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Estimation of Gingival Crevicular Fluid levels of Uric Acid and Xanthine Oxidase in Patients Diagnosed with Chronic Periodontitis: A Prospective Interventional Study

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Abstract: ***Background: Periodontitis is a chronic inflammatory disease characterized by progressive destruction of the supporting structures of teeth. Oxidative stress plays a crucial role in this process, with reactive oxygen species amplifying tissue damage. Uric acid and xanthine oxidase, important oxidative mediators, have established roles in systemic inflammation but remain under-investigated in periodontitis. Aim: To evaluate gingival crevicular fluid (GCF) levels of uric acid and xanthine oxidase in patients with periodontitis before and after non-surgical periodontal therapy (NSPT). Methods: Twenty adult periodontitis patients underwent comprehensive clinical assessment and GCF sampling at baseline and three months post-NSPT. Biomarker levels were quantified using ELISA. Clinical parameters assessed included Plaque Index, Gingival Index, Bleeding on Probing, Probing Pocket Depth, and Clinical Attachment Level. Results: Significant reductions in GCF uric acid and xanthine oxidase levels were observed following NSPT ($p < 0.001$). Concurrently, all clinical periodontal parameters showed marked improvement ($p < 0.001$). Regression analysis highlighted Probing Pocket Depth and Bleeding on Probing as key predictors of biomarker concentrations. Conclusion: Uric acid and xanthine oxidase are integral to periodontitis pathogenesis and serve as treatment responsive biomarkers. Their significant decline in post-therapy highlights their potential as clinical indicators for monitoring disease activity and guiding personalized periodontal management.***

Keywords: Uric Acid, Xanthine Oxidase, Periodontitis, Nonsurgical Periodontal Therapy, Oxidative Stress, Reactive Oxygen Species

1. Introduction

Periodontitis is a prevalent chronic inflammatory disease characterized by progressive destruction of the supporting structures of the teeth, including the gingiva, periodontal ligament, cementum, and alveolar bone. While microbial dysbiosis initiates the condition, it is the host immune-inflammatory response that largely dictates disease progression and severity. Among the host-mediated mechanisms implicated in periodontal destruction, oxidative stress has emerged as a critical factor. Reactive oxygen species (ROS), produced in excess during inflammatory responses, contribute to the breakdown of extracellular matrix components and alveolar bone resorption, thereby amplifying tissue damage [1].

Uric acid (UA) and xanthine oxidase (XO) are two key molecules associated with oxidative stress and inflammatory processes. UA is the product of purine metabolism and acts as a physiological antioxidant under normal conditions. However, under pathological states such as chronic inflammation, uric acid can function as a pro-oxidant and is considered a danger-associated molecular pattern (DAMP), capable of activating the NLRP3 inflammasome and triggering the release of interleukin-1β and other pro-inflammatory cytokines. XO, an enzyme involved in uric acid production, contributes directly to oxidative stress by generating ROS, including superoxide anions and hydrogen peroxide, during purine degradation [2].

The role of UA and XO has been extensively studied in systemic diseases such as cardiovascular disorders and gout, but their relevance in periodontal pathogenesis remains inadequately explored. Given their potential contribution to oxidative burden and inflammatory amplification, these biomarkers may serve as indicators of disease activity and therapeutic response in periodontitis [2].

Non-surgical periodontal therapy (NSPT), including scaling and root planing, remains the primary modality for managing periodontitis. While it is known to improve clinical outcomes and reduce inflammatory mediators, its effect on oxidative stress-related biomarkers such as UA and XO in gingival crevicular fluid (GCF) is not well established [3].

The present study aims to evaluate GCF levels of uric acid and xanthine oxidase in patients with chronic periodontitis before and after NSPT, to investigate their potential as diagnostic and treatment-responsive biomarkers in periodontal disease. Understanding the dynamics of these oxidative stress biomarkers in response to therapy may provide novel insights into the molecular resolution of periodontal inflammation and support their potential role as diagnostic or prognostic indicators in periodontal care [3,4,5].

2. Materials and Methodology

2.1 Study design

This prospective, interventional clinical study was carried out in the Department of Periodontology, Faculty of Dental

Sciences, Ramaiah University of Applied Sciences, Bengaluru, India, over a period of 9 months.

2.2 Study Population

A total of 20 participants were recruited for the study based on sample size estimation derived from the methodology of Lakshmi Priya et al. (2022). Patients were screened at the Department of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bengaluru, India and whoever met the inclusion criteria were screened for the study Following initial screening, 20 individuals who met the inclusion and exclusion criteria were enrolled [6]. The sample size estimation was done by maintaining 95% Confidence Interval & the study power at 80%.

- Group 1: Before NSPT [At baseline]
- Group 2: After NSPT [At 3 months interval]

2.3 Inclusion and Exclusion criteria

Inclusion Criteria:

- Subjects with age group of 25–65 years of age [7]
- Subjects having a minimum of ≥20 natural teeth.
- PPD ≥5 mm, bleeding on probing sites with clinical signs of inflammation [8]
- More than 7 mm of clinical attachment loss [9]
- Loss of periodontal attachment 4-5 mm radiographic bone loss [10].

Exclusion Criteria:

- Individuals with a history of smoking [11]
- Pregnant women [12]
- Subjects suffering from systemic diseases that could affect periodontal status [13].
- Subjects with previous history of periodontal therapy in the last six months or were on medication affecting periodontal status were excluded from the study [14]

2.4 Ethical Considerations

Prior to commencement, ethical approval for the study was granted by the Institutional Ethics Committee. All participants were thoroughly informed about the study objectives, procedures, potential risks, and benefits. Written informed consent was obtained from each participant, ensuring voluntary participation in accordance with the ethical principles outlined in the Declaration of Helsinki [15].

