

Evaluation of 8-Hydroxy Deoxyguanosine Levels, as a Marker of Oxidative Stress in Chronic Gingivitis and Chronic Periodontitis Individuals During the Course of Phase 1 Periodontal Therapy in Comparison with Periodontally Healthy Individuals.

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Abstract:

Aim: The Aim Was To Evaluate The Levels Of 8-Hydroxy Deoxyguanosine Levels, As A Marker Of Oxidative Stress In Chronic Gingivitis And Chronic Periodontitis Individuals During The Course Of Phase 1 Periodontal Therapy In Comparison With Periodontally Healthy Individuals.

Materials And Method: All The Individuals Were Divided Into 3 Groups Each Including 16 Subjects. Group 1 Included Healthy Subjects And Group 3 And 4 Included Subjects With Chronic Gingivitis And Chronic Periodontitis Respectively. Saliva Samples Were Collected And The 8-OHdg Levels Were Assessed.

Results: The Levels Reduced To 1.59 ± 0.50 In Chronic Periodontitis Group One Month After Scaling And Root Planing. A Statistically Non-Significant Value Of 0.09 Was Recorded. The Levels In Chronic Gingivitis Group Was 1.20 ± 0.89 .

Conclusion: From The Above Study It Can Be Concluded That 8-OHdg Is An Important Biomarker Of Oxidative Stress And Is Increased In Patients With Chronic Gingivitis And Chronic Periodontitis.

Key Words: 8-Hydroxy Deoxyguanosine, Gingivitis, Periodontitis

Introduction

Periodontal Disease (PD) Is A Chronic Inflammatory Disorder That Affects 10–15% Of The World Population And Is Considered The Greatest Cause Of Tooth Loss, Causing Damage To All The Structures That Support The Teeth: Periodontal Ligament, Root Cement, Alveolar Bone, And Gingival Tissues^{1,2,3,4}. Periodontal Disease Is The Result Of Complex Interactions Between Mechanisms Of Host Defence And Periodontal Pathogens Leading To Tissue Damage.¹

The Disease Progression Depends On Interaction Between The Host And Dental Plaque. Host Cells Respond To Dental Plaque Laden With Microbes By Both Cellular And Humoral Defence Mechanisms.⁵ In Response To Plaque, Polymorphonuclear Neutrophils Produce Increased Number Of Free Radicals Which Can Damage Cell Membranes And Associated Biomolecules. Thus, In Periodontitis, Polymorphonuclear Neutrophils Play A Vital Role In The Initial Host And Inflammatory Response To The Periodontal Pathogens.⁶ This Interaction Enhances Oxidative Stress During Periodontitis. Usually The Oxidative Stress That Includes DNA Damage Is The Derivative Of The Oxidised Nucleosides That Is Excreted In Body Fluids During DNA Repair.² As Oxidative Stress Increases, Neutrophils At The Site Increase And, Is Associated With Macrophages, Which Produce Cytosines Such As Tumour Necrosis Factor Alpha (TNF-A), Interleukin-1 (IL-1), And Prostaglandins.¹ During This Inflammatory Process, Fibroblasts Are Stimulated By Interleukin-1 And Extracellular

Matrix Metalloproteinases (Mmps) Are Secreted, Particularly Collagenase Produced By Polymorphonuclear Neutrophils. These Matrix Metalloproteinases (Mmps) Cause Collagen Degradation And TNF-A Is Responsible For Increased Osteoclast Activity, Leading To Bone Resorption.⁷ In Addition, Tlymphocytes Secrete The Receptor Activator Of Nuclear Factor K β Ligand (RANKL), Which, In Turn, Is Involved In Osteoclast Activity, Resulting In Bone Loss. Polymorphonuclear Lymphocytes (Pmnl) Are Believed To Produce Active Reactive Oxygen Species (ROS).³

