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Original Article

Serum Levels of MIP-1α as a Biomarker in Periodontal Disease with Post-Radiation Head and Neck Cancer

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ABSTRACT

Proinflammatory chemokines positivity is exhibited by Macrophage Inflammatory Protein -1α (MIP-1α) in the presence of inflammation and chronic infections. The present research included 150 individuals, including n=50 for patients who were affected by head and neck cancer, and who had radiation more than six months ago, n=50 patients with chronic periodontitis (CP), and n=50 controls. Clinical periodontal parameters were documented to record periodontal scores. An enzyme-linked immunosorbent test (ELISA) was developed to quantify blood MIPs-1α concentrations. Individuals diagnosed with chronic periodontitis following radiotherapy for head and neck cancer. They had a markedly elevated concentration of MIP-1α (102.65 pg/ml) compared to those with chronic periodontitis and the control group (63.25 and 46.09, respectively). The correlation between serum MIP-1α levels and clinical periodontal markers in chronic periodontal disease was found to be significantly positive. This case-control study suggests that elevated blood MIP-1α levels in head and neck cancers (HNCs) after radiotherapy may serve as indicators of periodontal tissue degradation.

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Keywords: Head and neck cancer, Chronic periodontitis, MIP-1α, Radiotherapy.

1 Introduction

Head and neck cancers (HNCs) encompass a variety of malignancies that commonly originate from epithelial tissues of the larynx, lips, oropharynx, nasopharynx, and oral cavity [1,2]. In 2020, around 890,000 original cases, besides 450,000 stated fatalities, HNC ranked as the seventh most prevalent malignancy worldwide [3]. Periodontal disease is a provocative state impacting the tissues that support the teeth, caused by the accumulation of anaerobic gram-negative bacteria under the gumline. It is marked by the progressive deterioration of periodontal tissues[4]. Currently, diagnostic criteria for periodontitis include clinical periodontal indices and radiographic assessments [5]. These measures often indicate historical periodontal disease rather than current disease activity. Consequently, when periodontal patients are exhibiting clinical enhancements, more diagnostic assessments are necessary to ascertain the disease's activity, its potential progression, and the patient's rate of response to periodontal treatment [6]. Radiotherapy serves as an essential treatment modality for head and neck cancers, utilized independently or in addition to other therapies to eliminate neoplastic cells [7]. Radiation can substantially affect the immunological milieu around the tumors, affecting both the quantity and types of immune cells that infiltrate the tumors, while also causing direct damage to tumor cells [8]. HNC radiation results in various adverse effects, notably a decrease in immune function within the periodontium and an increased vulnerability to attachment loss and periodontitis [9-12]. The severity of periodontitis may adversely impact the patient's quality of life in social, psychological, practical, and aesthetic dimensions [13,14]. Therefore, monitoring the oral health of the HNC patients suffering radiation treatment is crucial for both dental and medical professionals [10]. Various cells, containing immune cells, fibroblasts, keratinocytes, adipocytes, and muscle cells, secrete pro-inflammatory mediators [15-17]. Biomarkers, such as acute phase protein (APP) biomarkers, are molecules that may be applied to monitor health, the beginning of disease, a patient's reaction to therapy, and the results of that treatment [18]. Changes in APP concentrations are a physiological response to inflammation and tissue injury. Several diseases, including infection, trauma, infarction, inflammation, and different neoplasms, are linked to APPs [19,20]. Macrophages are considered the most common cells in impacted tissue; in addition, they play a role in infection clearance as well as immunomodulation of

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adaptive and innate immune responses [21]. Enzymes, cytokines, and inflammatory mediators like Macrophage Inflammatory Protein-1 alpha (MIP-1α/CCL3) are all found in them in significant amounts, and they are crucial in regulating bacterial diffusion in the connective tissue [22,23]. MIP-1α is considered a physiologically active chemokine that promotes the differentiation of monocytes or osteoclast progenitor cells into osteoclasts [24,25]. The side effects are considered to be increased MIP-1α levels in HNC post-RT patients, and the impact of periodontal infection on elevated MIP-1α levels. The study suggests that when periodontitis progresses to later stages, serum levels, which are known to be effective in releasing MIP-1α, may rise. The objective of this study is to perform comparative research on MIP-1α levels and clinical periodontal parameters in HNC patients' post-radiotherapy, patients without HNC, and periodontally healthy individuals.

2 Methods and Materials

2.1 Individuals selection

The Ethics Committee of the Al-Anbar Health Directorate, Ministry of Health, Iraq, granted clearance for this study (Ethics approval number: 2022057, Date: August 2, 2022). Three participant groups were involved in the study: 50 individuals had chronic periodontitis with head and neck cancer after radiotherapy (CP+HNC post-RT), 50 individuals had chronic periodontitis without head and neck cancer (CP without HNC), and 50 individuals in a control group who were periodontally healthy. Radiation-treated patients with CP+HNC were selected from those who visited the Anbar Cancer Centre (ACC) in Iraq. From August 2022 to February 2023, patients with CP, apart from those with HNCs, were selected from the Ramadi Specialized Dental Centre (RSDC), Iraq. Every participant gave written informed permission, and they were all briefed on the clinical assessment and sample procedures. According to the National Comprehensive Cancer Network (NCCN) guidelines, patients were diagnosed with HNC by oncologists at the Cancer Centre [26]. An expert dental hygienist assessed scores of periodontal characteristics.

2.2 Exclusion and Inclusion criteria

Patients who met the subsequent criteria for exclusion were not accepted: (a) a history of salivary gland or oral illness; (b) a conclusive diagnosis of systemic disease, xerostomia, or multiple sclerosis; and (c) a refusal to take part in the research. Additionally, those who satisfied one of the below inclusion requirements were accepted: (a) a head and neck cancer that is pathologically defined as a malignant neoplasm originating from epithelial cells; (b) no prior radiation exposure; (c) no distant metastases observed; (d) no prior surgical history involving the parotid, submandibular, or sublingual salivary glands; (e) a performance score between 0 and 1 point is deemed satisfactory, with a duration of survival expected to exceed one year.