2.5 Methodology

Comprehensive periodontal clinical examinations were performed at baseline and at 3 months following non-surgical periodontal therapy (NSPT). All assessments were conducted by a single calibrated examiner to minimize inter-examiner variability. Intra-examiner reliability was assessed prior to the study initiation using a kappa statistic, ensuring consistency in probing depth and attachment level measurements. Calibration of the examiner was performed prior to data collection by repeating measurements on 10 patients not included in the main study. A minimum intra-examiner agreement of >85% within ±1 mm was considered acceptable [16].

The following standardized periodontal parameters were recorded using a UNC-15 periodontal probe (Hu-Friedy, Chicago, IL, USA):

- P**laque Index (PI)**: Assessed according to the Silness and Löe criteria to evaluate the presence of supragingival plaque on the tooth surface.
- **Gingival Index (GI)**: Scored using the Löe and Silness index to assess gingival inflammation based on color, consistency, and bleeding on gentle probing.
- **Bleeding on Probing (BOP)**: Recorded as present or absent within 15 seconds of probing at each site and expressed as a percentage of total sites probed.
- **Probing Pocket Depth (PPD)**: Measured from the gingival margin to the base of the periodontal pocket in millimeters.
- **Clinical Attachment Level (CAL)**: Determined by measuring the distance from the cementoenamel junction (CEJ) to the base of the pocket.
- All measurements were recorded to the nearest millimeter. The full-mouth clinical assessment was completed in a single session at each time point. Oral hygiene practices were reinforced after the initial examination and during follow-up visits.

Following the acquisition of informed consent, eligible participants were enrolled in the study and assigned to two groups (Before NSPT & After NSPT). Comprehensive clinical evaluation was performed at baseline, including the measurement of key periodontal parameters such as Bleeding on Probing (BoP), Gingival Index (GI), Plaque Index (PI), Probing Pocket Depth (PPD), and Clinical Attachment Loss (CAL). These parameters were assessed using a calibrated Williams periodontal probe to ensure accuracy and consistency [17].

For gingival crevicular fluid (GCF) collection, the sites exhibiting the greatest probing pocket depth were identified. These sites were gently air-dried and isolated using sterile cotton rolls to minimize the risk of contamination from saliva. Once the sites were adequately isolated and dried, GCF collection was performed by positioning a calibrated microcapillary pipette or tubing at the entrance of the gingival sulcus [18,19]. GCF was collected by placing a calibrated microcapillary pipette at the entrance of the gingival sulcus, carefully avoiding contact with the marginal gingiva. Care was taken to avoid inserting the pipette into the sulcus, as this could provoke bleeding or induce an inflammatory response, thereby contaminating the sample [20]. The tip of the capillary tubing was gently placed in close proximity to the gingival margin, allowing the fluid to be drawn passively by capillary action without exerting pressure on the tissues [21]. The collected samples were immediately transferred into sterile, airtight plastic vials and stored at –80°C to preserve the integrity of the biomarkers until further biochemical analysis.

All participants received non-surgical periodontal therapy, including scaling and root planing, as part of the treatment protocol. After a three-month period following the initial therapy and baseline data collection, patients were recalled for follow-up [22].

At the three-month follow-up visit, clinical parameters (BoP, GI, PI, PPD, and CAL) were re-evaluated using the Williams

probe to assess the outcomes of the periodontal therapy. GCF samples were collected once again from the previously identified sites, following the same standardized procedure. These samples were also stored at –80°C until analysis.

The collected GCF samples from both baseline and the three-month follow-up were subsequently analyzed to determine the concentration of Uric acid and Xanthine oxidase. Quantification was carried out using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, (My BioSource) following the manufacturer's protocol to ensure precise and reliable results.

Gingival crevicular fluid (GCF) samples, standards, and reagents were equilibrated to room temperature (18–25°C) prior to assay setup to ensure uniform reagent performance. The ELISA was conducted using 96-well microplates pre-coated with specific monoclonal antibodies against uric acid and xanthine oxidase. Removable 8-well strips were labelled according to the assay plan. A total of 100 µL of either prepared standard or test GCF sample was added to each designated well, followed by incubation at room temperature for 2.5 hours on a gentle orbital shaker to facilitate optimal antigen-antibody interaction.

After the initial incubation, the wells were emptied and washed four times with 300 µL of 1X wash buffer using a multichannel pipette. Complete removal of wash solution was ensured by aspiration and blotting on lint-free absorbent paper. Subsequently, 100 µL of biotinylated detection antibody specific to each analyte was added to the wells, followed by 1 hour of incubation at room temperature with gentle agitation. A second identical washing step was performed to eliminate unbound antibodies.

Following this, 100 µL of horseradish peroxidase (HRP)-conjugated streptavidin was added to each well and incubated for 45 minutes at room temperature with gentle shaking to ensure binding to the biotinylated antibodies. The plate was then washed four times with 1X wash buffer to remove unbound conjugate.

For color development, 100 µL of TMB (3,3′,5,5′-tetramethylbenzidine) One-Step Substrate Reagent was dispensed into each well, and the plate was incubated in the dark at room temperature for 30 minutes with mild agitation. Finally, the enzymatic reaction was terminated by adding 50 µL of Stop Solution (0.2 M sulfuric acid), producing a yellow color. The absorbance was immediately recorded at 450 nm using a microplate reader [23].

2.6 Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analysis.