8-OHdg Is Commonly Used As A Marker To Evaluate Oxidative Damage In Chronic Inflammatory Diseases.⁸ At Present, 8-OHdg Is One Of The Most Commonly Used Markers For Evaluating Oxidative Damage In The Periodontal Tissue.⁴ Further, In Patients With Periodontitis, There Is An Increase In Concentration Of 8-OHdg In The Gingival Blood And Reduction In The Level Of Antioxidants (Konopka Et Al., 2007).⁹

Materials And Method

Individuals Visiting The Out-Patient Department Of Post Graduate Clinic Of Department Of Periodontology, Bharati Vidyapeeth (Deemed To Be University) Dental College And Hospital, Pune Will Be Explained The Need And Design Of The Study. 48 Individuals Who Are Willing To Participate Will Be Included In The Study And An Informed Written Consent Would Be Obtained. A Detailed Case History Of All 48 Individuals Will Be Recorded. A Precise Oral Examination Will Be Carried Out With Proper Illumination Using Mouth Mirror And UNC-15 Probe Calibrated In Millimetres. A Special Proforma Will Be Designed So As To Have A Systematic And Methodical Recording Of All The Observations Of The Study. A Precise Oral Examination Will Be Carried Out With Proper Illumination Using Mouth Mirror And UNC-15 Probe Calibrated In Millimetres. A Special Proforma Will Be Designed So As To Have A Systematic And Methodical Recording Of All The Observations Of The Study.

Study Design

Inclusion Criteria:

1. Systemically Healthy Individuals.
2. Individuals Between Age Group Of 20-50 Years
3. 16 Individuals With Healthy Periodontium
4. 16 Individuals With Generalized Chronic Gingivitis (Presence Of Bleeding On Gentle Probing)
5. 16 Individuals With Generalized Chronic Periodontitis (Probing Depth Of > 5mm).

EXCLUSION CRITERIA:

1. Individuals With History Of Smoking In The Past 6 Months
2. Individuals With History Of Use Of Vitamins Or Antioxidant Supplement.
3. Individuals With History Of Diabetes, Rheumatoid Arthritis
4. Individuals With History Of Periodontal Therapy In Past 6 Months.
5. Pregnant And Lactating Mothers
6. Individuals With History Of Use Of Antibiotics, Steroids Or Non-Steroidal Anti-Inflammatory Agents In Past 6 Months.

Study Group Individuals Participating In The Study Will Be Divided Into 3 Groups:

Group 1: Periodontally Healthy Individuals

Group 2: Generalised Chronic Gingivitis

Group 3: Generalised Chronic Periodontitis

Sampling Technique:

This Is A Single Centre Prospective Short-Term Study Was Of 1 Month's Duration And Comprised Of 16 Patients With Clinically Healthy Periodontium (Control Group) And 16 Patients With A History Of Chronic Gingivitis And 16 Patients With A History Of Chronic Periodontitis Who Received Scaling And Root Planning. The Same Experienced Examiner Recorded All Clinical Parameters To Ensure An Unbiased Evaluation Measurement With A UNC-15 (Hu-Friedy, Chicago, Illinois, USA) Manual Probe. The Patient Reporting To The Department Was Explained The Entire Procedure Of The Study And A Detailed Case History Was Recorded. The Patients Bleeding On Probing And Pocket Probing Depth

