Progression, As Well As Objective Measures For Response To Therapy, Are Being Sought.

Periodontal Disease Is The Result Of A Complex Interplay Among Bacterial Challenge, Host Response, And Other Modifying Factors. Clinical Measurements Used In Diagnosis Of Periodontal Diseases Are Often Of Limited Usefulness, In That They Are Indications Of Previous Periodontal Disease Rather Than The Present Disease Activity. Biochemical Mediators In Oral Fluids Like Saliva And Gingival Crevicular Fluid (GCF) Are Highly Beneficial In The Determination Of Current Periodontal Status. These Substances Are Known As Biomarkers. They Help In Determination Of Inflammatory Mediator Levels, As They Are Good Indicators Of Inflammatory Activity [1]. Biomarkers, Whether Produced By Normal Healthy Individuals Or By Individuals Affected By Specific Systemic Diseases, Are Telltale Molecules That Could Be Used To Monitor Health Status, Disease Onset, Treatment Response And Outcome. Informative Biomarkers Can Further Serve As Early Sentinels Of Disease [2]. Although A Single Specific Target Biomarker For Periodontal Disease Has Not Been Identified, Combinations Of Putative Biomarkers Of Disease Have Been Evaluated In Oral Fluids And Demonstrated Significant Potential As Panels Of Targets For The Development Of An Oral Fluid Fingerprint Of Periodontal Disease Status.

Oral Fluid Biomarkers That Have Been Studied For Periodontal Diagnosis Include Proteins Of Host Origin (E.G., Enzymes And Immunoglobulins), Phenotypic Markers, Host Cells (E.G., Pmns), Hormones, Bacteria And Bacterial Products, Ions, And Volatile Compounds [3,4,5,6,7]. Among These Enzymes Of Host Origin Are Further Divided Into Proteolytic And Hydrolytic Enzymes. Intracellular Enzymes Are Increasingly Released From The Damaged Cells Of Periodontal Tissues Into The Gingival Crevicular Fluid (GCF) And Saliva. Several Enzymes That Are Evaluated For The Early Diagnosis Of Periodontal Disease Are Aspartate And Alanine Aminotransferase (AST, ALT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK), Alkaline And Acid Phosphatase (ALP, ACP), And Gamma Glutamiltransferase (GGT)[8,9,10] .

Phosphatases Are Enzymes That Catalyze Reactions Which Remove The Phosphate Groups From Various Molecules. This Action Of These Enzymes Is Called Dephosphorylation. These Enzymes Remove A Phosphate Group From The Substrate Which Are Monoesters And Releases Phosphate Ions As Products. These

Reactions Take Place In Various Types Of Cells In The Body [11]. Of These Alkaline Phosphatase And Acid Phosphatase Are One Such Hydrolytic Enzymes That Are Of Critical Importance In Clinical Biochemistry.

Alkaline Phosphatase (ALP) Is A Hydrolase Enzyme Responsible For Removing Phosphate Groups From Many Types Of Molecules, Including Nucleotides, Proteins, And Alkaloids. Human Alkaline Phosphatases (Alps) Are Classified Into Three Main Types: Kidney/Liver/Bone, Or "Universal" Type, Intestinal Type, And Placental Type [12]. Alkaline Phosphatase Is A Membrane Based Glycoprotein Produced By Many Cells Within The Area Of The Periodontium And Gingival Crevice[13]. The Main Sources Of The Enzyme Are Polymorphonuclear Leukocytes (Pmns)[14,15],Osteoblast, Fibroblast Cells[16,17,15] And Gram Negative Anaerobic Bacteria Associated With Periodontal Disease. Bacterial Alkaline Phosphatase (B-AP) Aids In The Uptake And Metabolism Of Phosphorylated Organic Molecules Which Bacteria Require For Growth And Replication. The Presence Of B-AP Is Indicative Of Bacterial Infection At The Site.

Acid Phosphatase, A Lysosomal Enzyme (Except In Red Cells) Catalyses The Hydrolysis Of Orthophosphoric Monoester To An Alcohol And Orthophosphate. Zinc And Magnesium Are Cofactors. It Is Present In High Concentrations In The Prostate Gland, And Is Also Present In Red Cells, Platelets, Bone, Liver, And Spleen. Acid Phosphatase, Hyaluronidase, And Protease Are Also Found In Neutrophils[18] And Are Produced By Plaque Bacteria. Acid Phosphatase Is Also Present In The Vacuoles Found In The Cytoplasm Of Osteoclast Which Are Filled With Lysosomes[19,20]. Membrane-Coating Granules Present In Keratinized Epithelia Are Also Been Named Microgranules, Keratinosomes, And Lysosomes Contains Acid Phosphatase. Acid Phosphatase Has Been Widely Investigated Amongst The Lysosomal Enzymes And Has Often Been Used As An Lysosomal Marker [21].