Descriptive Statistics: Descriptive analysis of all the explanatory and outcome parameters was done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables.

Inferential Statistics: Independent Student t Test / Mann Whitney Test was used to compare the mean clinical parameters and GCF levels of Uric Acid and Xanthine Oxidase between 2 groups at different time intervals [Before & After NSPT].

Student Paired t test / Wilcoxon signed Rank post hoc test was used to compare the mean clinical parameters and GCF levels of Uric Acid and Xanthine Oxidase between Before and After NSPT procedure in Periodontitis group.

Pearson's correlation test / Spearman's correlation test was used to assess the relationship between clinical parameters and GCF levels of Uric Acid and Xanthine Oxidase in each group.

Multiple Stepwise Linear Regression analysis was performed to predict the GCF levels of Uric Acid and Xanthine Oxidase using clinical parameters in healthy group and periodontitis group [Before & After NSPT].

The level of significance was set at $P<0.001$.

3. Results

The baseline demographic and clinical characteristics of the study population, including age distribution, sex, and initial periodontal status, are summarized in (Table 1). These data provide a comprehensive profile of the cohort prior to intervention and serve as the foundation for evaluating therapeutic outcomes following non-surgical periodontal therapy.

i) Demographic Data Analysis: Demographic evaluation revealed an equal number of male and female participants, thereby maintaining gender parity across the study population. Statistical analysis confirmed the absence of any significant gender-related differences among the groups, ensuring that gender distribution did not act as a confounding variable in the interpretation of the study outcomes. The age distribution of the study participants ranged between 30 to 56 years, with a calculated mean age of 42.40 ± 8.85 years, reflecting a representative cross-section of the adult population.

A stratified analysis of the age data indicated that the largest proportion of participants (55.0%, n = 11) belonged to the 30–40-year, age group. This was followed by 25.0% (n = 5) of participants in the 51–60, year range, and 20.0% (n = 4) within the age range of 41–50 years. (table no 1 and graph no 1&2).

Table 1: Age and Gender distribution among study subjects

Variable	Category	n	%
Age	30-40 yrs.	11	55.0%
	41-50 yrs.	4	20.0%
	51-60 yrs.	5	25.0%
		Mean	SD
	Mean	42.40	8.85
	Range	30 - 56	
Gender	Males	10	50.0%
	Females	10	50.0%

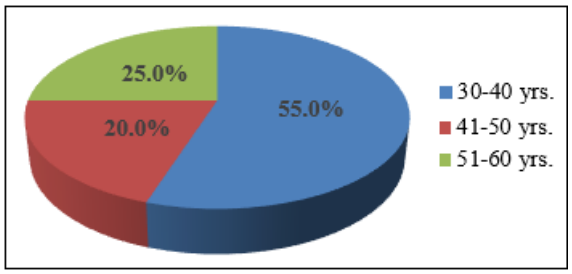


Figure 1: Distribution of study subjects based on their age groups

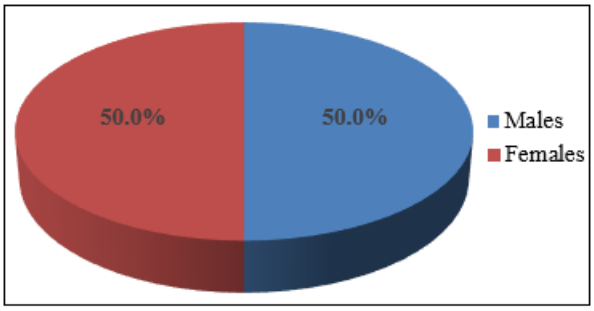


Figure 2: Distribution of study subjects based on their gender

The inclusion of participants across this broad age spectrum was intended to enhance the external validity of the study and ensure that the observed clinical outcomes were not unduly influenced by age-specific variables. The relatively uniform distribution across early to late adulthood supports the robustness of intergroup comparisons and minimizes the likelihood of age-related confounding effects.

ii) The inter group comparison of GCF levels of uric acid before and after NSPT: A statistically significant reduction in gingival crevicular fluid (GCF) uric acid levels was observed following non-surgical periodontal therapy (NSPT) over a three-month evaluation period. Prior to the initiation of treatment, the mean GCF uric acid concentration was recorded at 14,670.06 ng/ml, with a standard deviation of 4,386.11 ng/ml, post-treatment analysis revealed a marked decrease in mean uric acid levels to 8,372.77 ng/ml, accompanied by a reduced standard deviation of 2,058.68 ng/ml. (Table no 2 & Graph no 3)

Table 2: Comparison of mean GCF Uric Acid levels (in ng/ml) between Before & After NSPT (3 Months) using Paired Sample t Test

Parameter	Time	N	Mean	SD	Mean Diff	p-value
GCF Uric Acid	Before Rx	20	14670.06	4386.11	6297.29	<0.001*
	After Rx	20	8372.77	2058.68		

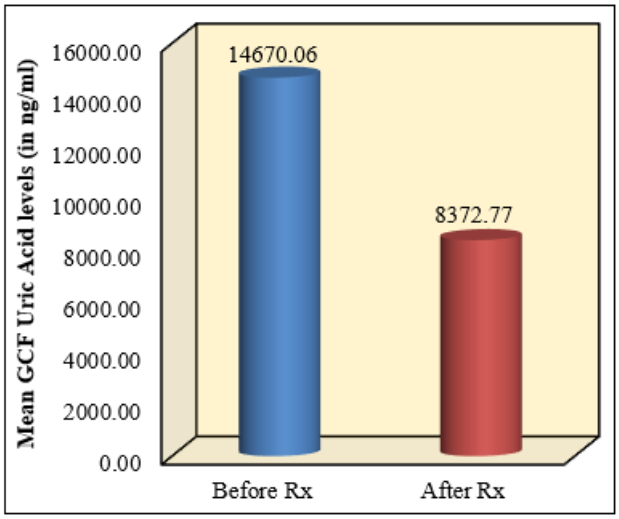


Figure 3: Mean GCF Uric Acid levels (in ng/ml) between Before & After NSPT (3 Months)

