Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 04-2023
DOI 10.17605/OSF.IO/8PZ4E

EXPRESSION OF IL-6, IL-8 & SOLCD44 IN PATIENTS WITH ORAL POTENTIAL MALIGNANT DISEASES AND ORAL SQUAMOUS CELL CARCINOMA

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Abstract

Background: The aim of the current study was to assess and compare the expression of IL6, IL8, CD44 in patients with oral potential malignant diseases and oral squamous cell carcinoma. **Methodology:** A total of 100 subjects were selected and divided into three groups: - Oral premalignant diseases (OPMD) group consisted of 40 patients, Oral squamous cell carcinoma (OSCC) group consisted of 40 patients who were recently diagnosed with OSCC and Healthy group consisted of 20 healthy volunteers as controls with no oral or systemic lesions. Demographic variables such as age, gender, site of the lesion & habits were recorded for all patients. Unstimulated saliva samples were collected and analyzed for the expression of salivary biomarkers namely IL-6, IL-8 and CD44 using ELISA and the readings were calculated with the UV Spectrophotometer at 450nm. **Results:** The results of the present study showed that the higher percentages of males had OSCC and OSMF. Among the various sites of the oral cavity, buccal mucosa showed a higher prevalence of OSCC. Tobacco chewing habit was seen to be prevalent in OSMF and OSCC groups when compared with the other habits. IL-6, IL-8 and CD44 expression was found to be elevated in the OSCC group followed by OPMD however IL-6 levels was found to be statistically significant (p value=0.005). **Conclusion:** From the results of the present study, we inferred that IL-6 can be used as a potential salivary biomarker in the early identification and prevention of precancerous lesions and can be used as a therapeutic tool in assessing the risk for the progression of oral squamous cell carcinoma.

Keywords: Oral premalignant lesions, Oral squamous cell carcinoma, Biomarker, Saliva, Cancer, Carcinogen.

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INTRODUCTION

Oral cancer is the most common form of cancer in India.[1] Approximately, 90% of oral cancer is squamous cell carcinoma which is seen typically on the lip or lateral part of the tongue usually as a lump or ulcer.[2] The reason for this high prevalence of oral cancer in India is primarily tobacco consumption in the form of gutka, quid, snuff or misri.[1] Some of the signs and symptoms of OSCC are proliferative ulcerative oral lesions with induration and the presence of regional node involvement. Human papillomavirus (HPV), poor oral hygiene, immunocompromised individuals including those suffering from acquired immunodeficiency syndrome (AIDS), infections such as syphilis and candidiasis, diseases including diabetes and low fruit and vegetable intake have also been identified as contributing factors to OSCC.[3]

Neoplastic progression accompanies molecular changes and over expression of certain biomarkers such as epidermal growth factor receptor, matrix metalloproteinase (MMPs), and vascular endothelial growth factor (VEGF) and also is generally proceeded by premalignant lesions or conditions such as lichen planus, erythroplakia, leucoplakia, oral sub mucous fibrosis (OSMF) and lichen planus.[4]In case of the OPMD like leukoplakia and OSMF the risk of progression into malignancy is typically determined based on clinical assessment and histopathological evaluation of biopsied materials. Therefore, there is a need to find predictive biomarkers that help in detecting the progression of a potentially malignant lesion into malignancy which is predominantly expressed in most of bodily fluids.[5]

The use of saliva comprises a non-invasive, easy, and rapid to collect, and yet, cost-effective specimen. There are shreds of evidence in recent decades that these biomarkers are produced in a dysregulated fashion in oropharyngeal squamous cell carcinomas and that they have roles in cell growth, invasion, immune status, interruption of tumour suppression, and even in cell survival.[6]

Salivary diagnostics for OSCC, would also comprise a suitable tool for population screening, monitoring of patients at risk of recurrent tumours, and consequently for improving the survival rate of patients with this disease. Some of the biomarkers which have been shown to be significantly altered in OSCC patients are SCC, TPS, LDH, IgG, IAP, CEA, IGF.[7,8] Some markers have been already shown as a diagnostic tool in Oral Squamous Cell Carcinoma (OSCC).[9] In the present study, we have focussed on IL-6, IL-8, CD44.

