suPAR is a potential biomarker of stage III-IV, grade C periodontitis through the impact of post-radiotherapy on head and neck cancer patients

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Research Article

Keywords: Chronic periodontitis, Head and Neck cancer, Radiotherapy, suPAR

Posted Date: February 2nd, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3902605/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Oral Health on September 27th, 2024. See the published version at https://doi.org/10.1186/s12903-024-04801-w.

Abstract

Background: The urokinase-type plasminogen activator receptor (uPAR) plays an essential function in leukocytes and endothelial homeostasis and, therefore, in the development of chronic periodontitis.

Methods: The study enrolled 150 participants, including 50 (CP+HNC post-RT) patients, 50 (CP) without HNC patients, and 50 healthy controls. Clinical Attachment Loss (CAL), Probing Pocket Depth (PPD), Plaque Index (PI), and Gingival Bleeding Index (GBI) were recorded. An enzyme-linked immunosorbent assay (ELISA) was constructed to quantify serum (suPAR) levels.

Results: Stage and grade of periodontitis were stage III-IV, grade C in patients (CP+HNC post-RT), stage I-III, grade A/B in patients (CP without HNC), and absent in (healthy). Chronic periodontitis with HNC post-RT patients presented a significantly higher proportion of suPAR levels (506.7 pg/ml) compared to chronic periodontitis without HNC and healthy controls (423.08 pg/ml and 255.9 pg/ml), respectively. A significant positive correlation was found between serum suPAR levels and CAL, PPD, PI, and GBI in the periodontal disease groups. ROC results of suPAR (AUC=0.976 for CP+HNC post-RT, AUC=0.872 for CP without HNC). Hyposalivation was decreased in patients (CP+HNC post-RT; 0.15 [0.11-0.23] ml/min, P=0.001) and (CP without HNC; 0.30 [0.25-0.41] ml/min, P=0.001), compared to healthy controls; 0.35 [0.28-0.54] ml/min, P=0.001).

Conclusion: The study showed a significant elevation in serum suPAR levels in CP+HNC post-RT patients compared to the CP without HNC and control groups.

1. Background

Head and neck cancers (HNCs) are diverse tumors usually generated by the epithelial cells of the larynx, lips, oropharynx, nasopharynx, and mouth (1). Squamous cell carcinomas account for over 90% of all head and neck cancers (HNSCC) (2). The progressive loss of periodontal tissues indicates periodontal disease, an inflammatory condition of the tooth-supporting structures caused by a subgingival accumulation of anaerobic gram-negative bacteria (3). Most HNC patients have radiotherapy as their main type of treatment, either by itself or in conjunction with other forms of therapy, to eradicate tumor cells (4). Nowadays, clinical parameters like clinical attachment level (CAL), probing depth (PD), bleeding on probing (BOP), and radiographic findings are used to diagnose periodontitis (5). These parameters frequently suggest prior periodontal disease instead of current disease activity. Therefore, because periodontitis patients are already seeing clinical improvements, additional diagnostic tests are required to identify if the disease is active, how it will progress in the future, and how rapidly the patient is responding to periodontal therapy (6). Radiation treatment may significantly impact the immunological environment surrounding the tumor, or the number and kind of immune cells invading the tumor, in addition to affecting tumor cells in a direct cytotoxic (4). HNC radiation has several adverse reactions, including a decline in the periodontium's immunological capacity and increased susceptibility to periodontitis and attachment loss (7, 8). Additionally, the severity of periodontitis may aggravate patients' quality of life in terms of emotional, functional, social, and aesthetic aspects (9). Thus, both