Was Recorded Using UNC -15 Probe. The Saliva Sample Of All The Patients Were Collected In Eppendorf Tubes Immediately After Scaling And Root Planning. For 8-Ohdg Assessment, Saliva Samples Were Collected From The Subjects At The Time Of Clinical Examination (Group 1, Group 2 And Group 3). Patients Were Seated With Head Slightly Down And They Were Asked Not To Swallow Or Move His/Her Tongue Or Lips During The Collection Period. After Clinical Examination, Paraffin Wax-Stimulated 2 MI Of Whole Saliva Was Collected In A Quiet Room Between 9:00 – 10 AM, At Least 8 Hours After Intake Of Food, By Expectorating Into Eppendorf Tubes From Control Group At Baseline And From The Test Group At Baseline. The Saliva Sample Was Again Collected At 1 Months After Scaling And Root Planning That Were Performed By Using Ultrasonic Scalers And Area-Specific Curettes. Saliva Samples Were Immediately Centrifuged At 10,000 × G For 10 Minutes To Remove Cell Debris And The Supernatant (50 µl Each) Was Stored At -80° C Until Analysed. The Supernatant Was Used To Determine 8-Ohdg Levels With A Commercially Available ELISA Kit (Highly Sensitive 8-Ohdg Check). The 8-Ohdg ELISA Kit Is A Competitive In Vitro Enzyme-Linked Immunosorbent Assay For Quantitative Measurement Of The Oxidative DNA 8- Ohdg. The Determination Range Of 8-Ohdg Levels Was 0.125 To 200 Ng/MI. Salivary Samples From Different Groups As Well As Different Time Points Were Tested At The Same Time On The Same Plate Using The Manufacturer's Instructions. The Operator Performing The ELISA Test Was Different From The Operator Who Collected The Salivary Samples In Order To Eliminate Bias.

Statistical Analysis

The One-Way ANOVA Test Was Applied To Test The Normality Of Data The Data Were Normally Distributed And Quantitative In Nature, Parametric Tests Were Applied. Differences In 8-Ohdg Levels, PPD, BOP And CAL Before And After Initial Periodontal Treatment And Between Test And Control Groups At Baseline Were Done By Unpaired And Paired T-Test. A P Value ≤ 0.01 Was Considered Statistically Significant. An SPSS Version 21 Software Program Analysed All The Data.

Results

Salivary 8-Ohdg Levels:

Mean Salivary 8-Ohdg Levels At Baseline Was Recorded In Healthy Gingiva Is 2.05±0.73 And The Level In Chronic Gingivitis Group Is 3.83±0.66. The Levels Were Higher In Chronic Periodontitis Group Which Was Recorded To Be 5.72±0.56. (TABLE1)

On Intergroup Comparison Of 8-Ohdg Levels Pre And Post Treatment The Levels Reduced To 1.20±0.89 And 1.59±0.50 In Chronic Gingivitis And Chronic Periodontitis Post Phase 1 Periodontal Therapy. Statistically Non-Significant Value Of 0.09 Was Recorded After Treatment In Both The Groups. (TABLE 2) (GRAPH 1).

On Intragroup Comparison Of 8-Ohdg Levels The Above Table Shows Intergroup Comparison In The Levels Of 8-Ohdg Pre-Treatment And Post-Treatment. The Levels Of 8-Ohdg In Chronic Gingivitis Group After 1 Month Of Scaling Was Recorded As 1.20±0.89. The Levels Of 8-Ohdg In Chronic Periodontitis Group Was Recorded As 1.59±0.50. A Statistically Non-Significant Value Of 0.09 Was Obtained. The F-Value Recorded Was 15.56. (TABLE 3)

Bleeding On Probing-

Mean BOP In Clinically Patients With Chronic Gingivitis Was 10.43± 0.94ng/MI And Chronic Periodontitis Patients (Test Group) Was 90.66 ± 4.92 At Baseline. Thus, At Baseline Mean BOP Was Significantly Higher In The Test Group (P ≤ 0.01). On Intergroup Comparison Mean BOP In The Chronic Gingivitis Group At 4 Weeks After Scaling And Root Planing Was 5.76 ± 0.64 Which Was Statistically Lower Than At Baseline (P ≤ 0.01) (TABLE 4). Mean BOP In Chronic Periodontitis Group At 4 Weeks After Scaling And Root Planing Was 5.90±1.65 Which Was Statistically Lower Than At Baseline And Showed Significant Difference Of 0.08 (TABLE 4). The F- Value Before Treatment Recorded Was 12.18 And Post- Treatment 13.91. The Pre-Treatment Values Of Bleeding On Probing In Chronic Gingivitis Was 10.43 ± 0.94 And The Values Significantly Reduced After 4 Weeks Of Scaling With The Values Of 5.76± 0.64. A Statistically Significant Difference Was Obtained Of P-