Moreover, the diagnostic utility of these salivary biomarkers such as IL6, IL8, and Sol CD44 in detecting OSCC / OPMD is essential for the early resumption of the course lesion.[10] IL-6 and IL-8 are cytokines that play important roles in inflammation and immune response, and their expression in saliva has been shown to be associated with oral cancer and oral potentially malignant disorders (OPMD).[10] Soluble CD44 (sCD44) is a protein that is involved in cell adhesion and is also implicated in cancer progression.[11] Hence the current study aimed to assess and compare the prevalence of age, sex and the various adverse habits such as tobacco chewing, alcohol consumption along with the expression of IL6, IL8, CD44 in patients with oral potential malignant

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diseases and oral squamous cell carcinoma in order to scrutinize their role in the progression of oral cancer.

2 METHODOLOGY

2.1 STUDY PROTOCOL

The present research was carried out from December 2019 to November 2020 in Chennai, Tamil Nadu, and India. 120 patients were selected for the study, of which 20 patients were eliminated as 10 refused to participate and 10 were not cooperative. Finally, after screening, 100 subjects were selected from the Department of Periodontology, Meenakshi Ammal Dental College and Hospital, Chennai. The study was approved by the Institutional Review Board, Meenakshi Academy of Higher Education and Research, Chennai. The study procedure was explained in detail and written informed consent was obtained from all the participants of the study (Figure 1).

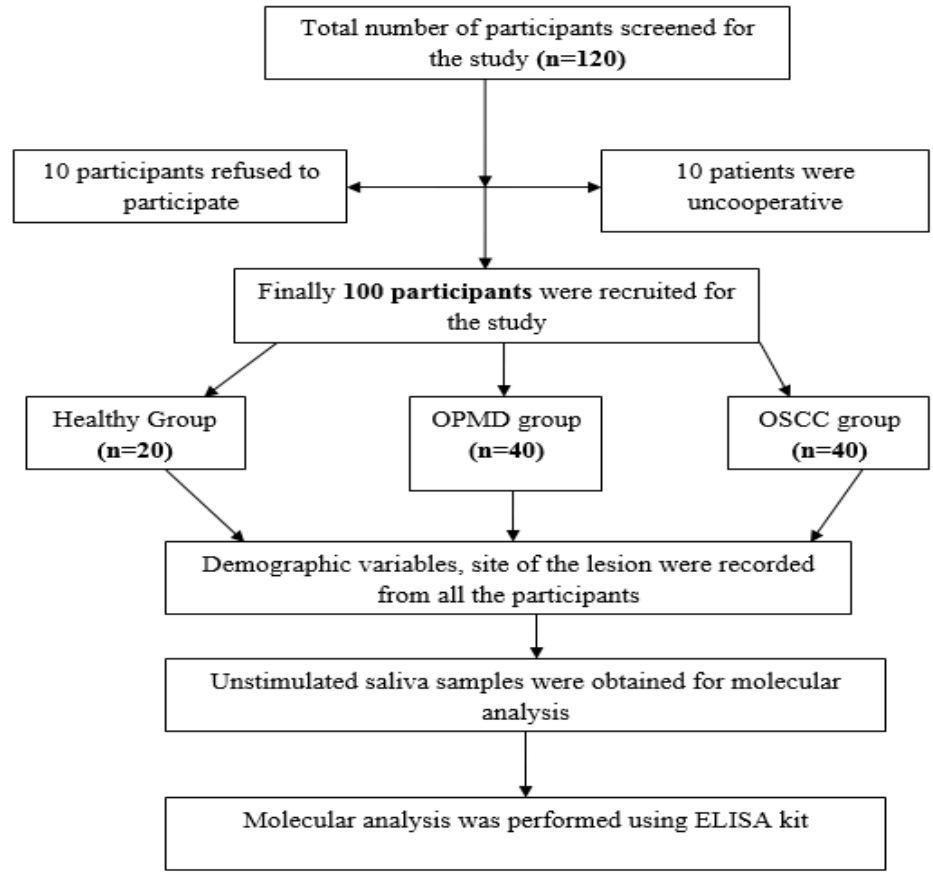

Figure 1: Study flow chart

The sample size required for the study was calculated by considering the prevalence of oral cancer and premalignant disease in the region from hospital records, and also

