**Serum level of 25-hydroxyvitamin D is associated**

**with chronic periodontitis in head and neck cancer post-radiotherapy patients**

***Ahmed A. Al-Kubaisi 1,[[1]](#footnote-1)\*, Zaizafoon Nabeel2, Ekram R. Aldelaimi3, Şevki Adem4***

*1 Al Maarif University College, Department of Medical Laboratory Techniques, Anbar, Iraq*

*2 College of Science, Department of Chemistry, Mustansiriyah University, Baghdad, Iraq*

*3* *Ramadi Specialized Dental Center, Department of Periodontology, Anbar, Iraq*

*4 Faculty of Sciences, Department of Chemistry, Çankırı Karatekin University, Çankırı, Turkey*

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| **Abstract** Vitamin D plays an essential role in bone metabolism and immunity. Hence, it might affect the development and extent of periodontal disease. This study aimed to assess Vitamin D status in periodontal disease. A total of 150 are participants in the current study, including 50 patients with head and neck cancer who received radiotherapy post-six months (HNC post-RT), 50 chronic periodontitis (CP) patients without HNC, and 50 periodontally healthy (control) enrolled. Clinical Attachment Loss (CAL), Probing Pocket Depth (PPD), Plaque Index (PI), and Gingival Bleeding Index (GBI) were recorded. An electrochemiluminescence immunoassay (eCLIA) was constructed to quantify serum Vitamin D levels. Chronic periodontitis with HNC post-RT patients presented a non-significant proportion of Vitamin D levels compared to chronic periodontitis without HNC(p>0.05) and patients with CP+HNC post-RT and CP without HNC exhibited significant Vitamin D levels compared to control (p<0.05). A significant negative correlation was found between serum Vitamin D levels and CAL, PPD, PI, and GBI in the periodontal disease groups. Moreover, a significant positive correlation was observed between serum Vitamin D levels and hyposalivation. Hyposalivation was increased 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). In this case-control study, Vitamin D deficiency is significantly associated with chronic periodontitis groups. The assessment of vitamin D levels in patients presenting with chronic periodontitis seems advisable, as vitamin D deficiency might be involved in the onset and progression of chronic periodontitis.  |
| Keywords: Head and Neck Cancer, Chronic periodontitis, Radiotherapy, Vitamin D |

1. **Introduction**

Head and neck cancers (HNC) are a varied collection of cancerous tumors that may develop in the larynx, oral cavity, pharynx, sinonasal cavities, oropharynx, and nasopharynx among other anatomical sites [1]. Squamous cell carcinomas (HNSCC) account for around 90% of all head and neck malignancies [2]. Periodontal disease is an inflammatory disorder of tooth-supporting structures induced by the subgingival buildup of anaerobic gram-negative bacteria and characterized by the gradual loss of periodontal tissues [3]. Nowadays, clinical parameters like clinical attachment level (CAL), probing depth (PD), bleeding on probing (BOP), and radiographic findings are used to diagnose periodontitis [4]. 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 [5]. 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 [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 [6]. 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]. Vitamin D got a vigorous increase in interest because of emerging evidence on new knowledge regarding the nonhormonal, intracrine, and paracine actions of 1-hydroxylated vitamin D metabolites and the world-wide trend to worsening nutritional vitamin D deficiency [10]. Vitamin D has a crucial function in skeletal mineralization but also plays an important role in immunity, diabetes, cancer, and the cardiovascular system [11]. Therefore, insufficient Vitamin D levels may result in an inadequate and prolonged immune reaction to periodontal pathogens which may lead to more severe periodontal destruction. The aim of this case-control study was the assessment of the association of chronic periodontitis groups and Vitamin D levels.

1. **Materials and Methods**

This work was approved by the Ethics Committee of Çankırı Karatekın University Scientific Research Evaluation Ethical Committee (No:41, Date:26.09.2022). The study's participants were CP+HNC post-RT (*n*=50), CP without HNC (*n*=50), and periodontally healthy as a control (*n*=50). CP+HNC post-RT patients were selected among those who received radiotherapy and attended to Cancer and Tumors Center/ 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. Exclusion and inclusion criteria were enrolled. Patients were diagnosed with HNC by an oncologist at Cancer and Tumors Center according to the National Comprehensive Cancer Network (NCCN)[12]. A single experienced oral hygienist (E. R.) examined clinical periodontal parameters. About (5-7ml) of venous blood was drawn by using a plastic syringe from all cases and control. Then, the sample was placed into a gel tube and left to clot at room temperature for about (15-20 minutes). The specimens were centrifuged at 3000 rpm for 10 minutes. Serum levels of Vitamin D were determined using modern electrochemiluminescence immunoassay technique (eCLIA), Nipigon Health corp, Ontario, Canada. The assay was performed according to the specifications of the manufacturer. When p <0.05, differences were deemed statistically significant. Both GraphPad Prism (version 9.5.1, La Jolla, California, USA) and IBM SPSS (version 27, NY, USA) were used to process all of the analyses.

1. **Results and Discussion**
	1. **Study population**

The features and demographics of all groups of study have been presented in (Table 2).Many CP+HNC post-RT patients had a history of smoking, and the mean patient age was 41.34 years (min-max: 28-62 years); 40 (80%) were men. Stage II periodontitis affected a total of 22 (44%) CP+HNC post-RT, stage III periodontitis affected nine (18%) CP+HNC post-RT, and stage IV periodontitis affected nineteen (38%) CP+HNC post-RT. The range of the total radiation dosage was 5700–7000 cGy, with a mean of 6350 cGy.