The absolute reduction in mean uric acid levels was 6,297.29 ng/ml. This change was found to be highly statistically significant, as indicated by a p-value of less than 0.001, thereby confirming the efficacy of NSPT in substantially lowering local biochemical markers of oxidative stress and inflammation in the periodontal milieu. These findings emphasize the therapeutic value of NSPT in modulating pathogenic biochemical pathways associated with periodontitis.

iii) The inter group comparison of GCF levels of xanthine oxidase before and after NSPT: A marked reduction in gingival crevicular fluid (GCF) xanthine oxidase levels was observed following non-surgical periodontal therapy (NSPT) over a three-month follow-up period. Baseline measurements revealed a mean xanthine oxidase concentration of 5.35 ng/ml, with a standard deviation of 0.68 ng/ml, reflecting a moderate degree of interindividual variability. Following NSPT, the mean levels significantly declined to 3.20 ng/ml, accompanied by a lower standard deviation of 0.43 ng/ml, indicating a more reliable response to therapy across the study population.

Table 3: Comparison of mean GCF xanthine oxidase levels (in ng/ml) between Before & After NSPT (3 Months) using Paired Sample t Test

Parameter	Time	N	Mean	SD	Mean Diff	p-value
GCF XDH	Before Rx	20	5.35	0.68	2.15	<0.001*
	After Rx	20	3.20	0.43		

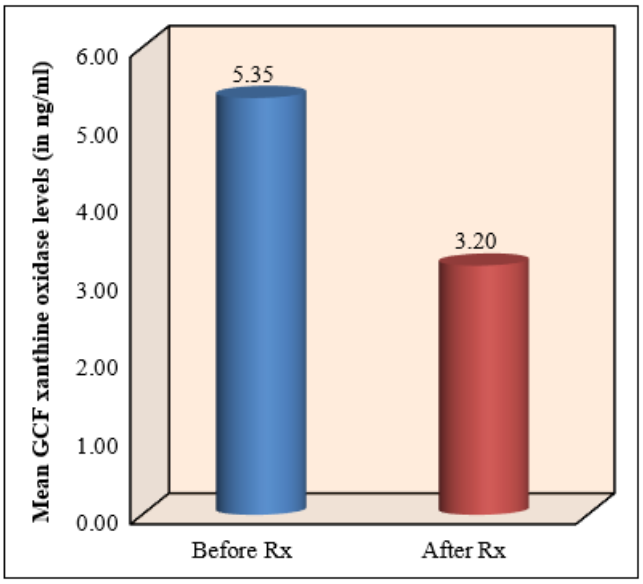


Figure 4: Mean GCF xanthine oxidase levels (in ng/ml) between Before & After NSPT (3 Months)

The absolute reduction in mean xanthine oxidase concentration was 2.15 ng/ml. This change was found to be highly statistically significant, with a p-value of less than 0.001, (table no 3, graph no 4) thereby confirming the efficacy of NSPT in attenuating local oxidative stress as reflected by decreased xanthine oxidase activity in the GCF. These findings further support the therapeutic potential of NSPT in modulating key biochemical markers implicated in periodontal pathogenesis.

iv) The inter group comparison of clinical parameters: The clinical parameters exhibited substantial improvements following NSPT over the three-month period as depicted in Table no 5.

The Plaque Index showed a marked reduction, with mean values decreasing from 2.33 ± 0.39 before treatment to 1.43 ± 0.37 after NSPT, with a mean difference of 0.90 and a highly significant p-value of less than 0.001. (Graph no 5)

Similarly, the Gingival Index demonstrated a considerable decline, shifting from a mean of 2.41 ± 0.38 before treatment to 1.34 ± 0.45 after NSPT, with a mean difference of 1.07, reflecting a statistically significant improvement. (Graph no 6)

Bleeding on Probing also reduced notably, with the mean value decreasing from 36.40 ± 5.84 before NSPT to 24.25 ± 5.68 post-treatment, demonstrating a mean difference of 12.15 and a p value of less than 0.001, indicating significant changes. (Graph no 7)

Probing Pocket Depth followed a similar trend, showing a reduction from a mean of 5.60 ± 0.75 before NSPT to 4.35 ± 0.75 after treatment, with a mean difference of 1.25, highlighting significant periodontal improvements. (Graph no 8)

Clinical Attachment Level reported a decrease in mean values from 3.60 ± 0.88 before NSPT to 2.45 ± 0.76 after treatment, resulting in a mean difference of 1.15, which was statistically significant. (Graph no 9)

Overall, these findings strongly suggest that NSPT led to considerable improvements in clinical parameters, reflecting enhanced periodontal health through significant reductions in plaque accumulation, gingival inflammation, bleeding, pocket depths & CAL.