This Study Was Done To Evaluate The Level Of ALP And ACP Activity As Potential Biochemical GCF Markers Among Chronic Periodontitis Patients.

II. Material And Methods

Examination Included 60 Male Patients, Aged 20 – 40 Years. The Study Population Was Further Divided Into 20 Patients With Gingivitis, 20 Patients With Periodontal Disease, And 20 Healthy Adult Volunteers. All Subjects Had Good General Health With No History Of Systemic Disease. Alcoholic, Pan Chewers, Drug Abuse , Smokers , Patients Who Had Periodontal Therapy Done 6 Months Prior To The Study, Patients Under Any Systemic Antibiotic And Or Anti Inflammatory Drug Therapy Within 3 Months Prior To This Study Were Excluded From This Study. Patients With Lesser Than 20 Permanent Teeth And Teeth With Fixed Or Removal Prosthetics Were Also Excluded. As The Initial Examination, Each Subject Completed A Detailed Medical Questionnaire And Received A Complete Periodontal Examination, Which Included: Plaque Index (PI), Gingiva Index (GI),Periodontal Disease Index (PI), Bleeding On Probing (BOP) And Probing Depth (PD). Clinical Periodontal Recordings Were Performed At Six Sites (Mesio-Buccal, Mid-Buccal , Disto-Buccal , Mesio-Lingual, Mid-Lingual And Disto-Lingual) On Each Tooth Using UNC – 15 Probe . The Study Protocol Was Explained To The Participants And Written Inform Consent Was Obtained.

The Study Population Was Divided Into:

- Group 1 - 20 Subjects With Clinically Healthy Periodontium With No Bleeding On Probing, No Radiographic Evidence Of Alveolar Bone Loss And Greater Than 90% Of The Sites Exhibiting PD Not Exceeding 3mm.
- Group 2 – 20 Subjects With Gingivitis With PD Not Exceeding 3mm, Presence Of Bleeding On Probing And No Radiographic Evidence Of Alveolar Bone Loss.
- Group 3 – 20 Subjects With Periodontitis With PD Exceeding Greater Than 5 Mm, Presence Of Bleeding On Probing And Presence Of Radiographic Evidence Of Alveolar Bone Loss.

Gingival Crevicular Fluid Sampling:

Gingival Crevicular Fluid Samples For Evaluating Acid And Alkaline Phosphatase Was Collected From The Same Site With Greatest Probing Depth After Isolation And Preparation Of The Concerned Tooth Using Calibrated Microcapillary Tubes As Per The Recommendation Of Cimasoni Et Al 1969 [22]. The Sampling Time Was 30 Seconds. GCF Samples Were Then Transferred With A Jet Pressure From The Capillary Tube Into Eppendorf Tube Containing 200 µl Of Normal Saline (Prepared By Using 0.85 Gms Of Nacl And In 100 Ml Of Distal Water) . A Total Of 60 GCF Samples Collected Were Stored At - 80 °C Until It Was Analyzed Using Spectrophotometer.

Determination Of Alkaline And Acid Phosphatase Activity*:*

ALP And ACP Activity Was Measured By Spectrophotometer Using An ALP And ACP Determination Kit (CORAL DIAGNOSIS) According To The Manufacturer's Instructions.

Determination Of Alkaline Phosphatase Activity:

After Homogenizing The Mixture, Working Reagent Was Added To The Distal Water In The Blank, Stand And Controls Test Tubes And Incubated For 37°C For 3 Minutes. After Which The Phenol Standard Was Added To The Stand And Then The Samples Were Added To The Test Tubes And Incubated At 37 °C For 15 Mins. After 15 Mins Colour Reagent Was Added And The Absorbance Was Red Using Spectrophotometer. Intensity Of The Colour Formed Is Directly Proportional To Activity Of ALP Present In The Sample.

Total ALP Activity In Each Group Was Estimated Using The Formula:

$$\text{Total ALP Activity In K.A Units} = \frac{\text{Abst} - \text{Absc}}{\text{Abst} - \text{Absc}} \times 10.$$

Determination Of Acid Phosphatase Activity:

After Homogenizing The Mixture, Working Reagent Was Added To The Distal Water In The Blank, Stand And Controls Test Tubes And Incubated For 37°C For 3 Minutes. After Which The Phenol Standard Was Added To The Stand And Then The Samples Were Added To The Test Tubes And Incubated At 37 °C For 60 Mins. After 60 Mins Colour Reagent Was Added And The Absorbance Was Red Using Spectrophotometer. Intensity Of The Colour Formed Is Directly Proportional To Activity Of ACP Present In The Sample.