clinical and dental professionals need to monitor the oral hygiene condition of head and neck cancer patients receiving radiation (10). Although radiotherapy-related oral complications are often seen in clinical practice, several questions, including the causes, methods of early detection, and preventative measures, remain unanswered (11, 12). Plasminogen is converted to plasmin by urokinase plasminogen activator (uPA), which sets off a proteolytic cascade linked to tissue damage during inflammation (13). The plasma in blood contains the protein suPAR (14), serum (15), other bodily fluids cerebrospinal fluid (CSF) (16), saliva (17), urine (14), and is the membrane-bound receptor uPAR in soluble form. By controlling the breakdown of extracellular matrix, uPAR, when expressed on the cell surface membrane, is a key mediator of fibrinolysis and plasminogen activation, as well as a number of essential cellular activities. Consequently, angiogenesis, migration, adhesion, proliferation, and the inflammatory response are all impacted by uPAR (18). The soluble form, suPAR, is released into the circulation by proteolytic cleavage of uPAR (19). The aim of this study to improve a soluble urokinase plasminogen activator receptor suPAR is a reliable biomarker of chronic periodontitis and may replace HNC post-RT as the gold standard for evaluating this condition.

2. Materials and methods

2.1. Participants selection

This study was approved by Scientific Research Evaluation Ethical Committee of Ministry of Health, Al-Anbar Directorate of Health, Iraq (NO:2022057, Date: Aug., 22, 2022). A total 150 participants included (*n*=50, CP+HNC post-RT), (*n*=50, CP without HNC), and (*n*=50, healthy) as a control. CP+HNC post-RT patients were selected among those who received radiotherapy after six months and attended Anbar Cancer Center (ACC), Iraq. CP without HNC patients was selected from Ramadi Specialized Dental Center (RSDC), Iraq was performed from September 2022 to January 2023. All participants provided a written informed consent, and all steps of the clinical examination and sampling procedures were explained to each participant. The National Comprehensive Cancer Network (NCCN) states that patients were diagnosed with HNC by an oncologist at the cancer and tumors center (20). An experienced dentist who examined clinical periodontal parameters.

2. 2. Exclusion and Inclusion Criteria

Patients with the following exclusion criteria were excluded: (1) previous oral disease or salivary gland disease history; (2) definitive diagnosis of multiple sclerosis, xerostomia, or systemic disease; and (3) refusal to participate in the study. Furthermore, those with one of the following inclusion criteria were enrolled: (1) a pathologically confirmed malignant neoplasm of HNC (derived from epithelial cells); (2) not having received radiation therapy previously; (3) no distant metastasis; (4) no history of salivary gland surgery (parotid, submandibular, or sublingual); (5) a generally satisfactory physical condition with a performance score of 0 to 1 point and a planned survival period of more than a year.

2. 3. Oral saliva collection

The procedure was performed the patient sat in the dental chair and asked to clean his mouth with water washing to avoid materials accumulated and debris. The patient was called to swallow a residue of saliva in his oral cavity, after which the timer was started. Unstimulated saliva flow was removed every minute into a beaker. The amount collected of saliva was placed in plastic Eppendorf tubes and stored at -20 ^{0}C before pH determination.

2. 4. Examination of Periodontal Indices

A single-trained dentist by (E.R.) performed post-radiation periodontal indices check six months after radiotherapy finished. Clinical measures including plaque index (PI), gingival bleeding index (GBI), clinical attachment loss (CAL), and probing pocket depth (PPD) were assessed. The third molars were included in the assessment of clinical characteristics for all of the present teeth. PPD, CAL, GBI, and PI data were collected at six locations, and four sites per tooth, respectively. From the intersection of the cement and enamel to the bottom of the periodontal pocket, CAL was measured. GBI was measured depending on whether there was bleeding for ten seconds after probing (0 or 1, respectively) (21). PI measured a (0-3) score (22). All periodontal measures were performed manually using a millimeter periodontal probe (Williams Periodontal Probe PW; Hu-FriedyR, Chicago, IL, USA). The revised periodontitis categorization system outlined by Tonetti *et al*. (2018) was used to diagnose patients (23).

2. 5. Determination of suPAR levels

From both the patient and control groups, whole blood was drawn. Using a plastic syringe, about 5–7 milliliters of venous blood were collected. After that, the sample was put into a gel tube and coagulated for 15 to 20 minutes at room temperature. The samples were centrifuged for ten minutes at 3000 rpm. An enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of suPAR in serum (Human suPAR ELISA kit, Elabscience, Texas, USA). The assay was carried out in compliance with the manufacturer's instructions.