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including evidence from previous literature. A power analysis was done which showed 95% power with a minimum sample of 20 subjects in all three groups.

The research was carried out in accordance with the 1975 Helsinki Declaration, as revised in 2013. The study population consisted of both male and female patients who were divided into three groups as follows: - OPMD group- 40 patients with oral premalignant diseases, OSCC group- 40 patients who were recently diagnosed and untreated OSCC and Healthy group -20 healthy volunteers as control with no oral lesions. All participants who were recruited for the study were within the age group of 30 to 75 years. For the OSCC and OPMD groups, subjects with confirmed histopathological diagnoses for OSCC and other OPMD from biopsy reports by the participating institutes were recruited. For the healthy control group, volunteers who were otherwise systemically healthy and who did not have a clinical or histopathological diagnosis of OSCC, or oral potentially malignant disorders, were selected. Volunteers who could not give their informed written consent, pregnant and lactating mothers, participants with immunosuppression, and subjects who suffered at the time, or previously from any other malignancies, were excluded.

2.2 PARAMETERS ASSESSED

Demographic information was recorded which included the age of the participants, gender, and habit history. The other details assessed included the site and distribution of the oral premalignant lesion in OPMD and OSCC groups.

2.3 COLLECTION OF UNSTIMULATED SALIVA SAMPLES

Unstimulated Saliva collection performed using the “draining (drooling)” method as described by Navazesh and Christensen. 5 mL of unstimulated saliva was taken from each subject for the examination. After the collection, the salivary samples were subjected to centrifugation at 3500 rpm for 15 minutes at 4°C. Samples contaminated with blood were discarded. The supernatants of the collected samples were separated and stored at −70 °C until analysis.

2.4 MOLECULAR ANALYSIS

The samples were subjected to molecular analysis using the ELISA method. Enzyme-linked immunosorbent assay (ELISA) kits for the biomarkers such as IL6, IL8, and soluble CD44 were used for detecting the protein concentration, wherein the detection range for all three ELISA kits was 5.6–450 pg/mL. Following optimization, a 1:10 dilution was used for the OSCC and OPMD groups. Each sample was tested and the readings were calculated with UV-Spectrophotometer at 450nm. The data were calculated from the mean of tests for each sample.

2.6 STATISTICAL ANALYSIS

Data collected were expressed in the form of mean and standard deviation. The statistical analysis was done by ANOVA using SPSS software. Two-way analysis of variance (ANOVA) was used to compare the OD450 obtained in ELISA with recombinant tM2e-MBP and M2e-MBP proteins and M2e antibody positive and negative sera. Mean comparison was also carried out by Tukey's test using MINITAB 16