Total ALP Activity In Each Group Was Estimated Using The Formula:

$$\text{Total ACP Activity In K.A Units} = \frac{\text{Abst} - \text{Absc}}{\text{Abst} - \text{Absc}} \times 5.$$

Statistical Analysis:

Data Was Presented As Mean And Standard Deviations. Data Analysis Was Performed By Using SPSS As Software For Statistics. For Comparison Between Groups Post Hoc ANOVA Test Was Used. Probabilities Of Less Than 0.05 Were Accepted As Significant.

III. Result

The Results Showed That GCF ALP And ACP Activity Was Higher In Patients With Gingivitis And Periodontitis When Compared With The Control. The ALP Activity Was Highest In Patients With Periodontitis Where As The ACP Activity Was Highest In Gingivitis Group .

Table No 1 : Comparison Between Experimental Group And Study Variable.

Groups	Number	Particulars	ALP Activity (Mean Values)	ACP Activity (Mean Values)
Group – 1 Clinically Healthy Periodontium	20	Mean	5.89	1.48
Group – 2 Gingivitis	20	Mean	12.1	3.68
Group – 3 Periodontitis	20	Mean	13.64	3.34

Table No 2: Correlation Between Plaque Index With ALP And ACP

Groups	Number	Particulars	Plaque Index	ALP (K.A. Units)	ACP (K.A. Units)	ALP Activity (K.A.UNITS) Significance	ACP Activity (K.A.UNITS) Significance
Group – 2 Gingivitis	20	Mean	1.9900	12.10	3.68	0.034 (S)	0.320 (NS)
Group – 3 Periodontitis	20	Mean	2.3260	13.68	3.34	0.037 (S)	0.314 (NS)

P - Value < 0.05 Statistically Significant

Table No 3: Correlation Between Gingival Bleeding Index With ALP And ACP

Groups	Number	Particulars	Gingival Bleeding Index	ALP (K.A. Units)	ACP (K.A. Units)	ALP Activity (K.A.UNITS) Significance	ACP Activity (K.A.UNITS) Significance
Group – 2	20	Mean	2.0380	12.10	3.68	0.035 (S)	0 .333 (NS)

Gingivitis							
Group – 3 Periodontitis	20	Mean	2.3260	13.68	3.34	0.037 (S)	0.314 (NS)

P - Value < 0.05 Statistically Significant

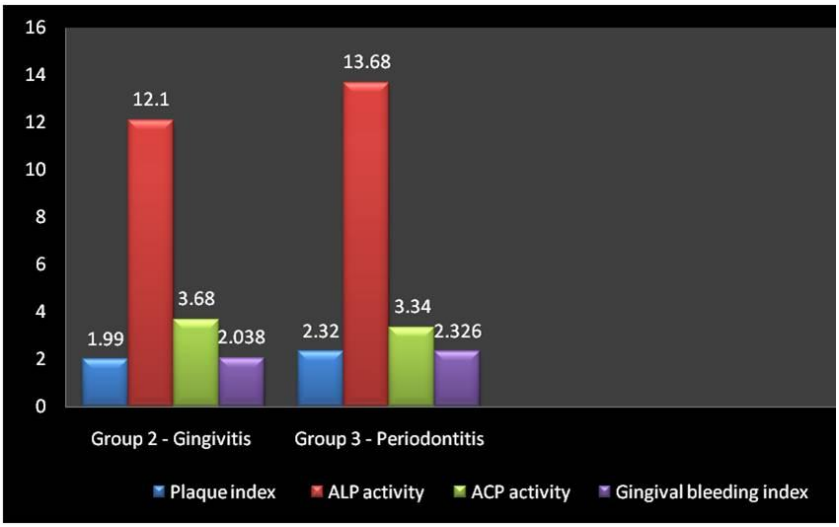

Figure 1: Bar Chart Which Compares The ALP And ACP Activity, Plaque And Gingival Bleeding Index Values Among Gingivitis And Periodontitis Groups.

IV. Discussion

Periodontitis Is One Of The Major Threats To Oral As Well As To Overall Health. The Process Involved In The Destruction Of The Periodontium Is Highly Complex And Vast Ranges Of Biological Substances Are Involved [23].This Study Was Done To Evaluate The Level Of Hydrolytic Enzymes ALP And ACP As Potential Biochemical GCF Markers Among Chronic Periodontitis Patients.This Comparative Study Was Done Among 60 Subjects In The Department Of Periodontology At S.R.M Dental College, Chennai. Systemically Healthy Male Patients Without Any Habits Were Included In This Study In Order To Prevent The Gender Difference Report As Well As The Influence Of Risk Elements Effect On ALP And ACP Activity.