2. 6. Statistical analysis

Frequency and percentage were used to characterize categorical variables. Kolmogorov-Smirnova and Shapiro-Wilk tests demonstrated that the data was abnormally distributed. Therefore, in the statistical analysis of this study, non-parametric tests were used. Kruskal-Wallis test between multiple groups or the Mann-Whitney test between two groups were used to analyze continuous variables having a skewed distribution. These variables were reported as the Mean±SD with the interquartile range. The Pearson correlation coefficient was used for correlation analysis. The effectiveness of serum indicators for (CP+HNC post-RT) and (CP without HNC) was assessed using the area under the receiver operating characteristic (ROC) curve analysis. Differences were considered statistically significant when $P < 0.05$. All of the analyses were processed using IBM SPSS (version 27, NY, USA) and GraphPad Prism (version 9.5.1, La Jolla, California, USA).

3. Results

3.1. Study population

The features and demographics of chronic periodontitis (CP) and head and neck cancer (HNC) patients are shown in (Table 1). In this research population, there were n=26 nasopharyngeal tumors (n=20 nonkeratinizing and n=6 keratinizing carcinomas), (n=6 oropharyngeal squamous cell carcinoma, SCC), (n=8 Laryngeal cancer), (n=6 tongue cancer), and (n=4 primary malignancies of unclear origin). Many patients had a history of smoking, and the mean patient age was 45.8 years (min-max: 28-72 years); 40 (80%) were men. The range of the total radiation dosage was 5700–7000 cGy, with a mean of 6350 cGy. Twenty-eight of the patients (or 56%) had a concomitant systemic therapy (2-3 doses) of cisplatin or cetuximab in addition to radiotherapy. In this research cohort, chronic periodontitis patients without HNCs included: Patients' average ages ranged from 28 to 65 years, with 40 (80%) of them being male. More than half of them had a history of smoking, with 31 (62%) and 5 (10) of them having used alcohol. A control group was used periodontally healthy as a controlled study, the mean age was 40.12 years (min-max: 29-60 years).

Table 1. Demographic and medical characteristics of cases and control

Variables		CP+HNC-post RT	CP without HNC	Control (Healthy)
Mean±SD;**Age, years** (min-max)		41.34±8.41; (28-62)	40.06±6.41; (28-60)	40.12±6.40; (29-60)
Gender, n (%)		-		
Male:		40 (80)	40 (80)	40 (80)
Female:		10 (20)	10 (20)	10 (20)
Body Mass Index Mean±SD**(Weight/height^2),**		26.01±5.74	27.91± 4.61	25.45±4.91
Stage of Tumor, n (%)	1-2	9(18)	N/A	N/A
	3-4	41(82)		
Smoking, n (%)				
Yes		36 (72)	31 (62)	0 (0.0)
No		14 (28)	19 (38)	50 (100)
Drinking, n (%)				
Yes		13 (26)	5 (10)	0 (0.0)
No		37 (74)	45 (90)	50 (100)
Type of treatment, n (%)	RT	22(44)		
	CT+RT	28(56)	N/A	N/A
RT, n (%)		22(44)	N/A	N/A
CT-RT, n (%)		28 (56)	N/A	N/A

Note: HNC-post RT: Head and Neck Cancer post-radiotherapy, CP: Chronic Periodontitis, SD: Standard Deviation, RT: Radiotherapy, CT+RT: Chemoradiotherapy, N/A: Not Applicable.

3.2. Clinical Periodontal Parameters, Oral pH, and Hyposalivation

The results noticed, as compared to the healthy group between CP+HNC post-RT and CP without HNC had larger Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), and greater Plaque Index (PI), and Gingival Bleeding Index (GBI) with a significant statistical difference of (P= 0.001). Moreover, Oral pH changed in groups (CP+HNC post-RT, CP without HNC, and control), the mean levels of Oral pH were (6.0), (7.77), and (7.12), respectively (P= 0.001). Furthermore, the mean levels of hyposalivation were 0.155 ml/min, 0.30 ml/min, and 0.35 ml/min, respectively. Comparing the chronic periodontitis with HNC

post-RT and chronic periodontitis without HNC groups to the healthy group, the groups with chronic periodontitis had substantially decreased hyposalivation levels (P= 0.001), as shown in (Table 2).