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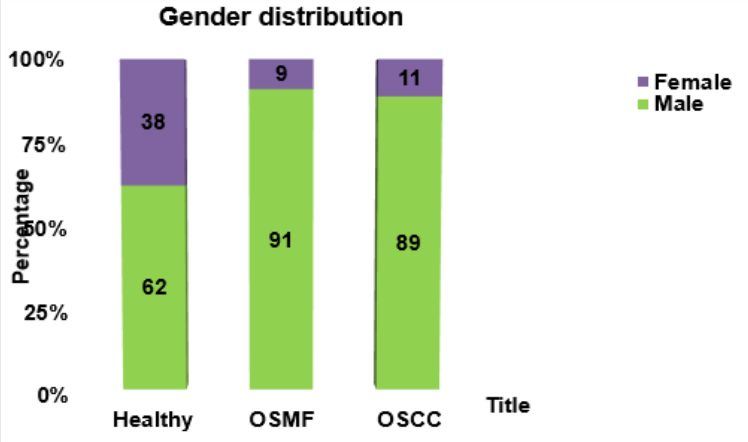
package. Regression modeling of ELISA OD450 with different dilutions of M2e-MBP and tM2e-MBP was performed using Microsoft Office Excel 2010. For applicability experiment, T-test (MINITAB 16 package) was used to compare the OD450 of tM2e-MBP versus M2e-MBP with different positive serum samples. Analysis of variance and mean comparison using Tukey's test was used to compare the effects of infected, negative and vaccinated field sera based on corrected OD450. p value ≤ 0.05 is considered to be statistically significant.

RESULTS

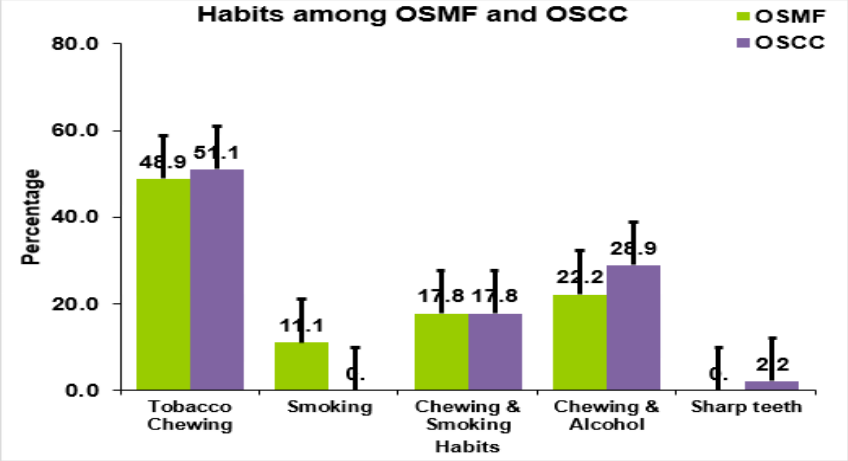
The results of the study showed that the majority of the participants included in the study were males and fell under the age range of 30 to 70 years (Graph 1).

Graph 1: Gender distribution of the participants in percentage among the groups



It was also observed that all the patients with OPMD and OSCC patients either smoked cigarettes or chewed tobacco, consumed areca nuts, or were alcoholic (Graph 2).

Graph 2: Various habits among OSMF and OSCC patients



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On assessing the site distribution of these OSCC lesions, it was found that the majority of these lesions (71%) were found in relation to the buccal mucosa. (Graph 3).

Graph 3: Percentage of presence of Oral squamous cell carcinoma among various sites in the oral cavity