2.3 Oral saliva collection

The collection samples began with the individual sitting in the dentist's chair and being instructed to rinse their mouth with water to remove any debris and contaminants that had accumulated. The timer started when an individual was told to swallow oral saliva from his mouth and throat. At one-minute intervals, unstimulated saliva was collected and placed in a beaker. Before being analyzed for pH, saliva samples were stored at -20 °C in plastic Eppendorf vials.

2.4 Measurement of periodontal parameters

A practiced dentist conducted post-radiation periodontal index assessments (6 months) following the completion of irradiation. Clinical parameters, including Plaque Index (PI), Gingival Bleeding Index (GBI), Clinical Attachment Level (CAL), and Probing Pocket Depth (PPD), were evaluated. The examination of clinical parameters included the third molars, as well as all existing teeth. The scores of (PI), (GBI), (CAL), and (PPD) were gathered at six places, with four sites per tooth, respectively. CAL was determined from the connection of the cementum and enamel to the periodontal pocket. The GBI was assessed in terms of the existence of bleeding for 10 s post-probing around 0 or 1, respectively) [27]. The principal investigator assessed a score ranging from 0 to 3 [28]. The updated periodontitis classification system proposed by Tonetti et al. (2018) was used for patient diagnosis [29].

2.5 Determination of MIP-1α levels

About (3–5mL) of venous blood was withdrawn from each case and control. Then, the sample was put in a gel tube and allowed to coagulate for 15 to 20 minutes at room temperature. Sera was isolated by using centrifugation at 3000 rpm for 15 minutes. An enzyme-linked immunosorbent test (ELISA) was used to evaluate the serum levels of MIP-1α (Human MIP-1α ELISA kit, Elabscience, Texas, USA). The test procedure was conducted according to the guidelines provided by the manufacturer.

2.6 The sample size calculation

The study's sample size analysis, which looked at two patient cohorts, had an effect size of 0.26 on the CAL and an expected SD of (0.5) [30], a statistical power of 80% and a two-tailed significance criterion of 0.05). A total of approximately 47 participants per group was deemed necessary to achieve sufficient statistical power. A total of roughly fifty patients were recruited in each group, yielding a power score of 80%.

2.7 Statistical data

The data of (frequency and percentage) were used to delineate category variables. The distribution tests indicated that the data had an abnormal distribution. Consequently, non-parametric tests were used in the statistical examination for the current study. The Kruskal-Wallis test was used for comparing various groups, while the Mann-Whitney test was used for evaluating individual groups to analyze continuous data with an unequal distribution.

The data were presented as the mean (standard deviation, SD) with the range of the interquartile. The efficacy of serum biomarkers for chronic periodontitis or the absence of HNC was evaluated by area under the curve (AUC) using the ROC curve analysis. When $p < 0.01$, the differences were considered statistically significant. All analyses were processed using GraphPad Prism (version 9) and IBM SPSS (version 27).

3 Results

3.1 The population of the study

The attributes and demographics of all groups in this research are presented in Table 1. Nasopharyngeal tumors (six keratinizing and twenty non-keratinizing carcinomas), squamous cell carcinoma (SCC), oropharyngeal tumors, laryngeal tumors, tongue tumors, and primary malignancies of unknown origin were all present in this population analysis. The overall radiation dose ranged from 5700 to 7000 cGy. In this instance, 28 patients (56%) received concurrent systemic treatment (2-3 doses) of intravenous cisplatin (300 mg/m² on days 1, 23, and 45) with radiation. This study group included chronic periodontitis patients who did not have head and neck cancers (CP without HNC) only, with an average age range of 28 to 60 years, of whom 40 (80%) were male. Over fifty percent had a smoking history, with 30 individuals (60%) and six individuals (12%) having used alcohol. The control group exhibited periodontal health.

Table 1: Characteristics and demographics of patients and control.

Parameter		CP+HNC-post RT	CP without HNC	Control	p value
Age, years Mean±SD; (min-max)		40.34±8.41; (28-62)	41.06±6.41; (28-60)	41.12±6.40; (29-60)	n.s*
Gender, n (%) Male: Female:		 40 (80) 10 (20)	 40 (80) 10 (20)	 40 (80) 10 (20)	n.s*
BMI (Weight/height^2), Mean±SD		25.01±5.73	27.91± 4.62	26.45±4.92	0.53
Stage of Tumor, n (%)	1-2	9(18)	N/A	N/A	-
	3-4	41(82)			
Smoking, n (%) Yes No		 37 (74) 13 (26)	 30 (60) 20 (40)	 0 (0.0) 50 (100)	0.002**
Alcohol abuse, n (%) Yes No		 14 (28) 36 (72)	 6 (12) 44 (88)	 0 (0.0) 50 (100)	0.004**
Type of medication, n (%)					
Radiotherapy, n (%) Chemoradiotherapy, n (%)		(42)21 (58)29	N/A N/A	N/A N/A	- -

N/A: Not Applicable, BMI: Body Mass Index, *: non-significant, **: Significant ($p<0.01$).

CP+HNC-post RT: Chronic periodontitis with head and neck cancer post-radiotherapy.

CP without HNC: Chronic periodontitis has no head and neck cancer.