**Table 1.** Demographic and medical characteristics of cases and controls

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| **Variables** | **CP+HNC-post RT** | **CP without HNC** | **Control****(Healthy)** |
| **Age, years** Mean±SD; (min-max) | 41.34±8.41; (28-62) | 40.06±6.41;(28-60) | 40.12±6.40;(29-60) |
| **Gender, n (%)**Male:Female: | 40 (80)10 (20) | 40 (80)10 (20) | 40 (80)10 (20) |
| **Stage of Tumor, n (%)** | 1-2 | 9(18) | N/A | N/A |
| 3-4 | 41(82) |
| **Smoking, n (%)**YesNo | 36 (72)14 (28) | 31 (62)19 (38) | 0 (0.0)50 (100) |
| **Stage of periodontitis, n (%)**Stage IStage IIStage IIIStage IV | 0 (0.0)22 (44)9 (18)19 (38) | 15 (30)24 (48)9 (18)2 (4) | Absent |
| **Grade of periodontitis, n (%)**Grade AGrade BGrade C | 0 (0.0)0 (0.0)50(100) | 19 (38)31 (62)0 (0.0) | Absent |
| **Type of treatment, n (%)** | RT | 22(44) | N/A | N/A |
| CT+RT | 28(56) |

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

* 1. **Clinical Periodontal Parameters 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). 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, hyposalivation levels were significantly higher in chronic periodontitis groups (*P*= 0.001), as shown in (Table 2).

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

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| --- | --- | --- | --- | --- |
| **Variables** | **CP+ HNC post-RT** | **CP without HNC**  |  **Healthy****(Control)** | ***P*** |
| CAL (mm) | Mean ± SD | 7.02 ± 0.43 | 6.34 ± 0.78 | - | 0.001 |
| (min-max) | (6.5-7.5) | (5.25-7.50) | - |
| PPD (mm) | Mean ± SD | 7.1 ± 0.46 | 6.12 ± 0.61 | 3.05 ± 0.15 | 0.001 |
| (min-max) | (6.00-7.50) | (4.50-7.25) | (3.00-3.50) |
| PI (mm) | Mean ± SD | 2.52 ± 0.61 | 1.94 ± 1.03 | 0.3 ± 0.46 | 0.001 |
| (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) |
| Hyposalivation(ml/min) | Mean ± SD | 0.15±0.04 | 0.30±0.04 | 0.35±0.05 | 0.001 |
| (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

Our results hyposalivation was increased in patients CP+HNC post-RT and CP without HNC, compared to healthy control. Hyposalivation and the protective effects of saliva loss may make periodontitis more likely by hyposalivation. Salivary changes in quantitative, qualitative and microbial composition occur during and after radiotherapy [13]. 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. Our results support and further extend the previous studies that have demonstrated significant damage to the periodontium and loss of periodontal attachment due to radiotherapy[14, 15]. According to the revised periodontitis categorization system, the most notable alterations in clinical periodontal measurements were seen in CAL, which correlated with a fast development of periodontitis (from grade A to grade C) [16].

* 1. **Serum Vitamin D levels are highly correlated with clinical activity**

The evaluation of serum Vitamin D was increased in patients with CP+HNC post-RT (12.43 [8.32-16.77] ng/mL, *p* < 0.001) compared to CP without HNC (14.30 [8.00-24.15] ng/ mL, *p <* 0.001), and healthy controls (19.80 [9.80-31.22] ng/mL, *p<* 0.001 (Fig. 2).



**Figure 2.** Violin plot of the mean, interquartile range, and upper and lower levels involved: serum Vitamin D levels in CP+HNC post-RT, CP without HNC, and Healthy (Mann-Whitny or Kruskal-Wallis test, ns: non-significant, \**p*<0.001).

The findings of the present study were those patients with CP+HNC post-RT exhibited non significant indicated of serum Vitamin D compared to CP without HNC. Furthermore, our results observed patients with CP+HNC post-RT and CP without HNC exhibited significantly lower serum Vitamin D compared to control. Our results were agreed with study reported by *Markus L. et al* data show that low serum-concentrations of Vitamin D are significantly associated with periodontal disease, suggesting that insufficient Vitamin D levels might be involved in periodontal disease progression [17].

**3.4. Serum Vitamin D levels are significantly correlated with periodontitis indices 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 Vitamin D levels. In addition, hyposalivation showed a statistically significant correlation with serum Vitamin D levels, as shown in (Table 3).

**Table 3** Correlation of serum Vitamin D level with CAL, PPD, PI, GBI, and Hyposalivation in the periodontal disease groups

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| **Variables** | **Correlation coefficient (p value)** |
| CAL | -0.442 (0.001) |
| PPD | -0.454 (0.001) |
| PI | -0.401 (0.001) |
| GBI | -0.465 (0.001) |
| Hyposalivation | 0.372 (0.001) |

The current study was noticed a significant negative correlation between serum Vitamin D levels and CAL, PPD, PI, and GBI in chronic periodontitis groups. Moreover, a significant positive correlation significantly was observed between serum Vitamin D levels and hyposalivation.

1. **Conclusion**

The findings from this comparative, periodontal indces, and biochemical trial revealed that serum Vitamin D level is an essential factor in the pathogenesis of chronic periodontitis. It can be used as a diagnostic or a prognostic marker for chronic periodontitis especially in patients underwent radiotherapy for head and neck cancer.

**Acknowledgement**

We are grateful to all medical oncologists and the medical laboratory department team at Anbar Cancer Center/Iraq as well as Ramadi Specialized Dental Center team for their efforts to come out with this study.

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1. \* Corresponding author. *e-mail address: Ahmed.amer@uoa.edu.iq* [↑](#footnote-ref-1)