Value ≤ 0.03 After Treatment As Compared To Pre-Treatment Value ≤ 0.01 . The Pre-Treatment Values Of Bleeding On Probing In Chronic Periodontitis Group At Baseline Was 90.66 ± 4.92 And The Values Reduced Significantly After 4 Weeks Of Scaling, It Was Recorded To Be 5.90 ± 0.95 With The P-Value Of 0.03 (TABLE 5) (GRAPH 2). Statistically Significant Difference Of 0.03 Was Obtained.

Pocket Probing Depth-

On Intergroup Comparison Mean PPD In Chronic Gingivitis (Group 2) Was 3.01 ± 0.65 Mm And In Chronic Periodontitis Patients (Test Group) Was 5.26 ± 0.91 mm At Baseline. The Difference Between The Two Groups Was Statistically Significant, With Greater PPD Observed In The Test Group ($P \leq 0.01$). Mean PPD In The Chronic Gingivitis Group Following Scaling And Root Planing At 4 Weeks Was 2.70 ± 0.35 Mm. Mean PPD In Chronic Periodontitis Was 1.95 ± 0.95 . Therefore, A Significant Reduction In PPD Was Observed In The Both Group After Scaling And Root Planing As Compared To Baseline ($P \leq 0.01$). The P-Value Post-Treatment Was 0.27 Which Is Statistically Non-Significant (TABLE 6). On Intragroup Comparison The Pocket Probing Depth Values Before Treatment For Chronic Gingivitis Group Was 3.01 ± 0.94 With Statistically Significant Value Of 0.01. The Values Reduced To 1.65 ± 0.47 . The Pocket Probing Depth Values For Chronic Periodontitis Before At Baseline 5.26 ± 0.91 And The Values Reduced To 1.95 ± 0.95 Which Appears To Be Statistically Significant With The P Value Of ≤ 0.01 (TABLE 7) (GRAPH 3).

Discussion

In The Present Study Salivary Levels Of 8-OHdg Were Recorded In Healthy Patients, And Chronic Gingivitis And Chronic Periodontitis. Oxidative Stress Due To The Formation Of ROS, Which Is Stimulated By PMNL Through Oxygen-Related Mechanisms, Causes Periodontal Tissue Damage.⁹ The Increase In ROS Formation, The Reduction Of Antioxidant Enzyme Levels, And The Defects In DNA Restoration Mechanism Increase The Risk For Oxidative DNA Damage. Conditions That Lead To An Increase Of Oxidative Stress Cause Early Oxidative Damage On DNA Molecules And ROS Has Been Shown To Induce Excessive DNA Damage In Periodontal Tissues.⁹

The Salivary Levels Of 8-OHdg Was Evaluated In Subjects With Chronic Gingivitis And Chronic Periodontitis And The Levels Were Significantly Higher In Subjects With Chronic Periodontitis.¹⁰ The Parameters Bleeding On Probing And Pocket Probing Depth Were Also Recorded In Chronic Gingivitis And Chronic Periodontitis Group. The Levels Were Higher In Both The Groups At Baseline Which Reduced After Treatment. The Levels In Chronic Periodontitis Group Was Still High After Treatment Which Revealed That The Inflammation Was Higher As Compared To Control Group. These Results Are In Accordance With The Study Done By Takane Et Al In 2005.¹⁰ Also, The 8-OHdg Levels Were Significantly Higher In The Whole Saliva Of Subjects With Periodontitis Than In Subjects With Healthy Periodontium. Another Study Conducted By Takane Et Al In 2005 It Was Evaluated For The First Time 8-OHdg Levels In Whole Saliva Of Patients With Periodontitis And Assessed Changes After Initial Periodontal Treatment.¹⁰ This Study Indicated That 8-OHdg Levels In Saliva Appear To Reflect The Status Of Periodontal Health. The Patients In The Chronic Periodontitis Group Are Individuals With Generalized Destruction. Higher BOP Levels In The Chronic Periodontitis Group After Treatment Show That Inflammation In The Pocket After Treatment Is Still Higher Than The Control Group.