3.2 Periodontal markers, Oral pH, and Hyposalivation

According to the observed results, a significant statistical difference ($p = 0.001$) was found between the clinical periodontal parameters of chronic periodontal patients with HNC post-RT and the healthy group. Additionally, there was a statistically significant difference in the oral pH values reported ($p = 0.001$) between the three groups. The average levels of hyposalivation were altered in the recorded values, compared to the CP+HNC post-RT and CP without HNC groups. In healthy subjects, hyposalivation levels were considerably elevated in the chronic periodontitis groups ($p = 0.001$), as shown in Table 2.

Table 2: Periodontal parameters in all subjects.

Parameter	CP+ HNC post-RT	CP without HNC	Control	p value
CAL (mm)	7.02 ± 0.42^a	6.34 ± 0.77^b	-	0.001^*
PPD (mm)	7.1 ± 0.45^a	6.12 ± 0.62^b	3.05 ± 0.14^c	0.001^*

PI (mm)	2.52 ± 0.62[a]	1.94 ± 1.02[b]	0.3 ± 0.45[c]	0.001*
GBI (%)	90.38 ± 0.57[a]	63.12± 0.61[b]	4.25 ± 0.38[c]	0.001*
Oral saliva pH	6.0±0.66[a]	7.77±0.27[b]	7.12±0.15[c]	0.001*
Hyposalivation (ml/min)	0.15±0.04[a]	0.30±0.04[b]	0.35±0.05[c]	0.001*

Values are expressed as mean±SD. Values with non-identical superscripts (a,b,c) are significantly different within the same parameter (*: significant at p<0.01, Mann-Whitney test).

3.3 Stage and grade indicated of periodontitis

Nine patients (18%) had stage II periodontitis, nineteen patients (38%) had stage III periodontitis, and twenty-two patients (44%) had stage IV periodontitis among the patients of CP+HNC post-RT with grade C periodontitis, which impacted fifty (100%) patients. In patients with chronic periodontitis, fifteen (30%) have stage I periodontitis, 24 (48%) have stage II periodontitis, 9 (18%) have stage III periodontitis, and 2 (4%) have stage IV periodontitis. In contrast, grade periodontitis was A/B, which was indicated by 0 (0.0%); the staging and grading of periodontitis variances were statistically significant at p = 0.001, as shown in Table 3.

Table 3: Classification of periodontitis in medical cases: categorization based on stage and grade.

Parameters		**CP+HNC post-RT**	**CP without HNC**	**p value**
Stage of periodontitis, n (%)	Stage I	0 (0.0)	15 (30)	-
	Stage II	9 (18)	24 (48)	0.0001*
	Stage III	19 (38)	9 (18)	0.0001*
	Stage IV	22 (44)	2 (4)	0.0001*
Grade of periodontitis, n (%)	Grade A	0 (0.0)	19 (38)	-
	Grade B	0 (0.0)	31 (62)	-
	Grade C	50 (100)	0 (0.0)	-

*: Statistically significant at p<0.01.

3.4 Serum MIP-1α levels are highly correlated with clinical activity

The assessment of serum MIP-1α was elevated in patients have CP+HNC after RT (102.65 [49.32-160.88] pg/mL: p < 0.0001) in comparison to CP without the HNC (63.25 [31.22-103.45] pg/mL, p < 0.0001) besides healthy cases (46.09 [29.36-66.26] pg/mL, p < 0.001), as seen in Figure 1.

3.5 Receiver Operating Curve Characteristic (ROC)

The results of Receiver Operating Characteristic (ROC) shown in Figure 2 and Table 4 indicate that serum MIP-1α (AUC = 0.990) may be more effectively determined in individuals with CP+HNC post-RT. At the same time, the indicator in CP without HNC is AUC = 0.723. In subjects with CP+ HNC after radiotherapy, MIP-1α levels were elevated (67.744 pg/ml), indicating the capacity to detect clinical improvement with a sensitivity of 94% and specificity of 100%, p = 0.001. Whereas, in patients with chronic pain without head and neck cancer, the MIP-1α levels were decreased (61.297 pg/ml), which might indicate clinical improvement, with a sensitivity of 60% and specificity of 96%, p = 0.001.

3.6 Serum MIP-1α levels exhibit a substantial correlation with periodontitis indicators, saliva pH, and hyposalivation

The coefficient of Pearson's correlation indicated a favorable statistical significance between periodontal measurement scores: (PI), (GBI), (PPD), and (CAL), and blood MIP-1α levels.

Furthermore, hyposalivation and saliva pH exhibited a statistically significant negative connection with serum MIP-1α levels, as seen in Figure 3 A-F).

4 Discussion

This study aimed to evaluate the relationship between blood levels of MIP-1α and clinical periodontal indicators in patients with head and neck cancer after radiotherapy. Consequently, we investigated the diagnostic and measurement of serum MIP-1α levels using ELISA for potential periodontal tissue deterioration induced by HNC radiation. To our knowledge, no previous studies have examined serum levels of MIP-1α in chronic periodontal patients with or without head and neck cancer after radiation. Our findings corroborate and enhance prior research. Few studies have been reported about the relationship between head and neck cancer (HNC) and oral complications across various countries using diverse methodologies [3]. Clinical