Table 2. Clinical features of head and neck cancer post-RT on periodontal health

Variables		CP+ HNC post-RT	CP without HNC	Healthy (Control)	*P*
CAL	Mean ± SD	7.02 ± 0.43	6.34 ± 0.78	-	0.001
(mm)	(min-max)	(6.5-7.5)	(5.25-7.50)	-	
PPD	Mean ± SD	7.1 ± 0.46	6.12 ± 0.61	3.05 ± 0.15	0.001
(mm)	(min-max)	(6.00-7.50)	(4.50-7.25)	(3.00-3.50)	
PI	Mean ± SD	2.52 ± 0.61	1.94 ± 1.03	0.3 ± 0.46	0.001
(mm)	(min-max)	(1.00-3.00)	(0.00-3.00)	(0.00-1.00)	
GBI	Mean ± SD	90.38 ± 0.58	63.12± 0.60	4.25 ± 0.39	0.001
(%)	(min-max)	(89.37-91.65)	(60.99-63.88)	(3.50-4.90)	
Oral saliva pH	Mean ± SD	6.0±0.67	7.77±0.28	7.12±0.16	0.001
	(min-max)	(4.66-7.11)	(7.00-8.10)	(6.90-7.50)	
Hyposalivation	Mean ± SD	0.15±0.04	0.30±0.04	0.35±0.05	0.001
(ml/min)	(min-max)	(0.11-0.23)	(0.25-0.41)	(0.28-0.54)	

Note: CAL: Clinical attachment loss, PPD: periodontal pocket depth, PI: plaque index, GBI: gingival bleeding index

3.3. Stage and grade of periodontitis

In the CP+HNC post-RT patients, stage II periodontitis affected a total of nine (18%) patients, stage III periodontitis affected nineteen (38%) patients, and stage IV periodontitis affected twenty-two (44%) patients. In CP without HNC group stage I periodontitis affected fifteen (30%) patients, stage II periodontitis affected a total of twenty-four (48%) patients, stage III periodontitis affected nine (18%) patients, and stage IV periodontitis affected two (4%) individuals. Grade A and B periodontitis affected 0(0.0), and grade C affected a total of 50 (100%) in CP+HNC post-RT group. Furthermore, grade A periodontitis affected nineteen (38%), grade B affected thirty-one (62%) and grade C affected 0(0.0) in CP without HNC group. At P = 0.001, The value of the variance of stage and grade of periodontitis was significant statistically as can be seen from (Table 3 and Figure 1).

Table 3 Stage and grade of periodontitis in clinical cases.

Variables		CP+HNC post-RT	CP without HNC	*P
Stage of periodontitis, n (%)	**Stage I**	0 (0.0)	15 (30)	0.001
	Stage II	9 (18)	24 (48)	
	Stage III	19 (38)	9 (18)	
	Stage IV	22 (44)	2 (4)	
Grade of periodontitis, n (%)	**Grade A**	0 (0.0)	19 (38)	0.001
	Grade B	0 (0.0)	31 (62)	
	Grade C	50 (100)	0 (0.0)	

*: Significant <0.05, Mann-Whitney test or Wilcoxon W test

3. 3. Clinical activity and serum suPAR levels are closely related.

Estimation of serum suPAR was increased in patients with CP+HNC post-RT (506.7 [305.0-991.12] pg/mL, P <0.001) compared to CP without HNC (423.08 [205.0-735.00] pg/ mL, P <0.001), and healthy controls (255.9 [65.00-368.33] pg/mL, P <0.001 (Fig. 2).

3. 4. Receiver Operating Curve Characteristic (ROC)

Receiver Operating Curve Characteristic (ROC) analyses shown in (Table 4 and Figure 3) suggested that a higher ability to identify active patients with CP+HNC post-RT might be achieved by serum suPAR (AUC = 0.976) than CP without HNC (AUC = 0.872). In patients with CP+ HNC post-RT, suPAR level was increased (371.665 pg/ml), which could identify clinical abatement with a sensitivity= 86% and specificity= 100%, P= 0.001. In patients with CP without HNC suPAR level was decreased (371.225 pg/ml), which could identify clinical abatement with a sensitivity= 62%, and specificity= 100%, P= 0.001).