Table 4: Comparison of mean values of clinical parameters between Before & After NSPT (3 Months) using Paired Sample t Test

Parameter	Time	N	Mean	SD	Mean Diff	p-value
PI	Before Rx	20	2.33	0.39	0.90	<0.001*
	After Rx	20	1.43	0.37		
GI	Before Rx	20	2.41	0.38	1.07	<0.001*
	After Rx	20	1.34	0.45		
BOP	Before Rx	20	36.40	5.84	12.15	<0.001*
	After Rx	20	24.25	5.68		
PPD	Before Rx	20	5.60	0.75	1.25	<0.001*
	After Rx	20	4.35	0.75		
CAL	Before Rx	20	3.60	0.88	1.15	<0.001*
	After Rx	20	2.45	0.76		

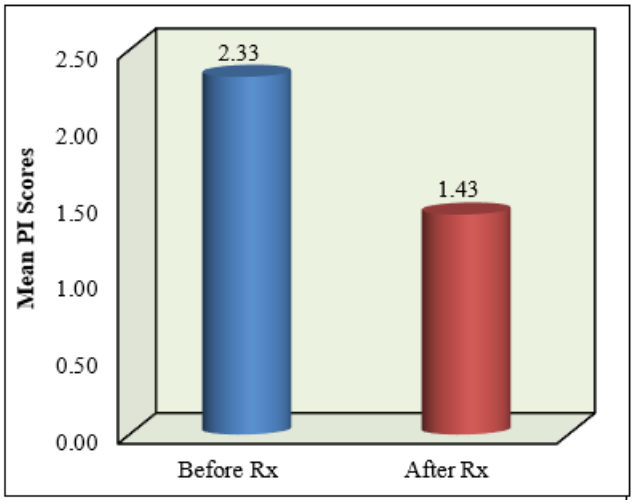


Figure 5: Mean PI Scores between Before & After NSPT (3 Months)

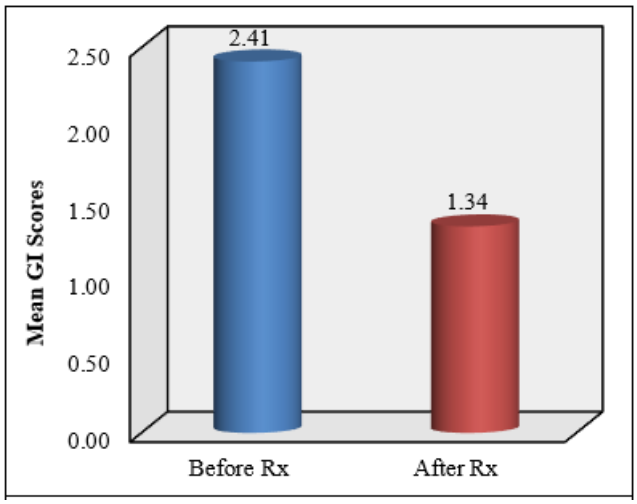


Figure 6: Mean GI Scores between Before & After NSPT (3 Months)

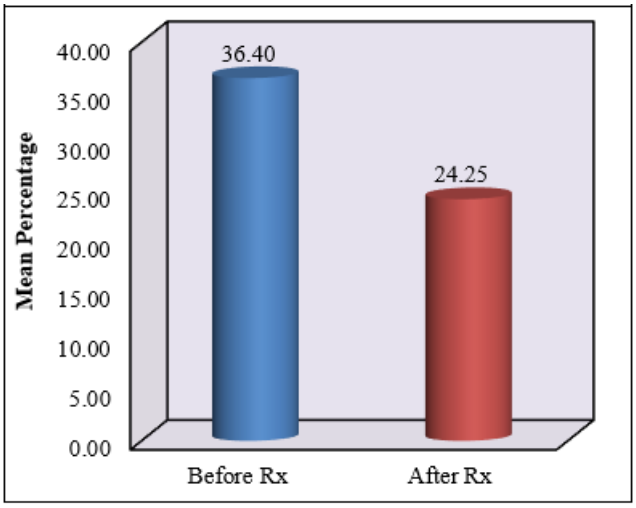


Figure 7: Mean BOP between Before & After NSPT (3 Months)

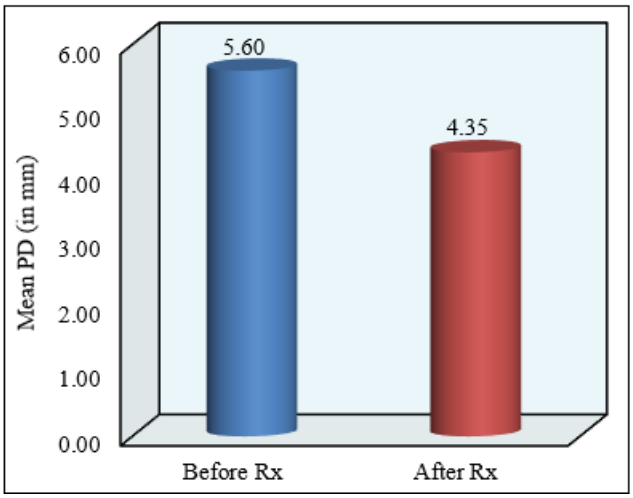


Figure 8: Mean PD between Before & After NSPT (3 Months)