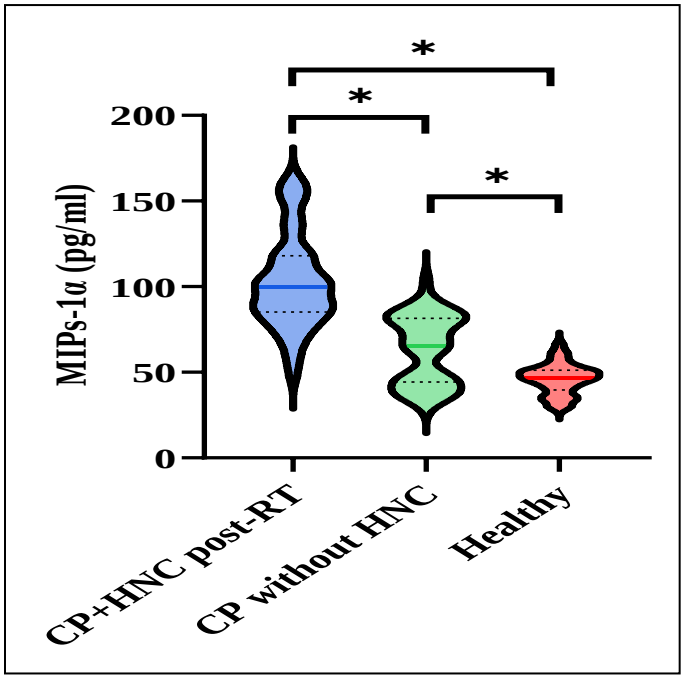

Figure 1: The mean changes of MIP-1α levels in different groups as compared between individuals. (Mann-Whitney or Kruskal-Wallis test, *p<0.0001).

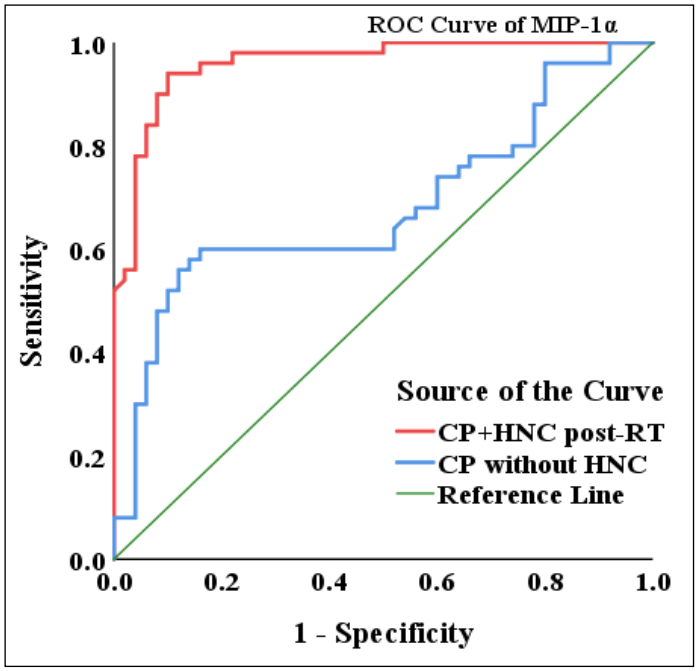

Figure 2: The (ROC) pattern of MIP-1α prediction in the selected groups.

Table 4: The (ROC) analyses of MIP-1α levels to estimate clinical abatement.

Variables	Groups	AUC	Cut-off value	Sensitivity	Specificity	p value
MIP-1α (pg/ml)	CP+HNC post-RT	0.990	67.744	94%	100%	0.001*
	CP without HNC	0.723	61.297	60%	96%	0.001*

*: Statistically significant at p<0.01.

features indicated that different types of periodontitis had different immune responses, even though many cytokines and chemokines need to be further studied [4,31,32]. Saliva pH levels in subjects with CP+HNC post-RT were altered; conversely, patients with chronic periodontitis devoid of HNC showed an increase, in contrast to the generally neutral pH of healthy controls. These results correspond with previous studies demonstrating that persons who have undergone radiation have moderately acidic saliva due to diminished buffering capacity, mainly arising from radiation-induced damage to serous acini [5]. Radiotherapy leads to alterations in saliva, affecting its volume, characteristics, and microbial makeup both during and following treatment [33]. The findings corroborate and expand upon prior research indicating a substantial breakdown of the periodontium tissues, besides the destruction of periodontal attachment, owing to radiation [12,34]. The first exposure to radiation correlates with a decline in periodontal health, which is dose-dependent [35]. Furthermore, the oral microbiota undergoes alterations due to the use of radiotherapy in the head and neck regions, transitioning to microorganisms associated with periodontitis [36]. The updated periodontal classification system indicated that the majority of significant changes in periodontal metrics were seen in Clinical Attachment Level (CAL), which was associated with a fast advancement of periodontitis from grade A/C [29]. It is a chemokine that attracts inflammation to the affected region, enhances the production of MIP-1 alpha through epithelial cells of the gingiva to facilitate an immediate inflammatory outcome, and recruits B lymphocytes during the latter periods of periodontal disease [37,38]. Previous investigations indicated that the expressions of MIP-1 alpha and its corresponding receptor CCR5 in gingival tissue were increased in both periodontitis groups, compared to the control group, with elevated levels seen in the severe periodontitis group, as opposed to the chronic periodontitis group [39]. A research study by Subramanyam, Cheppali et al estimated the levels of MIP-1α in gingival crevicular fluid and serum in periodontally healthy and diseased, besides aggressive periodontitis, and found that MIP-1α levels were considerably greater in the diseased subjects, compared to the healthy group [40]. According to previous literature [41], the salivary MIP-1 alpha levels were assessed in different stages of periodontitis and noted that the level of MIP-1 alpha elevated in accordance with the progression of periodontitis. In addition, [42]