Table 4. Receiver Operating Curve Characteristic (ROC) analyses of serum suPAR to identify clinical abatement.

Variables	Groups	AUC	Cut-off value (pg/ml)	Sensitivity %	Specificity %	*P*
suPAR (pg/ml)	CP+HNC post-RT	0.976	371.665	86	100	0.001
	CP without HNC	0.872	371.225	62	100	0.001

3. 5. Serum suPAR levels are significantly correlated with Periodontitis Indices, Oral pH, and Hyposalivation

Pearson's correlation coefficient was observed, and the clinical periodontal measurements scores: Plaque Index (PI), Gingival Bleeding Index (GBI), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) revealed a statistical significance with serum suPAR levels. In addition, hyposalivation and oral pH showed a statistically significant correlation with serum suPAR levels, as shown in (Fig. 4 A, B, C, D, E, F).

4. Discussion

The main goal of the current study was to investigate the association between serum levels of suPAR and clinical periodontal indices activity in HNC post-RT. In light of this, we studied here the diagnostic and characterization use of serum suPAR levels (ELISA) for possible oral and periodontal tissue degradation brought on by HNC radiation. To our knowledge, no previous study has explored concentrations of suPAR in serum of CP patients with or without HNC post-RT. The findings of the present study were those patients with CP+HNC post-RT exhibited significantly higher serum suPAR compared to CP without HNC and controls. The current study validates and expands upon earlier research; few findings about the correlation between oral health status linked to periodontitis (OHS-P) and head and neck cancer (HNC) have been published in various nations using various methodologies (24).In the current study, the focus of attention was on periodontal indices and saliva-pH changes to identify periodontium-damaging adverse effects of HNC radiation. Oral saliva pH was changed in patients CP+HNC post-RT and CP without HNC was increased, compared to healthy control was neutral approximately. These results are consistent in good agreement with other studies which have shown articles stating that individuals exposed to radiation have mildly acidic saliva due to the reduced buffering capacity resulting from radiation injury mainly on serous acini (25). Hyposalivation and the protective effects of saliva loss may make periodontitis more likely by hyposalivation. Findings appeared hyposalivation was decreased in patients CP+HNC post-RT and CP without HNC, compared to healthy control. Background details are provided, and it is investigated here whether periodontal indices and hyposalivation may be used to influence and characterize any possible degradation of oral and periodontal tissues driven on by HNC radiation. The results further support earlier research showing substantial radiotherapy-induced periodontium damage and loss of periodontal attachment. The findings are generally consistent with the main trends (26, 27). Following the start of radiotherapy, the impact of radiotherapy on periodontal health is related to poorer periodontal health and dose dependence (28). Additionally, the oral microbiota changes as a result of RT usage in the head and neck area, shifting to bacteria linked with periodontal disease (29). According to the new periodontitis categorization system, the most notable alterations in clinical periodontal measurements were seen in CAL, which was linked to the fast development of periodontitis (from grade A to grade C) (23).

The results indicated that chronic periodontitis with or without HNC had greater usPAR expression than periodontally healthy. It has been proposed that soluble urokinase-type plasminogen activator receptor, or suPAR, may serve as a biomarker for both periodontal health and disease. A study by Isola et al. assessed salivary and serum concentrations of suPAR in children with gingivitis and healthy subjects (30).

Data of Receiver Operating Curve Characteristic (ROC) analyses for suPAR appeared that it is a potentially useful diagnostic biomarker for CP+HNC post-RT. The sensitivity and specificity of suPAR in serum were found to be 86% and 100%, respectively, for differentiating between CP+HNC post-RT patients and healthy subjects, and 62% and 100%, respectively, for CP without HNC patients and healthy. Additionally, suPAR has demonstrated potential in predicting flare outcomes in CP+HNC post-RT patients.