Rai Et Al. Evaluated That Decreased Salivary Level Of 8-OHdg After 3 Weeks After SRP. 8-OHdg Levels Were Significantly Higher In Patients With Periodontitis Compared To Gingivitis And Measurement Of 8-OHdg Was In Identifying Periodontal Disease Which Is Similar To The Post Treatment Values Of Our Present Study.¹¹

Saliva Is Easy To Collect And A Useful Biomarker, But Its Flow Rate, Amount, And Contents May Change Because Of Many Factors. For Example, Stimulating Saliva Flow Has Been Demonstrated To Increase Saliva Volume And Disrupt The Concentration. In A Study It Was Reported That In Chronic Periodontitis Patient The Levels Of 8-OHdg, Porphyromonas Gingivalis And Tannerella Forsythia In Saliva Were Higher Than In Healthy Individual.¹² A Statistical Correlation Between The 8-OHdg And P. Gingivalis Levels In Saliva Has Been Reported.¹³ The Salivary Levels Of 8-OHdg Were

Reported To Be Significantly Higher In Patients Positive For Streptococcus Anginosus Than In Patients Negative For The Bacterium And A Significant Decrease In S. Anginosus And 8-OHdg Levels Were Observed After Initial Periodontal Treatment.¹³

This Study Is In Accordance With Takane Et Al Where There Was Significant Reduction In Pocket Probing Depth After 2 And 4 Weeks Of Initial Periodontal Therapy.⁹

From The Overall Results It Can Be Seen That Salivary Levels Of 8-OHdg Can Be Used As A Biomarker To Assess The Progression Of Periodontal Disease. Periodontal Therapy Decreases Oxidative Stress Biomarkers, And The Levels May Be Similar To Those Found In Periodontally Healthy Individuals.^{14,15} An Increasing Number Of Studies Report A Correlation Between Periodontal Tissue Initiation And Development And Increased Levels Of ROS. Hence One Of Most Used Biomarkers To Evaluate Oxidative Damage In Both Local And Systemic Disorders Is 8-OHdg.^{16,17,18}

Conclusion

The Following Conclusions Can Be Drawn:

- 1) Oxidative Stress Plays An Important Role In The Pathology Of Periodontitis;
- 2) 8-OHdg GCF Levels Can Be A Biomarker To Detect DNA Damage Caused By Oxidative Stress;
- 3) 8-OHdg Is Important And May Reveal The Severity Of Periodontal Disease And The Effect Of Periodontal Therapy

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TABLES

TABLE 1: Intergroup Comparison At Baseline Between The 3 Groups

8OHD	N	Mean±SD	F-Value	P-Value
Group 1	16	2.05±0.73	123.61	0.01*
Group 2	16	3.83±0.66		
Group 3	16	5.72±0.56		

P≤0.05 *Significant Difference

TABLE 2: Intergroup Comparison In 8OHG Pretreatment And Post Treatment Groups

Post Treatment	N	Mean±SD	F-Value	P-Value
Group 2	16	1.20±0.89	15.56	0.09
Group 3	16	1.59±0.50		

P≤0.05 *Significant Difference

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TABLE 3: Intragroup Comparison Of 8-OHDG Pretreatment And Post Treatment

Group 2	N	Mean±SD	P- Value
Pre-Treatment	16	3.83±0.66	0.01*
Post Treatment	16	1.20±0.89	
Group 3			
Pre-Treatment	16	5.72±0.56	0.01*
Post Treatment	16	1.59±0.50	

P≤0.05 *Significant Difference

TABLE 4: Inter Group Comparison Of BOP In Pre -Treatment And Post- Treatment Groups

Pre-Treatment	N	Mean±SD	F-Value	P-Value
Group 2	16	10.43±0.94	12.18	0.01*
Group 3	16	90.66±4.92		