ALP And ACP Are Intracellular Enzymes Present In Most Of The Tissues, And Organs Particularly In Bones. The Mean ALP Levels Of Group 1(Clinically Healthy Periodontium) Was Found To Be Lower Than Other Two Groups (Gingivitis And Periodontitis).In Support Of This Finding, Lilja E Et Al Et Al 1984 [24] Have Shown Through Histochemical Techniques That ALP Is Present In The Normal Periodontal Membrane In Rats. Lindhe J Et Al 2008 [25] Also Stated That Certain Leukocytes Can Be Seen In Junctional Epithelium Of Healthy Gingiva .This Supports The Presence Of ALP And ACP Even In Clinically Healthy Periodontium In The Present Study.

The Results Of The Present Study Shows That The Level Of ALP And ACP Are Significantly Elevated In Group 2 (12.10 K.A Units , 3.68 K.A Units) Group 3 (13.68 K.A Units , 3.34 K.A Units) When Compared To The Clinically Healthy Subjects (Group 1) Which Signifies The Value Of ALP And ACP As A Markers Of Periodontal Inflammation. Figure 1 Showed That Periodontitis Patients Had Significantly Higher Level Of ALP Activity Which Was In Agreement With The Studies Conducted By Chapple Et Al 1994 [26], Totan Et Al 2006 [27] . Table 2 And 3 Shows A Positive Correlation Between The ALP And ACP Activity And Values Of The Gingival Index And Periodontal Disease Index Which Is In Concordance With The Study Done By Todorovic Et Al 2006 [28]. The Possible Explanation For The Increase In The Level Of ALP In Group 2 And Group 3 Is That During The Progression Of The Periodontal Disease, Enzymes Are Released From The Pmnls, Inflammatory, Epithelial And Connective Tissue Cells And Also From The Dead And The Dying Cells Of The Periodontium Of The Affected Sites [29]. As Pmnls Are The Major Source Of ALP, They Could Have Contributed To The Increased Levels Of ALP In GCF Through Secondary Granules Release [26]. Microorganisms Like Prevotella Intermedia And Streptococcus Sanguis Also Shows Higher ALP Activity [30]. Thus Increase In Activity Of ALP In Group 2 (Gingivitis) Could Be Due To Increase In The Number Of Pnmls And Bacteria In The Gingival Sulcus.

Among The Three Groups The Mean Value Of ALP Is Highest In The Periodontitis Group (Group 3). Periodontal Pockets Are Chronic Inflammatory Lesions And Are Constantly Undergoing Repair. Complete Repair Does Not Occur Because Of Persistent Irritant And These Irritant Continues To Stimulate Fluid And Cellular Exudates Which In Turn Causes Degeneration Of New Tissue Elements. Apart From Increase In The

Number Of Pmnls And Bacterial Activity In Periodontitis, One Of The Major Mechanism Of Collagen Loss Is That Fibroblast Phagocytise Collagen Fibres. Increase In Fibroblast Activity Contributes To The Total ALP Level [29,30]. Both Histochemical And Biochemical Studies Have Shown That Periodontal Cells Have Intense ALP Activity, Unlike Gingival Cells .This Could Be The Possible Reason For The Highest Level Of ALP Activity In Group 3 When Compared To The Other Groups.

Chronic Periodontitis Is Associated With Elevated Levels Of Systemic Inflammatory Markers And Production Of Several Lysosomal Enzymes. Acid Phosphatase Is An Intracellular Enzyme Present In Most Of The Tissues And Is An Indicator Of Increased Cellular Damage In The Soft Tissue Of Periodontium And Inflamed Gingival Tissues .Clinical And Microbiological Studies Have Identified Increased Activity Of ACP Might Be A Consequence Of Destructive Processes In Alveolar Bone And Associated With Advance Stage Of Periodontal Disease. In The Present Study Acid Phosphatase Activity Had Non- Significant Difference Between Periodontitis And Gingivitis Patients Which Is In Contrast With The Study Done By Pushparani DS Et Al 2015 [31] Where The Level Of ACP Enzyme In The Gingival Tissue Correlated With The Severity Of Periodontitis .

V. Conclusion

Within The Limitation Of This Study It Can Be Concluded That GCF Level Of Alkaline Phosphatase Was Significantly Higher In Patients With Gingivitis And Periodontitis And Highest In Periodontits Group. Thus ALP Can Be Considered As A Potential Biomarker For The Detection And Progression Of Periodontal Disease Where As The Evaluation Of Acid Phosphatase Does Not Seem To Have Any Significant Diagnostic Value.

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