The results of this investigation demonstrated a statistically significant negative link between oral pH and hyposalivation and a positive correlation between the levels of suPAR and the clinical periodontal parameters assessed. The previous study found that salivary suPAR levels were significantly higher in patients with gingivitis compared to healthy subjects, and the levels correlated with periodontal clinical parameters (30). Another study by Kozakiewicz et al. investigated the impact of periodontitis and cardiovascular disease on elevated suPAR levels. The study found that suPAR levels were higher in patients with periodontitis and cardiovascular disease, suggesting that suPAR could be a valuable biomarker for risk stratification in these patients. In summary, soluble urokinase-type plasminogen activator receptor (suPAR) has been correlated with periodontal indices in children with gingivitis (31) and adults with periodontitis and cardiovascular disease. These findings suggest that suPAR measurements may be useful in the early assessment of gingivitis and the risk stratification of patients with periodontitis and cardiovascular disease.

However, the study’s sample size was limited, and the short time frame, and the inability to rule out some confounding variables, such as chemotherapy used, are the limitations of the study. Finally, Last but not least, a large body of evidence supports links between periodontal disease, HNC post-RT, and inflammatory contributions to each ailment. On the putative causative link between chronic periodontitis (CP) with HNC post-RT and chronic periodontitis (CP) without HNC (alone), however, there is no information currently available on the function of biomarkers. This is brought about by the large degree of variation among the study's design, populations who were included, assay techniques, and analyzed biomarkers. The causal relationship between periodontal inflammation and HNC post-RT must be established by future randomized control and prospective studies with standardized clinical and biological measurements, and the significance of biomarkers in tying these illnesses together must also be further examined.

5. Conclusion

In concluded, a good sensitive biomarker suPAR to improve the early diagnosis of periodontal tissue loss of periodontium as a side effect of irradiation.

Abbreviations

HNCs	Head and neck cancers
CP+HNC post-RT	Chronic periodontitis with head and neck cancer post-radiotherapy
suPAR	Soluble urokinase plasminogen activator receptor
CAL	Clinical attachment level
PPD	Periodontal pocket depth
PI	Plaque index
GBI	Gingival bleeding index
RSDC	Ramadi Specialized Dental Center
ACC	Anbar cancer center
ROC	Receiver operating characteristic
SCC	Squamous cell carcinomas
NCCN	National Comprehensive Cancer Network
AUC	Area under curve
CT+RT	Chemoradiotherapy
N/A	Not applicable
ELISA	Enzyme-linked immunosorbent assay

Declarations

Ethics approval and consent to participate:

All participants understood the nature of the research project and provided written informed consent to participate in this study. Permission for this study was obtained from Scientific Research Evaluation Ethical Committee of Ministry of Health, Al-Anbar Directorate of Health, Iraq (NO:2022057, Date: Aug., 22, 2022).

Consent for publication: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable

Authors' contributions:

AA Al-Kubaisi: Conceptualization, Methodology, Investigation, Writing- Original draft, Project administration, **MAG:** Resources**,** Software, Formal analysis, Data curation, **NSM:** Conceptualization, Methodology, Investigation, Writing- Review & editing. **ER:** Formal analysis, Data curation, Methodology, and Investigation, **HHE:** Conceptualization, Project administration, and Supervision. All authors read and approved the final manuscript.