Post Treatment				
Group 2	16	5.76±0.64	13.91	0.08*
Group 3	16	5.90±1.65		

P≤0.05 *Significant Difference

TABLE 5: Intragroup Comparison Of BOP Pre-Treatment And Post-Treatment:

Group 2	N	Mean±SD	P- Value
Pre-Treatment	16	10.43±0.94	0.01*
Post Treatment	16	5.76±0.64	
Group 3			
Pre-Treatment	16	5.26±0.91	0.03*
Post Treatment	16	5.90±0.95	

P≤0.05 *Significant Difference

TABLE 6: Inter Group Comparison Of PPD In Pretreatment And Post Treatment Groups

Pre-Treatment	N	Mean±SD	F-Value	P-Value
Group 2	16	3.01±0.65	2.89	0.01*
Group 3	16	5.26±0.91		
Post Treatment				
Group 2	16	1.65±0.47	2.70	0.27
Group 3	16	1.95±0.95		

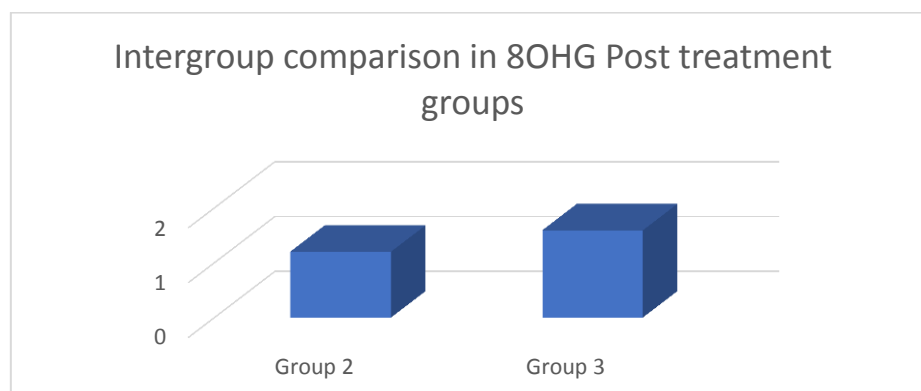
P≤0.05 *Significant Difference

TABLE 7: Intragroup Comparison Of PPD Pre- Treatment And Post- Treatment:

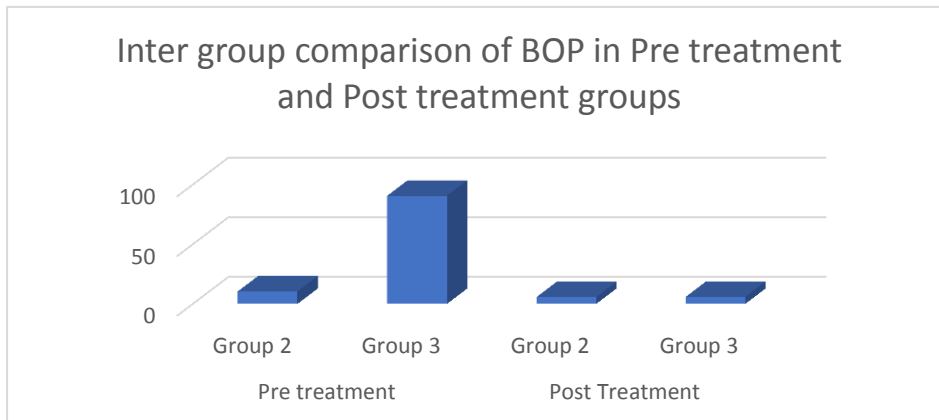
Group 2	N	Mean±SD	P- Value
Pre-Treatment	16	3.01±0.65	0.01*
Post Treatment	16	1.65±0.47	
Group 3			
Pre-Treatment	16	5.26±0.91	0.01*
Post Treatment	16	1.95±0.95	

P≤0.05 *Significant Difference

GRAPH 1



GRAPH 2



GRAPH 3