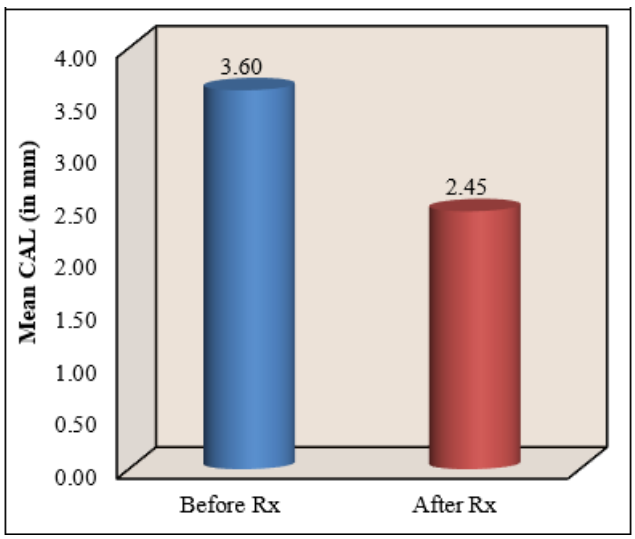


Figure 8: Mean CAL between Before & After NSPT (3 Months)

v) Assessment of relationship between GCF levels of Uric Acid and XDH with clinical parameters before and after NSPT: Correlation analysis revealed distinct and evolving patterns in the relationships between gingival crevicular fluid (GCF) biomarkers and clinical periodontal parameters before and after non-surgical periodontal therapy (NSPT).

At baseline, GCF uric acid demonstrated moderate positive correlations with several clinical indices, including Plaque Index ($r = 0.45$, $p = 0.04$), Gingival Index ($r = 0.50$, $p = 0.03$), Bleeding on Probing ($r = 0.46$, $p = 0.04$), and Probing Pocket Depth ($r = 0.68$, $p = 0.001$), (Table no 6) with the strongest association observed with Probing Pocket Depth. However, its correlation with Clinical Attachment Level ($r = 0.40$, $p = 0.08$) did not reach statistical significance.

Similarly, GCF xanthine oxidase exhibited significant moderate correlations with Gingival Index ($r = 0.47$, $p = 0.04$) and Probing Pocket Depth ($r = 0.57$, $p = 0.009$). Correlations with Plaque Index ($r = 0.36$, $p = 0.24$), Bleeding on Probing ($r = 0.31$, $p = 0.18$), and Clinical Attachment Level ($r = 0.25$, $p = 0.52$) were weaker and statistically non-significant.

Post-NSPT, GCF uric acid maintained significant positive correlations with Gingival Index ($r = 0.48$, $p = 0.03$), Bleeding on Probing ($r = 0.56$, $p = 0.01$), Probing Pocket Depth ($r = 0.59$, $p = 0.006$), and Clinical Attachment Level ($r = 0.46$, $p = 0.04$). Notably, the correlation with Bleeding on Probing strengthened following treatment, underscoring its sensitivity to changes in periodontal status.

GCF xanthine oxidase continued to demonstrate significant associations with Gingival Index ($r = 0.47$, $p = 0.04$), Probing Pocket Depth ($r = 0.60$, $p = 0.005$), and Clinical Attachment Level ($r = 0.50$, $p = 0.03$), further supporting its role as a biomarker of periodontal health. Correlations with Plaque Index ($r = 0.32$, $p = 0.17$) and Bleeding on Probing ($r = 0.43$, $p = 0.06$) remained moderate but did not achieve statistical significance.

Collectively, these findings highlight the dynamic interplay between GCF biomarkers and clinical periodontal parameters, illustrating how NSPT modulates these relationships and reflects improvements in periodontal health.

Table 5: Pearson correlation Test to assess the relationship b/w GCF Uric Acid, XDH levels with clinical parameters before & after NSPT among study subjects

Time	Variable	values	PI	GI	BOP	PPD	CAL
Before Rx	GCF Uric Acid	r	0.45	0.50	0.46	0.68	0.40
		p-values	0.04*	0.03*	0.04*	0.001*	0.08
	GCF XDH	r	0.36	0.47	0.31	0.57	0.25
		p-values	0.24	0.04*	0.18	0.009*	0.52
After Rx	GCF Uric Acid	r	0.41	0.48	0.56	0.59	0.46
		p-values	0.07	0.03*	0.01*	0.006*	0.04*
	GCF XDH	r	0.322	0.47	0.43	0.60	0.50
		p-values	0.17	0.04*	0.06	0.005*	0.03*

vi) Assessment of stepwise multiple linear regression analysis to predict GCF levels of Uric Acid and XDH with clinical parameters before treatment:

The regression analysis demonstrated that Probing Pocket Depth (PPD) served as a significant predictor of both gingival crevicular fluid (GCF) uric acid and xanthine oxidase concentrations prior to treatment.

Specifically, for GCF uric acid, the regression coefficient was 3,979.11, indicating that with each 1 mm increase in PPD, the GCF uric acid level increased by 3,979.11 ng/ml. The model explained 44% of the variance in uric acid levels, as shown by an R^2 value of 0.44. The predictor was highly significant, with a t-value of 3.978 and a p-value of 0.001.

Similarly, GCF xanthine oxidase levels increased by 0.518 ng/ml for every 1 mm increase in PPD, corresponding to a regression coefficient of 0.518. This model accounted for 29% of the variability in xanthine oxidase levels ($R^2 = 0.29$) and yielded a statistically significant association with a t-value of 2.946 and a p-value of 0.009.

These findings highlight the substantial influence of Probing Pocket Depth on GCF biomarker concentrations, emphasizing its importance as a clinical parameter in periodontal disease assessment and management.

Table 6: Stepwise Multiple Linear Regression Analysis to Predict GCF Uric Acid and XDH levels with clinical parameters before treatment

Time	DV	IV	Unstd. Coefficient.		t	p-value	R^2
			β	SE			
Before Rx	GCF Uric Acid	Constant	-7612.96	5649.77	-1.347	0.20	0.44
		PPD	3979.11	1000.31	3.978	0.001*	
	GCF XDH	Constant	2.451	0.993	2.468	0.02*	0.29
		PPD	0.518	0.176	2.946	0.009*	

vii) Assessment of stepwise multiple linear regression analysis to predict GCF levels of Uric Acid and XDH with clinical parameters after treatment:

The stepwise multiple linear regression analysis identified Bleeding on Probing (BOP) and Probing Pocket Depth (PPD) as significant predictors of gingival crevicular fluid (GCF) uric acid and xanthine oxidase levels following non-surgical periodontal therapy (NSPT).