Acknowledgements: Not applicable

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Figures

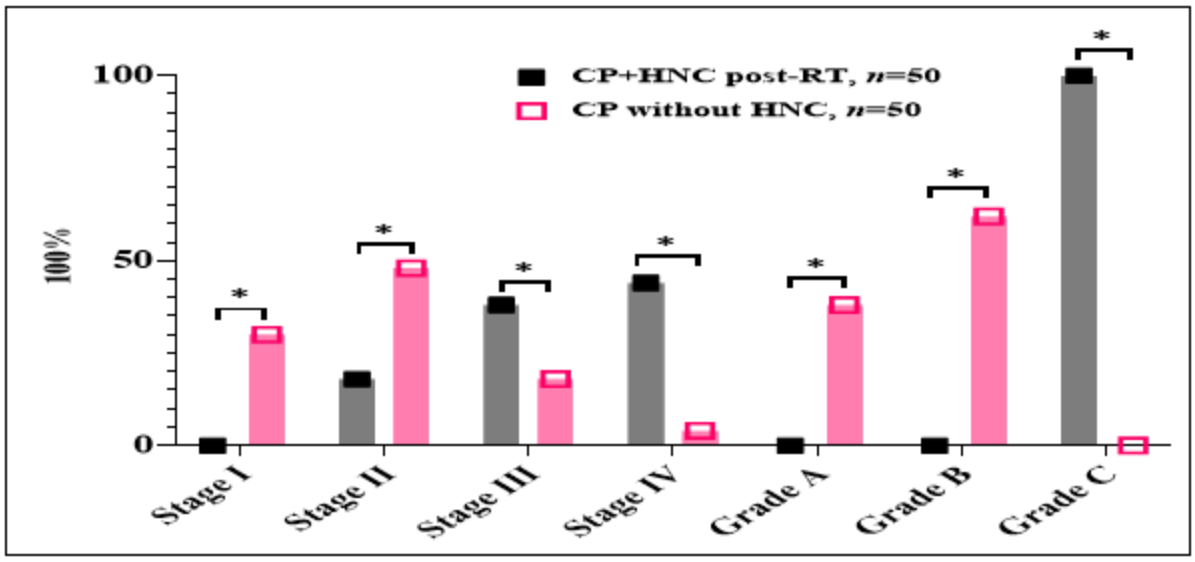

Figure 1

Stage and grade of periodontitis compared between two groups CP+HNC post-RT and CP without HNC (*: Significant<0.05).

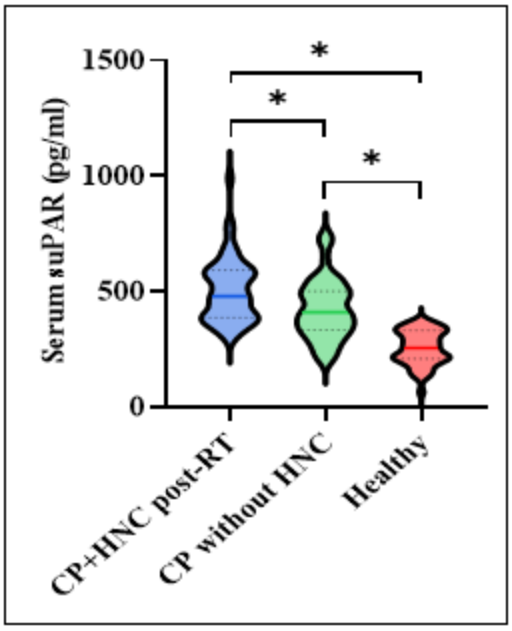

Figure 2

Violin plot of the mean, interquartile range, and upper and lower levels involved: serum suPAR levels in CP+HNC post-RT, CP without HNC, and Healthy. (*: Significant, P <0.05 ,Mann-Whitney or Kruskal-Wallis test).

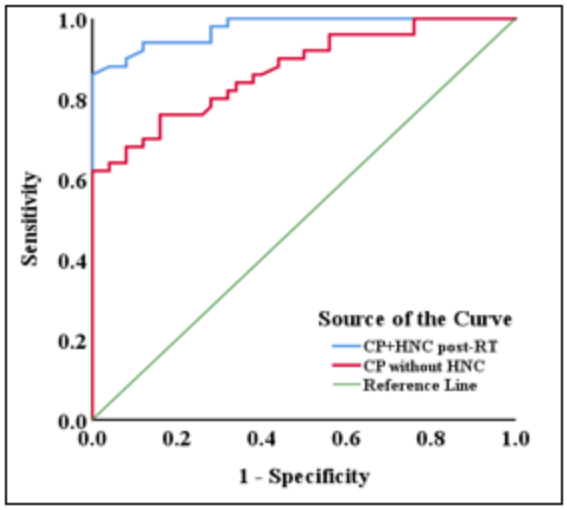

Figure 3

Receiver Operating Curve Characteristic (ROC) of suPAR prediction for CP+HNC post-RT and CP without HNC patients.