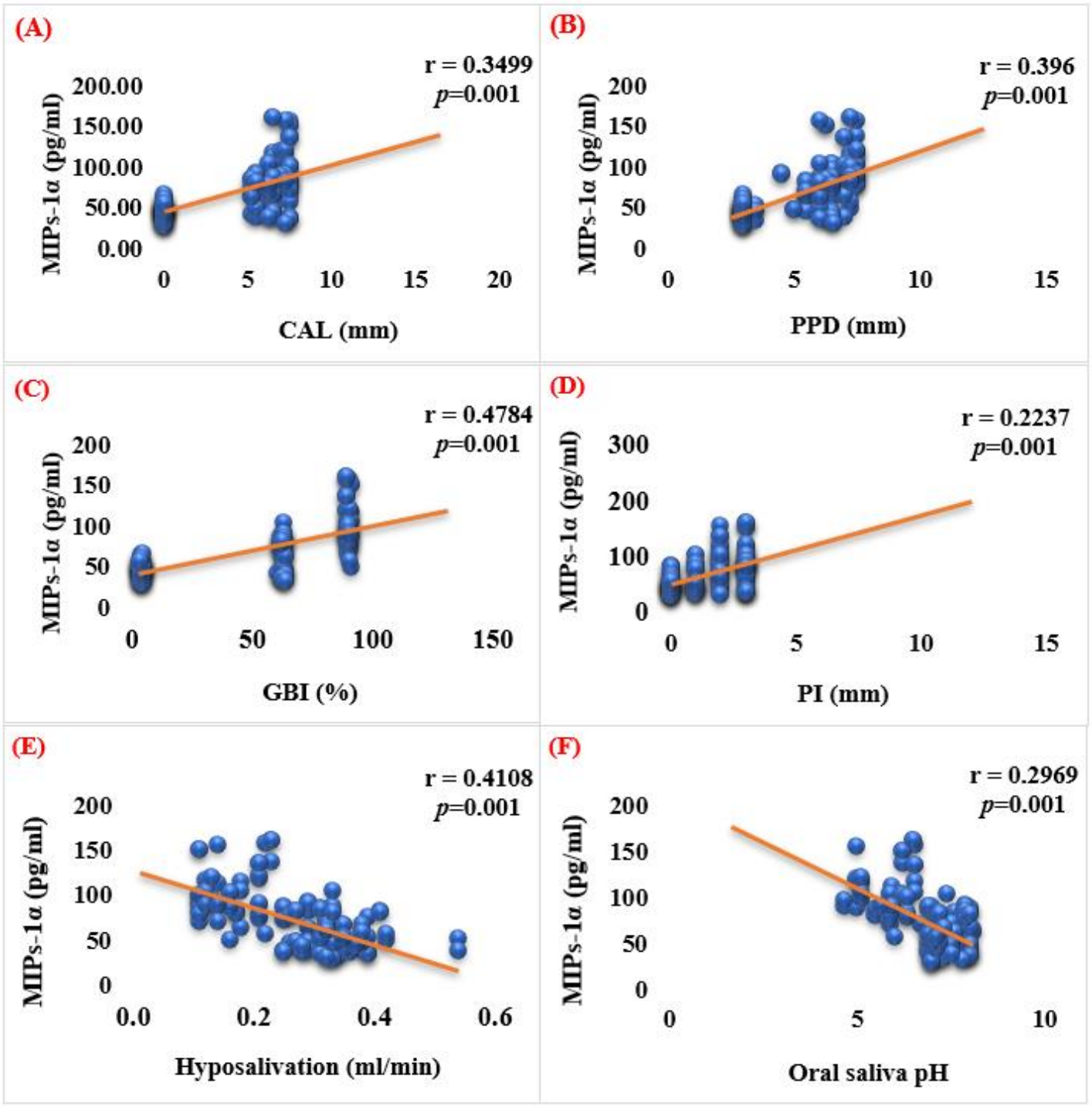

Figure 3: Serum levels of MIP-1α (pg/ml) correlated with: (A) CAL Clinical Attachment Level (mm), (B) PPD Probing Pocket Depth (mm), (C) GBI Gingival Bleeding Index (%), (D) PI Plaque Index (mm), (E) Hyposalivation (ml/min), (F) Oral saliva pH.

A pilot study identified the inflammatory profile of oral cancer patients and compared it with that of individuals with periodontal disease and healthy controls, analyzing salivary cytokine levels, including MIP-1α. Its expression is restricted to the tissue that connects close to the pocket cells of the afflicted gingival tissues [43]. A previous study showed that MIP-1α has a function in macrophage migration to periodontal tissues [44,45].

However, this clinical study's significance won't be taken into account until the biomarker's sensitivity and specificity for identifying the right disease are assessed [46]. The Receiver

Operating Characteristic (ROC) analysis for MIP-1α indicates its potential for use in diagnostics in subjects CP+HNC after radiotherapy. It was demonstrated that the sensitivity and specificity of MIP-1α in sera were useful indicators for distinguishing between chronic periodontitis without HNC outcomes and CP+HNC post-RT. Previously, the study found MIP-1α to be a more accurate biomarker for differentiating between gingivitis and healthy tissue, particularly in terms of sensitivity and specificity. However, MIP-1α and MCP-1 both demonstrated a sensitivity and specificity of 100% in distinguishing periodontitis [45]. A study by Fine et al. (2009) found that salivary MIP-1α has a sensitivity of 90.3% and specificity of 85.7% when used to diagnose advanced periodontitis. Subsequently, the same authors found that MIP-1α in GCF had a sensitivity of 93.33% and a specificity of 98.73% [47]. The results of this investigation showed that saliva pH and hyposalivation were statistically significantly negatively correlated, whereas MIP-1α levels were positively correlated with the clinical periodontal features evaluated. Moreover, previous studies evaluating the MIP-1α biomarker in serum, GCF, or saliva have demonstrated a comparable association with clinical features of periodontal disease [48,49]. A previous study by de Lima, Acevedo et al. indicates that MIP-1α is the most prevalently secreted chemokine in periodontitis cells. Its expression is limited to the connective tissue close to the pocket cells of the afflicted gingival tissues [43]. Nonetheless, the study's shortcomings include a restricted sample size, a brief duration, and the inability to entirely exclude several confounding variables, such as the type of chemotherapy administered. Lastly, a substantial body of evidence supports the correlation between periodontitis, HNC after radiation therapy (RT), and the inflammatory components of each condition. Currently, there is little evidence available on the role of biomarkers in the proposed causal relationship between chronic periodontitis (CP) associated with head and neck cancer (HNC) post-radiotherapy and chronic periodontitis (CP) without HNC. This arises from considerable variability in the investigation's design, participant demographics, assay methods, and evaluated biomarkers. In the future, a prospective study and a randomized controlled trial with biological data are needed to establish the causal relationship between periodontal inflammation and head and neck cancer after radiation. Further inquiry is necessary to elucidate the function of biomarkers in connecting these disorders.