For GCF uric acid, the regression model accounted for 46% of the variance in biomarker levels, as indicated by an R^2 value of 0.46. The regression coefficient for Bleeding on Probing was 198.91, suggesting that for each 1% decrease in BOP, GCF uric acid levels decreased by 198.91 ng/ml. Additionally, Probing Pocket Depth exhibited a regression coefficient of 1,222.82, indicating that a 1 mm reduction in PPD corresponded to a decrease of 1,222.82 ng/ml in GCF uric acid concentration.

Both predictors demonstrated statistical significance, with p-values of 0.01 and 0.02, respectively. The model's constant term, 553.34, was not statistically significant. (Table no 8) Regarding GCF xanthine oxidase levels, Probing Pocket Depth remained a significant predictor with an R^2 value of 0.20, explaining 20% of the variability in enzyme levels. The regression coefficient for PPD was 0.282, meaning that each 1 mm decrease in PPD was associated with a reduction of 0.282 ng/ml in GCF xanthine oxidase levels.

This relationship was statistically significant ($p = 0.03$). The constant term of 2.508 showed high significance ($p = 0.001$). These results emphasize the critical roles of Bleeding on Probing and Probing Pocket Depth as key clinical indicators predictive of reductions in GCF biomarker levels after NSPT, thereby underscoring their importance in evaluating periodontal treatment efficacy.

Table 7: Stepwise Multiple Linear Regression Analysis to Predict GCF Uric Acid and XDH levels with clinical parameters after treatment

Time	DV	IV	Unstd. Coefficient.		t	p-value	R^2
			β	SE			
After Rx	GCF Uric Acid	Constant	553.34	1871.50	0.296	0.77	0.46
		BOP	198.91	61.17	3.252	0.01*	
		PD	1222.82	457.93	2.67	0.02*	
	GCF XDH	Constant	2.508	0.299	8.393	0.001*	0.20
		PPD	0.282	0.117	2.419	0.03*	

4. Figures

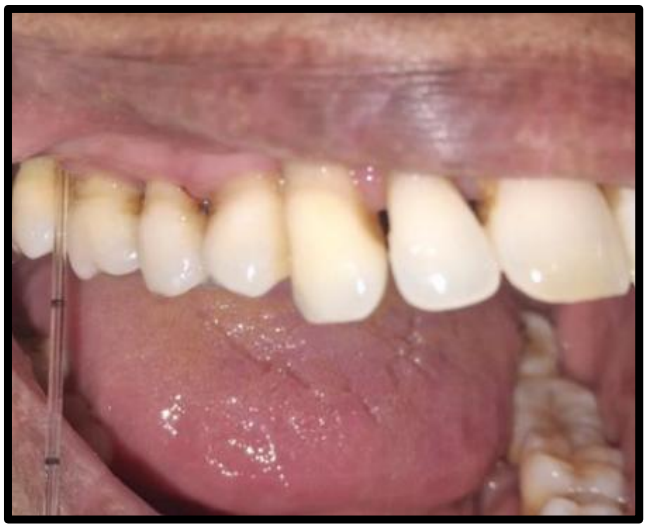
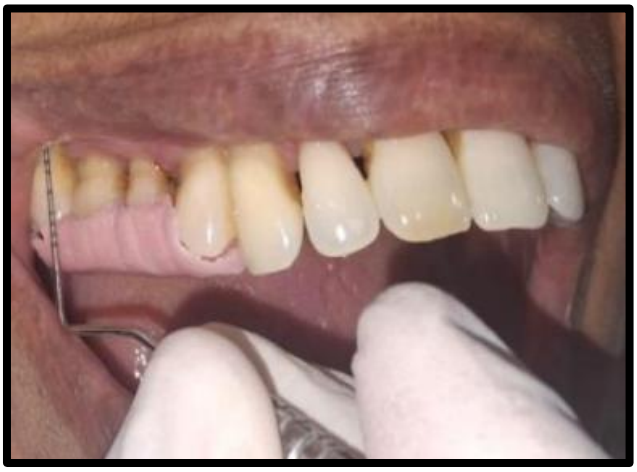