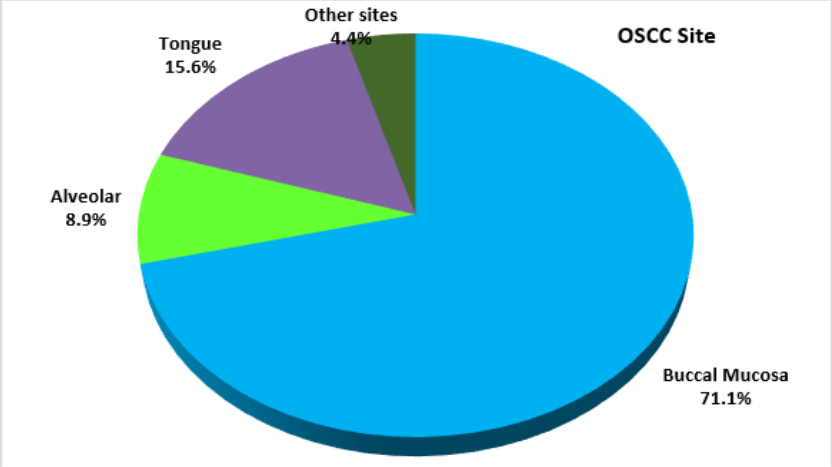

Table 1 depicts the percentage prevalence of age, gender, adverse habits and distribution of the lesion among the groups. We found that 33% of the population examined were under the age group of 40 years in all three groups. The majority of the population in the OSCC group (88.9%) and OMPD (91.2%) group were males. On comparing the distribution of these lesions, most of them were found in the buccal mucosa of OMPD patients (100%) and OSCC patients (71.1%). On examining the prevalence of adverse habits, it was found that most of the patients who had OSMF (48.9%) and OSCC (51.1%) had the habit of tobacco chewing followed by tobacco chewing and alcohol consumption in both groups respectively. (22.2% and 28.9%) (Table 1).

Table 1: Comparison of demographic variables and distribution of lesions among all the three groups

		Group							
		Healthy		OSMF		OSCC		Total	
		N	%	N	%	N	%	N	%
Gender	Male	28	62.2	41	91.1	40	88.9	109	80.7
	Female	17	37.8	4	8.9	5	11.1	26	19.3
Age group	<= 40 years	15	33.3	15	33.3	15	33.3	45	33.3
	> 40 years	30	66.7	30	66.7	30	66.7	90	66.7
Site	No	45	100	0	0	0	0	45	33.3
	Buccal Mucosa	0	0	45	100	32	71.1	77	57
	Alveolar	0	0	0	0	4	8.9	4	3
	Tongue	0	0	0	0	7	15.6	7	5.2
	Other sites	0	0	0	0	2	4.4	2	1.5
Habit	No	45	100	0	0	0	0	45	33.3

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	Tobacco Chewing	0	0	22	48.9	23	51.1	45	33.3
	Smoking	0	0	5	11.1	0	0	5	3.7
	Chewing & Smoking	0	0	8	17.8	8	17.8	16	11.9
	Chewing & Alcohol	0	0	10	22.2	13	28.9	23	17
	Sharp teeth	0	0	0	0	1	2.2	1	0.7

On assessing and comparing the levels of biomarkers among all the three groups, all three biomarkers (IL-6, IL-8 and CD44) were found to be elevated in both OMPD and OSCC subjects of which the results of IL-6 was statistically significant (p value=0.0005) when compared between the groups. (Table 2).

Table 2 : Expression of IL-6, IL-9 and CD-44 in all the three groups

		Group			p-value
		Healthy	**OSMF**	**OSCC**	
IL-6	N	45	45	45	0.005^{*}
	Median	9.629	8.525	17.641	
	1st Quartile	1.869	-1.894	7.079	
	3rd Quartile	17.319	17.761	37.151	
	Mean	9.016	8.239	23.346	
	Std. Dev.	13.569	15.810	26.349	
CD44	N	45	45	45	0.069^{NS}
	Median	22.170	9.090	11.865	
	1st Quartile	4.490	6.300	4.340	
	3rd Quartile	27.670	30.470	20.150	
	Mean	16.788	17.460	12.922	
	Std. Dev.	12.062	12.609	9.819	
IL-8	N	45	45	45	0.727^{NS}
	Median	797.480	667.420	657.860	
	1st Quartile	25.481	420.000	627.450	
	3rd Quartile	1078.810	959.560	1027.920	
	Mean	650.201	626.382	699.281	
	Std. Dev.	452.187	414.432	339.769	

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On multiple pairwise comparisons of IL-6 levels among the groups, a statistically significant difference was found between OSMF to OSCC group and the healthy to OSCC group (p value=0.008, 0.023 respectively) (Table 3).

Table 3: Multiple pairwise comparison of the biomarker IL-6 between the three groups

Pair	p-value
OSMF-Healthy	0.999^{NS}
OSMF-OSCC	0.008*
Healthy-OSCC	0.023*

On correlating all the three biomarkers with the study variables, we found a positive correlation between most of the variables with the biomarkers except for IL-6 vs CD44 and IL-8 vs IL-6 in the OSMF group. All the correlations among the groups were found to be statistically insignificant (Table 4).