Conclusions

In conclusion, serum macrophage inflammatory protein-1α (MIP-1α) is a promising biomarker for detecting chronic periodontitis with head and neck cancer after radiotherapy (CP+HNC post-RT). It was highly expressed in HNC patients and showed a positive correlation with periodontal disease severity. Elevated MIP-1α levels were associated with damaged periodontium response to radiation.

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Authors contribution

AA Al-Kubaisi: Conceptualization, Methodology, Investigation, Writing-Original draft, Project administration, SAA: Resources, Software, Formal analysis, Data curation, MYM: Conceptualization, Methodology, Investigation, Writing- Review & editing. AAF: Formal analysis, Data curation, Methodology, and Investigation, ERA: Conceptualization, Project administration, and Supervision. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that they have no known competing financial interests.

References

[1] El-Mahdy, H. A., Mohamadin, A. M., Abulsoud, A. I., Khidr, E. G., El-Husseiny, A. A., Ismail, A., Elsakka, E. G., Mokhlis, H. A., El-Husseiny, H. M., and Doghish, A. S. miRNAs as potential game-changers in head and neck cancer: Future clinical and medicinal uses. *Path Res Pract* **245**, 154457, doi.org/10.1016/j.prp.2023.154457 (2023).

[2] Khidhir, K.G., Expression Profile of PBRM1, PLAU and CLEC3B Genes in Head and Neck Squamous Cell Carcinoma. *Passer J Basic Appl Sci* **5**, 128-133, doi: 10.24271/psr.2023.376917.1195 (2023).

[3] Vu, H., Shin, Y.-J., Kong, M.-S., and Kim, H.-D. Smoking and drinking adjusted association between head and neck cancers and oral health status related to periodontitis: a meta-analysis. *J Korean Med Sci* **36**, 1-16, doi: 10.3346/jkms.2021.36.e98 (2021).

[4] Kawamoto, D., Amado, P. P. L., Albuquerque-Souza, E., Bueno, M. R., Vale, G. C., Saraiva, L., and Mayer, M. P. A. Chemokines and cytokines profile in whole saliva of patients with periodontitis. *Cytokine* **135**, 155197, doi:10.1016/j.cyto.2020.155197 (2020).

[5] Tiwana, M. S., Mahajan, M. K., Uppal, B., Koshy, G., Sachdeva, J., Lee, H. N., and Talole, S. D. Whole saliva physico-biochemical changes and quality of life in head and neck cancer patients following conventional radiation therapy: a prospective longitudinal study. *Indian J Cancer* **48**, 289-295, doi: 10.4103/0019-509X.84918 (2011).

[6] Müller, V. J., Belibasakis, G. N., Bosshard, P. P., Wiedemeier, D. B., Bichsel, D., Rücker, M., and Stadlinger, B. Change of saliva composition with radiotherapy .*Arch Oral Biol* **106**, 104480, https://doi.org/10.1016/j.archoralbio (2019).

[7] Muftuoglu, C., Mert, U., Akagunduz, O. O., Tavlayan, E., Al-Omar, A., Asadi, M., and Caner, A. Profiling of circRNA expressions in radiation-treated head and neck cancer cells and the potential role of circPVT1. *Arch Oral Biol* **150**, 105690, doi: 10.1016/j.archoralbio (2023).

[8] Karam, S.D. and D. Raben, Radioimmunotherapy for the treatment of head and neck cancer. *The Lancet Oncol* **20**, 404-416, (2019).

[9] Schuurhuis, J. M., Stokman, M. A., Witjes, M. J. H., Reintsema, H., Langendijk, J. A., Vissink, A., and Spijkervet, F. K. L. Patients with advanced periodontal disease before intensity-modulated radiation therapy are prone to develop bone healing problems: a 2-year prospective follow-up study. *Support Care Cancer* **4**, 1133-1142, doi: 10.1007/s00520-017-3934-y (2018).

[10] Irie, M.-S., Mendes, E.-M., Borges, J.-S., Osuna, L.-G.-G., Rabelo, G.-D., and Soares, P.-B.-F. Periodontal therapy for patients before and after

radiotherapy: A review of the literature and topics of interest for clinicians. *Med Oral, Patol Oral Cir Bucal* **23**, 524-530, doi:10.4317/medoral.22474 (2018).

[11] Sroussi, H. Y., Epstein, J. B., Bensadoun, R.-J., Saunders, D. P., Lalla, R. V., Migliorati, C. A., Heaivilin, N., and Zumsteg, Z. S. Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions ,dental caries, periodontal disease, and osteoradionecrosis. *Cancer Med* **6**, 2918-2931, doi: 10.1002/cam4.1221 (2017).

[12] Ammajan, R. R., Joseph, R., Rajeev, R., Choudhary, K., and Vidhyadharan, K. Assessment of periodontal changes in patients undergoing radiotherapy for head-and-neck malignancy. *Journal of Radiation and Cancer Research* **9**, 630-637, 10.4103/0973-1482.126461 (2020).

[13] Ferreira, M. C., Dias-Pereira, A. C., Branco-de-Almeida, L. S., Martins, C. C., and Paiva, S. M. Impact of periodontal disease on quality of life: a systematic review. *J Periodontal Res* **52**, 651-665, doi: 10.1111/jre.12436 (2017).