Figure 1: Collection of GCF sample



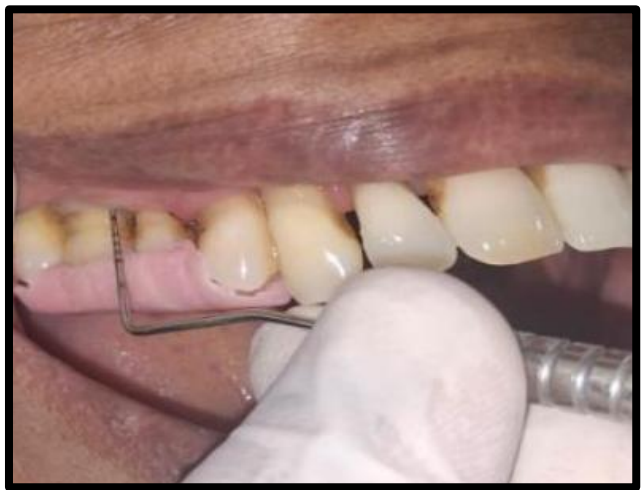


Figure 2: Measuring the probing pocket depth

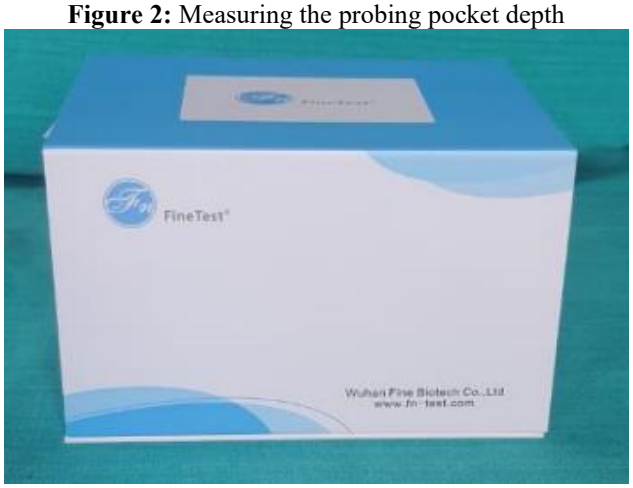


Figure 3: ELISA Kit used to assess gingival crevicular fluid levels of uric acid and xanthine oxidase

5. Discussion

Periodontitis is a chronic, multifactorial inflammatory disease characterized by the progressive destruction of tooth-supporting structures, in which host-derived oxidative stress plays a central pathogenic role. The excessive generation of reactive oxygen species (ROS) contributes to connective tissue degradation and alveolar bone loss, linking redox imbalance to clinical disease severity [26]. Among oxidative stress-related molecules, uric acid (UA) and xanthine oxidase (XO) have gained attention due to their dual antioxidant–pro-oxidant behaviour and capacity to influence immune responses within the periodontal microenvironment [24].

Uric acid functions as a major extracellular antioxidant under physiological conditions, particularly in saliva and gingival crevicular fluid (GCF). However, in chronic inflammation, it may act as a pro-inflammatory agent by engaging danger-associated molecular pattern (DAMP) receptors and promoting inflammasome activation [25]. Xanthine oxidase, the enzyme catalyzing the final steps of purine metabolism,

generates superoxide radicals and hydrogen peroxide during UA production, thereby amplifying oxidative damage. The upregulation of XO activity within inflamed periodontal tissues further contributes to tissue destruction through lipid peroxidation, protein denaturation, and DNA damage [25].

In the present study, significant reductions in GCF levels of UA and XO were observed following non-surgical periodontal therapy (NSPT), along with corresponding improvements in clinical periodontal parameters. These findings reinforce the hypothesis that elevated UA and XO levels reflect oxidative and inflammatory burden in periodontitis and are responsive to mechanical debridement [26]. Regression analysis identified probing pocket depth (PPD) as the most consistent predictor of both biomarkers at baseline and after therapy, suggesting that pocket depth is closely associated with local oxidative status. Post-treatment, bleeding on probing (BOP) also emerged as a significant predictor of UA levels, highlighting a link between vascular inflammatory activity and local redox imbalance [26].

These results align with earlier studies reporting elevated salivary or GCF XO levels and altered UA concentrations in patients with periodontitis. The dynamic reduction of both markers after NSPT underscores their potential as treatment-responsive biomarkers. Unlike systemic measurements, GCF levels offer site-specific insights and may reflect localized disease activity more accurately, particularly in systemically healthy individuals [27].

However, interpreting these biomarkers requires caution. UA levels can be influenced by systemic factors such as renal function, diet, and comorbid conditions, while XO activity may vary with medication use or individual oxidative capacity. Hence, localized biomarker analysis should be considered complementary to clinical and systemic evaluation.

From a clinical perspective, the integration of oxidative biomarkers like UA and XO into routine periodontal assessment could enhance the precision of disease monitoring, facilitate early detection of subclinical inflammation, and support personalized therapeutic strategies. The findings also contribute to the growing body of evidence connecting periodontal inflammation to systemic oxidative stress, suggesting broader implications for systemic health [28].

Further longitudinal and multicenter studies are warranted to validate UA and XO as reliable biomarkers and to explore their utility across diverse populations and therapeutic protocols. Understanding the molecular dynamics of oxidative stress in periodontal healing may ultimately improve diagnostic accuracy and guide targeted interventions [28].

6. Conclusion

This study highlights the significant role of oxidative stress in the pathogenesis of periodontitis, as evidenced by the modulation of gingival crevicular fluid levels of uric acid and xanthine oxidase in response to non-surgical periodontal therapy. Uric acid, though physiologically antioxidant, may exhibit pro-inflammatory properties under chronic oxidative stress [30]. Xanthine oxidase, a key ROS-generating enzyme, further contributes to periodontal tissue destruction. The observed reduction in biomarker levels post-therapy supports their potential utility in monitoring disease activity and therapeutic outcomes. Given their site-specific expression and non-invasive accessibility, these biomarkers may enhance diagnostic precision and contribute to a more individualized approach in periodontal care [30].

7. Clinical Relevance

Scientific rationale for the study:
Uric acid and xanthine oxidase are oxidative stress-related molecules implicated in inflammatory conditions, yet their diagnostic significance in periodontitis remains underexplored.

Principal findings:
Both biomarkers showed significant reduction following non-surgical periodontal therapy, paralleling clinical improvement in periodontal parameters.

Practical implications:
GCF uric acid and xanthine oxidase levels may serve as sensitive, treatment-responsive biomarkers for periodontal disease monitoring and could potentially bridge the link between oral and systemic inflammatory conditions.

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