Table 4: Overall and groupwise spearman's correlation among the groups

			CD44	IL-8
All the groups taken together	IL-6	Correlation	-.067	-.011
		p-value	.439^{NS}	.895^{NS}
		N	135	135
	CD44	Correlation		.087
		p-value		.317^{NS}
		N		135
Healthy	IL-6	Correlation	.016	.102
		p-value	.914^{NS}	.504^{NS}
		N	45	45
	CD44	Correlation		.086
		p-value		.574^{NS}
		N		45
OSMF	IL-6	Correlation	-.013	-.004
		p-value	.932^{NS}	.980^{NS}
		N	45	45
	CD44	Correlation		.172

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		p-value		.259^{NS}
		N		45
OSCC	IL-6	Correlation	.014	.013
		p-value	.929^{NS}	.933^{NS}
		N	45	45
	CD44	Correlation		-.007
		p-value		.962^{NS}
		N		45

DISCUSSION

In recent years, many researchers have focussed on finding a biomarker for screening OSCC in saliva, however their validity for routine usage as a diagnostic/screening marker has not been elucidated.[12] The current research investigations have almost solely focused on one or a few markers in studies with small sample numbers, leaving them with limited literature on varied OSCC population. In light of this perspective, the current study looked into whether the above-mentioned markers could be used as a new OSCC diagnostic tool and aid in distinguishing between premalignant oral lesions and OSCC, which is a particular challenge in the tobacco smokers/pan-chewing population. As a result, this study was done to determine the biomarkers' specificity and sensitivity.

Most of the participants in our study were males more than 40 years of age and who chewed tobacco for more than 5 years. This was in accordance with the studies done by Pires FR et al and Wolfer S et al, who examined the gender differences and patterns of occurrence of oral squamous cell carcinoma and pre malignant lesions in South Indian population and concluded that males predominantly above 40 years of age with any one of the risk factors present are at higher chances of developing cancer.[13,14] This could be attributed to the fact that men exhibit a higher percentage of risk behaviour such as alcohol and tobacco consumption at the time of diagnosis than women.[15] Moreover, Poveda-Roda et al, observed that there exists no difference of gender in the number of cigarettes smoked daily in OSCC patients, but the higher prevalence could be accredited to shorter duration of smoking in females than males before the occurrence of cancer.[16]

In the present study on assessing and comparing the levels of biomarkers among all three groups, all three biomarkers (IL-6, IL-8 and CD44) were found to be elevated in both OMPD and OSCC subjects of which the results of IL-6 were found to be statistically significant. These results were in accordance with studies done by Nadhisha S Piyarthe et al, Gleber Natto et al and Lee L et al who reported higher concentrations of IL-6 and IL-8 biomarkers in oral squamous carcinoma lesions when compared with the oral submucous fibrosis lesions.[17,18,19] A study done by Essa AA et al has revealed

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the fact CD-44 protein can be used as a potential diagnostic marker for measuring the relationship of tumour angiogenesis with oral squamous cell carcinoma lesion wherein the author found higher levels of this protein in OSCC lesions which were in accordance with our study.[20] (Table 3). The two interleukins assessed in the present study has been demonstrated to possess several roles in carcinogenesis in different cancer types. In OSCC, interleukins are mainly involved in the invasion and migration of cancer cells, while these oral squamous carcinomatous cells were shown to induce stromal cells to produce IL-6, accelerating bone destruction by osteoclast formation. Interleukins were also reported to promote tumourogenesis by facilitating inflammation, invasion, and angiogenesis. IL8 has a shown to promote the invasion and metastasis in the progression cancerous disease through promoting and prolonging the inflammatory reactions in early-stage.[21] There was a positive correlation between IL-6 levels and OSCC histopathologic grade in correlation studies. The level of IL-6 in saliva was shown to be increasing from highly differentiated to moderately differentiate to poorly differentiated OSCC lesions. This shows that, as shown in other studies, IL-6 can be utilized as a marker for disease aggressiveness and severity. Despite the fact that previous research has linked IL-6 levels to OSCC clinical staging, the current study found no link or increase in IL-6 levels with OSCC clinical staging. This could be because the vast majority of OSCC patients are in stages I and II rather than later stages. With OPML grading and staging of PMC, however, there was no increase in IL-6 levels.[22]