[14] Rasheed, H.H. and N.A. Hama Amin, Reliability of CBCT Dataset in Determination of Surgical Approaches of Maxillary Impacted Canine Teeth. *Passer J Basic Appl Sci* **5**, 1-6, 10.24271/psr.2022.356606.1155 (2023).

[15] Scheller, J., Chalaris, A., Schmidt-Arras, D., and Rose-John, S. The pro-and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* **1813**, 878-888, doi.:10.1016/j.bbamcr.2011.01.034 (2011).

[16] Murakami, T., Takahata, Y., Hata, K., and Nishimura, R. Role of interleukin-1 and inflammasomes in oral disease. *J Oral Biosci* **62**, 242-248, doi: 10.1016/j.job.2020.07.003 (2020).

[17] Mustafa, M.S. and R.M. Abdullah, Predictive Role of Key Pro-Inflammatory Cytokines in Iraqi Patients Developed Acute Myeloid Leukemia. *Passer J. Basic Appl Sci* **7**, 623-629, doi: 10.24271/psr.2025.485642.1806 (2025).

[18] Zia, A., Khan, S., Bey, A., Gupta, N., and Mukhtar-Un-Nisar, S. Oral biomarkers in the diagnosis and progression of periodontal diseases. *Biology and Medicine* **3**, 45-52, 2011.

[19] Kushner, I., The phenomenon of the acute phase response. *Ann N Y Acad Sci* **389**, 39-48, doi: 10.1111/j.1749-6632.1982.tb22124.x (1982).

[20] Al-Kubaisi, A. A., Nabeel, Z., Adem, Ş., and Mula-Hussain, L. Changes and Roles of IL-6, hsCRP, and proCT in Patients with Chronic Periodontitis in Head and Neck Cancer Pre/Post Radiotherapy. *Al-Rafidain J Med Sci* **7**, 248-254, doi: 10.54133/ajms.v7i1.1248 (2024).

[21] Hancková, M., Miháliková, L., Pastoreková, S., and Betáková, T. Hypoxia alters the immune response in mouse peritoneal macrophages infected with influenza a virus with truncated NS1 protein. *Cytokine* **164**, 156138, do:10.1016/j.cyto.2023.156138 (2023).

[22] Cafiero, C., et al., Predictive periodontitis: The most promising salivary biomarkers for early diagnosis of periodontitis. *J Clin Med* **10**, 1488, doi.org/10.3390/jcm10071488 (2021).

[23] Al-Kubaisi, A. A., Ghazi, M. A., Majeed, N. S., Aldelaimi, E. R., and Enezei, H. H. Soluble urokinase plasminogen activator receptor (suPAR) is a potential biomarker of stage III-IV, grade C periodontitis through the impact of post-radiotherapy on head and neck cancer patients. *BMC Oral Health* **24**, 1144, doi.org/10.1186/s12903-024-04801-w (2024).

[24] Wu, X., et al., MIP-1α induces inflammatory responses by upregulating chemokine receptor 1/chemokine receptor 5 and activating c-Jun N-terminal kinase and mitogen-activated protein kinase signaling pathways in acute pancreatitis. *J Cellular Biochem* **120**, 2994-3000, doi.org/10.1002/jcb.27049 (2019).

[25] Subramanyam, M. B. D., Cheppali, S. R., Anumla, D., Sighinam, B., Prasuna, E., and Reddy, R. N. Estimation of macrophage inflammatory protein-1α (MIP-1 α) levels in serum and gingival crevicular fluid in periodontal health, disease, and after treatment—A clinico-biochemical study. *Journal of Dr. YSR University of Health Science* **8**, 107-113, doi: 10.4103/JDRNTRUHS.JDRNTRUHS_87_18 (2019).

[26] Colevas, A. D., Yom, S. S., Pfister, D. G., Spencer, S., Adelstein, D., Adkins, D., Brizel, D. M., Burtness, B., Busse, P. M., and Caudell, J. J. NCCN guidelines insights: head and neck cancers, version 1.2018. *J Nati Compr Canc Netw* **16**, 470-490, doi.org/10.6004/jnccn.2018.0026 (2018)

[27] Barnes, C. M., Russell, C. M., Reinhardt, R. A., Payne, J. B., and Lyle, D. M. Comparison of irrigation to floss as an adjunct to tooth brushing: effect on bleeding, gingivitis, and supragingival plaque. *J Clin Dent* **16**, 71-7, 2005.

[28] Silness, J. and H .Löe, Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* **22**, 121-135, doi: 10.3109/00016356408993968 *(*1964).

[29] Tonetti, M.S., H. Greenwell, and K.S. Kornman, Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol* **89**, S159-S172, doi: 10.1002/JPER.18-0006 (2018).

[30] Isola, G., Lo Giudice, A., Polizzi, A., Alibrandi, A., Murabito, P., and Indelicato, F. Identification of the different salivary Interleukin-6 profiles in patients with periodontitis: A cross-sectional study .*Arch Oral Biol* **122**, 104997, doi: 10.1016/j.archoralbio.2020.104997 (2021).

[31] Albukhalefah, A.K.M. and H.A.N. Nayyef, Association between biochemical leptin and some biochemical markers in Iraqi patients with obesity and periodontal. *Rom J Diabetes Nutr Metab Dis* **5**,1-8, 10.46389/rjd-2024-569 (2024).

[32] Al-Kubaisi, A. A., Mssdf, A. K., Awad, S. A., Ibrahim, K. H., Mukhlif, M. Y., and Aldelaimi, E. R. Assessment of Serum Ferritin Levels and Periodontal Indices in Patients with Chronic Periodontitis Post/Pre Radiation Treatment of Head and Neck Cancer. *Odovtos-Int J Dent Sc* **27**, 464-475, doi: 10.15517/kvtbk628 (2025).

