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Assessment of vitamin D levels in patients with oral potentially malignant disorders and oral squamous cell carcinoma-A cross-sectional study

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ABSTRACT

Objectives: The aim of this study was to assess and compare the serum and salivary levels of Vitamin D in patients with oral potentially malignant disorders (OPMD) and Oral squamous cell carcinoma (OSCC) with healthy controls.

Method: This cross-sectional study was carried out among 60 patients reporting to the department of Oral Medicine and Radiology, and included patients with OPMDs, OSCC and healthy controls. The Vitamin D levels were estimated using the chemiluminescence immunoassay. One-way ANOVA was used to compare mean and statistical difference between the groups. Tukey's post HOC test calculated for inter group difference. Serum and salivary Vitamin D levels were corelated with Pearson's coefficient. The values of $p < 0.005$ was considered as significant.

Results: The levels of Vitamin D were decreased in study group as compared to controls both in serum and saliva. ($p < 0.001$). Between serum and salivary Vitamin D levels, a strong association was discovered using the Pearson's coefficient value of 0.737 ($p < 0.001$)

Conclusion: In this study, patients with both OPMDs and OSCC had Vitamin D insufficiency & deficiency. Vitamin D level assessment should be included as a standard component of routine investigations for these individuals.

1. Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent type of head and neck cancer, representing over 90 % of cases.[1] Approximately 30 % of all cancers in India are oral cancers. Globocan's estimate in 2018 indicates that there were 119,992 newly reported cases of oral cancer, resulting in an estimated 72,616 deaths. The high prevalence of alcohol, tobacco, and areca nut consumption in India is a contributing factor to this issue.[2] The most prevalent oral potentially malignant disorders (OPMDs) with a high risk of developing OSCC are oral submucous fibrosis (OSMF) and oral leukoplakia (OL). They are often asymptomatic and may go unnoticed in the early stages as most patients report in the advanced stages, leading to an unfavourable outcome. OSCC is known to have a poor prognosis due to delayed diagnosis and lack of disease-related biomarkers or other cost-effective treatments.[3]

The multistep carcinogenesis model emphasises how crucial it is to continue exploring for new biomarkers for early OSCC diagnosis and as an initial step towards better treatment.[4,5] Translation oncology places a strong emphasis on the creation of novel molecular tools.[6,7] Both healthy and cancerous tissues undergo apoptosis, which is a type of programmed cell death. Apoptosis resistance, which is linked to tumour recurrence, radiation resistance, and chemotherapy resistance, is widely acknowledged to have a substantial role in the development of OSCC.[8] Therefore, the management of OSCC will benefit from therapeutic strategies that induce cancer cell death.

In numerous cell lines, Vitamin D has been demonstrated to have anticancer properties, including the prevention of cancer cells' angiogenesis, metastasis, and invasiveness. Since Vitamin D regulates cell differentiation and proliferation in a variety of tissues, including keratinocytes of squamous epithelial cells, it may contribute to the development of OSCC.[9] As a result, it has been suggested that Vitamin D may provide defence against cancer at a number of different places.[10] Numerous in vitro and in vivo studies on various cancer types have demonstrated the anticancer properties of calcitriol. Vitamin D insufficiency has been identified as a risk factor for several malignancies, including prostate, ovarian, colon, breast, and rectum.[11]

Few epidemiological studies[12,13] have been conducted in India to support the concept that low Vitamin D levels are linked to an increased

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risk of head and neck cancer, especially OSCC and OPMDs. The relationship between Vitamin D and OSCC has been extensively investigated in various quantitative studies worldwide, as reported in a systematic review by Mäkitie An et al.[14] Further investigation is required in this direction. The literature is severely lacking in information regarding Vitamin D deficiency in OPMDs, specifically OSMF and oral leukoplakia.

Saliva is a non-invasive medium that can measure OPMD and OSCC-related biomarkers in close proximity to oral lesions. The role of saliva in identifying Vitamin D deficiency in OSCC has not been researched, with the exception of a single study by Bahramian A et al.[15] Therefore, the present study aims to assess and compare the serum and salivary levels of Vitamin D in patients with OPMDs and OSCC associated with tobacco use with healthy controls to ascertain any variations between the various study groups.

2. Method

The primary objective of study was to assess and compare serum and salivary levels of Vitamin D in Oral Leukoplakia, Oral submucous fibrosis and Oral squamous cell carcinoma and healthy controls. The secondary objective is to evaluate if saliva can be used as a medium to estimate Vitamin D levels in the patients.