The study's limitations include that it didn't consider additional premalignant illnesses in the premalignant group, such as erythroplakia and oral lichen planus. The basis for their omission is their uncommon incidence, and moreover leukoplakia and OSMF are OPMLs that have been shown to progress to malignant form at a faster rate than others. However, our study's findings should not be overlooked. It's also worth noting that tobacco smoking/chewing has been shown to increase the risk of OSCC. Our study explained the difference in IL-6 levels between cancer patients and PMD patients inspite of the fact that the number of smokers in these two groups were similar. Further experimental research is needed to show and validate the present results of the study.

CONCLUSION

Our findings indicate that inspite of IL-6, IL-8 and CD44 being elevated in the OSCC and OPMD groups when compared with the healthy controls, we found that only IL-6 levels were statistically significant thus demonstrating the fact that this biomarker has a definitive and potential role in the pathogenesis of the cancerous progression thus holding its place as a promising biomarker for detecting oral premalignant and squamous cell carcinoma lesions. This can form the base for developing a saliva-based diagnostic kit with IL-6 biomarker which could be considered as a cost-effective adjunct diagnostic tool in the postoperative management of these patients. In addition, the assessment of such biomarkers in saliva and serum must eventually be tested in a prospective, blinded fashion in clinical settings requiring actual cancer detection.

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Funding

The study did not receive any funding from institutional, Government and/or non-profit sectors.

Conflict of Interest

The authors report no conflicts of interest related to this study.

References

1. Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. *Sensors International*. 2020; 1:100046.
2. Bugshan A, Farooq I. Oral squamous cell carcinoma: metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. *F1000Research*. 2020; 9.
3. Paz AR, Cavalcanti YW, Godoy GP, Alves PM. Clinical findings and risk factors to oral squamous cell carcinoma in young patients: A 12-year retrospective analysis. *Medicina oral, patologia oral y cirugia bucal*. 2016; 21(2):e151.
4. Condurache Hritcu OM, Botez AE, Olinici DT, Onofrei P, Stoica L, Grecu VB, Toader PM, Gheucă-Solovăstru L, Cotrutz EC. Molecular markers associated with potentially malignant oral lesions. *Experimental and Therapeutic Medicine*. 2021; 22(2):1-5.
5. Muthu K, Narayanan M. Recognizing the salivary panomics for the clinical application in oral potentially malignant disorders. *Journal of Oral and Maxillofacial Pathology*. 2021; 25(2):332.
6. Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DT. Salivary biomarkers: toward future clinical and diagnostic utilities. *Clinical microbiology reviews*. 2013; 26(4):781-91.
7. Riccardi G, Bellizzi MG, Fatuzzo I, Zoccali F, Cavalcanti L, Greco A, Vincentiis MD, Ralli M, Fiore M, Petrella C, Minni A. Salivary Biomarkers in Oral Squamous Cell Carcinoma: A Proteomic Overview. *Proteomes*. 2022; 10(4):37.
8. Pillai J, Chincholkar T, Dixit R, Pandey M. A systematic review of proteomic biomarkers in oral squamous cell cancer. *World Journal of Surgical Oncology*. 2021; 19:1-28.
9. Nguyen TT, Sodnom-Ish B, Choi SW, Jung HI, Cho J, Hwang I, Kim SM. Salivary biomarkers in oral squamous cell carcinoma. *Journal of the Korean Association of Oral and Maxillofacial Surgeons*. 2020; 46(5):301-12.
10. Cristaldi M, Mauceri R, Di Fede O, Giuliana G, Campisi G, Panzarella V. Salivary biomarkers for oral squamous cell carcinoma diagnosis and follow-up: current status and perspectives. *Frontiers in physiology*. 2019; 10: 1476.
11. Čēma I, Dzudzilo M, Kleina R, Franckevica I, Svirskis Š. Correlation of soluble CD44 expression in saliva and CD44 protein in oral leukoplakia tissues. *Cancers*. 2021; 13(22):5739.
12. Dikova V, Jantus-Lewintre E, Bagan J. Potential non-invasive biomarkers for early diagnosis of oral squamous cell carcinoma. *J Clin Medicin*. 2021; 10(8):1658.
13. Pires FR, Ramos AB, Oliveira JB, Tavares AS, Luz PS, Santos TC. Oral squamous cell carcinoma: clinicopathological features from 346 cases from a single oral pathology service during an 8-year period. *J Appl Oral Sci*. 2013; 21: 460-7.
14. Wolfer S, Kunzler A, Foos T, Ernst C, Leha A, Schultze-Mosgau S. Gender and risk-taking behaviors influence the clinical presentation of oral squamous cell carcinoma. *Clin Exp Dent Res*.2022;8(1):141-51
15. Moyses, R. A., López, R. V. M., Cury, P. M., Siqueira, S. A. C., Curioni, O. A., de Gois Filho, J. F., Figueiredo, D. L. A., Head and Neck Genome Project GENCAPO, Tajara, E. H., & Michaluart, P. (2013). Significant differences in demographic, clinical, and pathological features in relation to

ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 04-2023
DOI 10.17605/OSF.IO/8PZ4E

smoking and alcohol consumption among 1,633 head and neck cancer patients. *Clinics*, **68**(6), 738–744

16. Poveda-Roda, R., Bagan, J. V., Jimenez-Soriano, Y., Margaix-Munoz, M., & Sarrion-Perez, G. (2010). Changes in smoking habit among patients with a history of oral squamous cell carcinoma (OSCC). *Medicina Oral, Patologia Oral, Cirugia Bucal*, **15**(5), e721–e726.
17. Piyarathne NS, Weerasekera MM, Fonseka PF, Karunatilleke AH, Liyanage RL, Jayasinghe RD, De Silva K, Yasawardene S, Gupta E, Jayasinghe JA, Abu-Eid R. Salivary Interleukin Levels in Oral Squamous Cell Carcinoma and Oral Epithelial Dysplasia: Findings from a Sri Lankan Study. *Cancers*. 2023; 15(5):1510.
18. Gleber-Netto, F.O.; Yakob, M.; Li, F.; Feng, Z.; Dai, J.; Kao, H.-K.; Chang, Y.-L.; Chang, K.-P.; Wong, D.T. Salivary Biomarkers for Detection of Oral Squamous Cell Carcinoma in a Taiwanese Population. *Clin. Cancer Res.* **2016**, *22*, 3340–3347
19. Lee, L.; Wong, Y.; Hsiao, H.; Wang, Y.; Chan, M.; Chang, K. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. *Int. J. Oral Maxillofac. Surg.* **2018**, *47*, 699–707
20. Essa AA, Deraz EM. Expression of CD44 (NKI-P1) in oral squamous cell carcinoma associated vascular endothelial cells: A relationship to tumor angiogenesis. *The Saudi Dent J*. 2022; 34(1):21-6.
21. Briukhovetska D, Dörr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in cancer: from biology to therapy. *Nat Rev Cancer*. 2021; 21(8):481-99.
22. Dineshkumar T, Ashwini BK, Rameshkumar A, Rajashree P, Ramya R, Rajkumar K. Salivary and serum interleukin-6 levels in oral premalignant disorders and squamous cell carcinoma: diagnostic value and clinicopathologic correlations. *Asian Pac. J. Cancer Prev*.2016; 17(11):4899.