[33] Müller, V. J., Belibasakis, G. N., Bosshard, P. P., Wiedemeier, D. B., Bichsel, D., Rücker, M., and Stadlinger, B. Change of saliva composition with radiotherapy. *Arch Oral Biol* **106**, 104480, doi: 10.1016/j.archoralbio (2019).

[34] Schuurhuis, J. M., Stokman, M. A., Witjes, M. J. H., Reintsema, H., Langendijk, J. A., Vissink, A., and Spijkervet, F. K. L. Patients with advanced periodontal disease before intensity-modulated radiation therapy are prone to develop bone healing problems: a 2-year prospective follow-up study. *Support Care Cancer* **26**, 1133-1142, doi: 10.1007/s00520-017-3934-y (2018).

[35] H Hommez, G. M. G., De Meerleer, G. O., De Neve, W. J., and De Moor, R. J. G. Effect of radiation dose on the prevalence of apical periodontitis—a dosimetric analysis. *Clin Oral Invest* **16**, 1543–1547, doi: 10.1007/s00784-011-0665-1 (2012).

[36] Laheij, A.M. and J.J. de Soet, Can the oral microflora affect oral ulcerative mucositis? *Curr Opin Support Palliat Care* **8**, 180-7, doi: 10.1097/SPC.0000000000000053 (2014).

[37] Bhavsar ,I., C.S. Miller, and M. Al-Sabbagh, Macrophage inflammatory protein-1 alpha (MIP-1 alpha)/CCL3: as a biomarker. *General Methods in Biomarker Research and Their Applications* **27**, 223-249, doi: 10.1007/978-94-007-7696-8 (2015).

[38] Grande, M. A., Belstrøm, D., Damgaard, C., Holmstrup, P., Könönen, E., Gursoy, M., and Gursoy, U. K. Salivary concentrations of macrophage activation-related chemokines are influenced by non-surgical periodontal treatment: a 12-week follow-up study. *J Oral Microbiol* **12**, 1694383, doi: 10.1080/20002297.2019.1694383 (2020).

[39] Ramadan, D. E., Hariyani, N., Indrawati, R., Ridwan, R. D., and Diyatri, I. Cytokines and chemokines in periodontitis. *Eur J Dent* **14**, 483-495, doi: 10.1055/s-0040-1712718 (2020).

[40] Subramanyam, M. B. D., Cheppali, S. R., Anumla, D., Sighinam, B., Prasuna, E., and Reddy, R. N. Estimation of macrophage inflammatory protein-1a (MIP-1 a) levels in serum and gingival crevicular fluid in periodontal health, disease, and after treatment—A clinico-biochemical study. *J Dr. NTR University of Health Sci* **8**, 107-113, doi: 10.4103/JDRNTRUHS.JDRNTRUHS_87_18 (2019).

[41] Muhammed, R.M., ASSESSMENT OF SALIVARY IL-6, IL-12, and MIP-1α LEVELS IN DIFFERENT STAGES OF PERIODONTITS. *University of Baghdad* (2022),

[42] Laliberté, C., Ng, N., Eymael, D., Higgins, K., Ali, A., Kiss, A., Bradley, G., and Magalhaes, M. A. Characterization of oral squamous cell carcinoma associated inflammation: a pilot study. *Front Oral Health* **2**, 740469, doi: 10.3389/froh (2021).

[43] de Lima, C. L., Acevedo, A. C., Grisi, D. C., Taba Jr, M., Guerra, E., and De Luca Canto, G. Host-derived salivary biomarkers in diagnosing periodontal disease: Systematic review and meta-analysis. *J Clin. Periodontol* **43**, 492-502, doi: 10.1111/jcpe.12538 (2016).

[44] Gemmell, E., C. Carter, and G. Seymour, Chemokines in human periodontal disease tissues. *Clin Exp Immuno* **125**, 134-141, doi: 10.1046/j.1365-2249.2001.01511.x (2001).

[45] Nisha, K. J., Suresh, A., Anilkumar, A., and Padmanabhan, S. MIP-1α and MCP-1 as salivary biomarkers in periodontal disease. *Saudi Dent J* **30**, 292-298, doi: 10.1016/j.sdentj.2018.07.002 (2018).

[46] Fine, D. H., Markowitz, K., Furgang, D., Fairlie, K., Ferrandiz, J., Nasri, C., McKiernan, M., Donnelly, R., and Gunsolley, J. Macrophage inflammatory protein-1α: a salivary biomarker of bone loss in a longitudinal cohort study of children at risk for aggressive periodontal disease*? J Periodontol* 80, 106-113, doi: 10.1902/jop.2009.080296 (2009).

[47] Fine, D. H., Markowitz, K., Fairlie, K., Tischio-Bereski, D., Ferrandiz, J., Godboley, D., Furgang, D., Gunsolley, J., and Best, A. Macrophage inflammatory protein-1α shows predictive value as a risk marker for subjects and sites vulnerable to bone loss in a longitudinal model of aggressive periodontitis. *PloS one* **9**, 98541, doi: 10.1371/journal.pone.0098541 (2014).

[48] Pradeep, A., H. Daisy, and P. Hadge, Serum levels of monocyte chemoattractant protein-1 in periodontal health and disease. *Cytokine* **47**, 77-81, doi: 10.1016/j.cyto.2009.05.012 (2009).

[49] Sexton, W. M., Lin, Y., Kryscio, R. J., Dawson Iii, D. R., Ebersole, J. L., and Miller, C. S. Salivary biomarkers of periodontal disease in response to treatment. *J Clin Periodontol* **38**, 334-341, doi: 10.1111/j.1600-051X.2011.01706.x (2011).