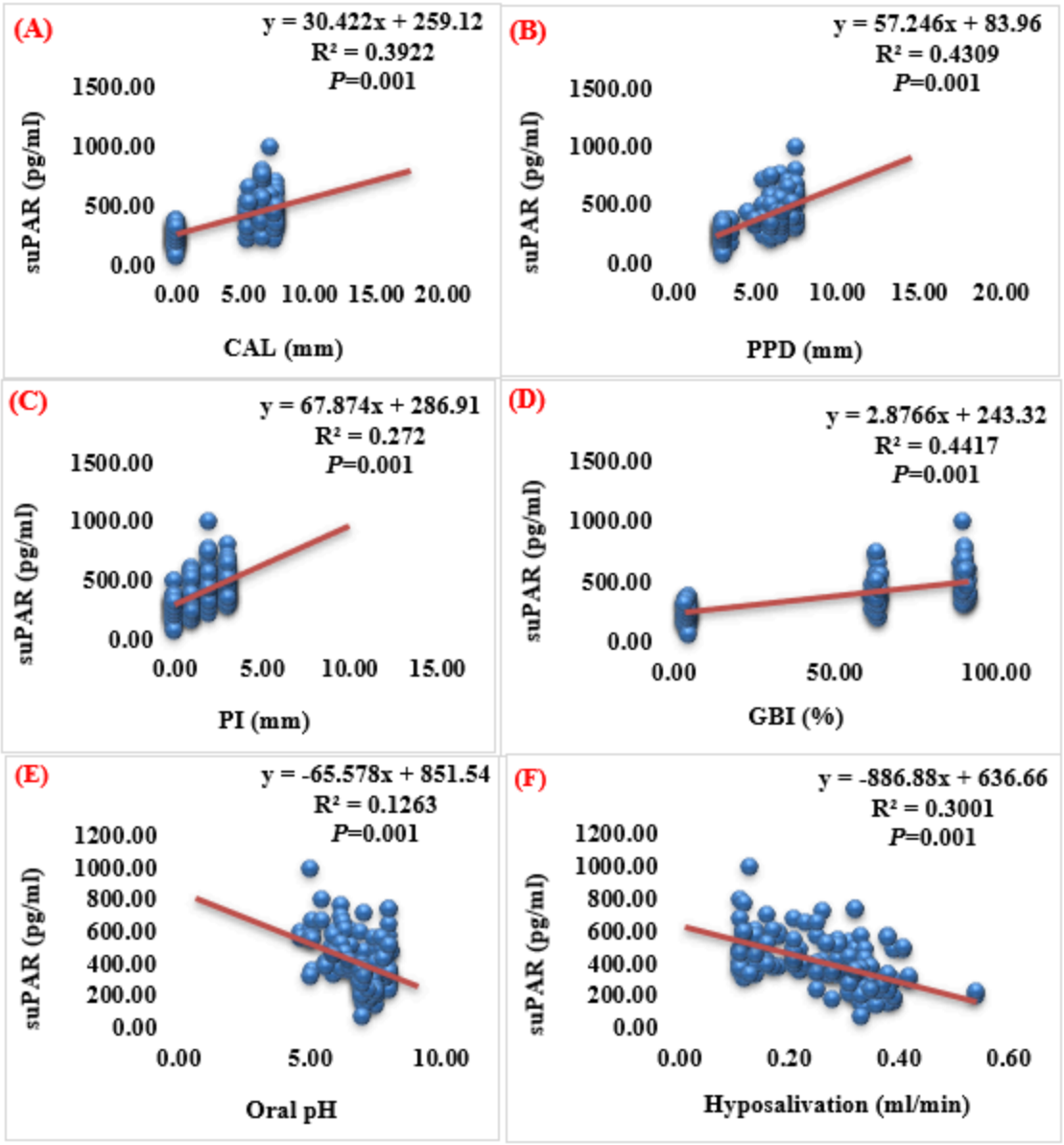

Figure 4

Serum concentrations of suPAR (pg/ml) correlated with: **(A)** CAL Clinical Attachment Level (mm), **(B)** Probing Pocket Depth PPD (mm), **(C)** PI Plaque Index (mm), **(D)** GBI Gingival Bleeding Index (%), **(E)** Oral pH, **(F)** Hyposalivation(ml/min).