2.1. Ethics statement

This cross-sectional study was commenced after approval from the institutional ethics committee (SVIEC/ON/DENTSRP/22071). After receiving study information, all participants gave informed permission to participate in this study.

2.2. Sample size & patient selection

This study was performed as a short-term research project, and in accordance with the guidelines, the study had to completed within six months. Thus, the number of patients reporting to the outpatient department was taken into consideration. The sample size for this study was determined using the following formula: $n = [(Z/2 + Z)2 (p1 (1-p1) + (p2 (1-p2))]/(p1 - p2)$. At 80 % power for the 0.05 level of significance, each study group needed a minimum of 14 individuals (14 per group).

Duration of study was six months from April 2022–October 2022.The research study involved 60 participants, who were divided into 4 groups with 15 participants in each group. The study included four groups of participants: those with Oral leukoplakia, Oral submucous fibrosis, oral squamous cell carcinoma associated with any type of tobacco and areca nut and related habit irrespective of duration were included in study, and healthy participants without any habit and lesions formed the control group. The study included participants who were at least 18 years old and had been diagnosed with oral squamous cell carcinoma, oral submucous fibrosis, or leukoplakia based on clinical and histopathological evidence.

In order to control the effect of predictors and confounding factors on the assessment of Vitamin D levels, patients with the following conditions were excluded from the study. The exclusion criteria for all participants consisted of participants with chronic morbidity, muscle weakness, systemic diseases such as parathyroid and thyroid disorders, cardiovascular diseases, autoimmune disorders, diabetes mellitus, chronic kidney and liver disease. Additionally, individuals who were taking Vitamin D and calcium supplementation or calcium channel blockers within the last six months, as well as those who had undergone any type of cancer treatment, were also excluded.

The details of patient's demographic data like occupation, geographic location, history of exposure to habits like consumption of tobacco and areca nut and its products, presence of medical disorders, history of medication use was recorded in a specially designed case history proforma. The patients were examined and based on the criteria mentioned below they were included in the four study groups. The primary outcome of the study is to measure the serum and salivary Vitamin D levels.

The diagnosis and the staging of each lesion was made according to the following criteria-

I. Oral Leukoplakia: White non-scrapable lesion with no other aetiology than tobacco was classified as homogenous leukoplakia. According to the van der Waal criteria, a non-scrapable mixed red and white lesion with no other known cause than tobacco was classified as non-homogenous leukoplakia.[16]
II. Oral Sub mucous Fibrosis: Classification by More CB[17] et al., divided into four stages Stage I, II, III & IV
III. Oral squamous cell carcinoma: Ulceroproliferative/fungating growth with central necrosis and rolled borders. Following a clinical examination and palpation, the growth was classified using the TNM classification.[18] Histopathological proven cases were included in the study.

2.3. Blood and saliva collection

After recording the study details in the case history proforma, the patients were instructed to report the following morning on an empty stomach. A sterile syringe was used to draw 5 ml of venous blood from each participant, and all necessary aseptic measures were taken. Five millilitres of entire, unstimulated saliva were collected from the patient when they were relaxed and comfortable. The patients were asked to stay still for a half-hour without eating or drinking before saliva was taken. The samples were centrifuged after being maintained at room temperature.[15]

2.4. Vitamin D estimation in serum and saliva

The serum and saliva were transferred to a centrifuge tube and centrifuged at 1000 rcf (relative centrifugal force) for 15 min.[15] The resultant supernatant was subjected to measurement in the fully automatic chemiluminescence immunoassay analyser. The MAGLUMI 25-OH Vitamin D assay kit (Shenzhen,518057CHINA) was used for the quantitative determination of total 25-OH Vitamin D in serum and saliva.

100 μL of sample was taken with 10 μL of calibrator and 50 μL of magnetic microbeads and displacing reagent. After incubating for 20 min, the sample was tagged with ABEI (n-4-aminobutyl-*n*-ethyl-isoluminol) and incubated further for 10 min. Following a wash cycle, the analyzer used a calibration curve produced by a 2-point calibration master curve technique to automatically determine the 25-OH Vitamin D concentration in each sample. The results were displayed in ng/mL.[19]

Patients were categorised according to the levels of Vitamin D concentration as follows[19]

Normal- 30–100 ng/mL
Insufficiency -29-10 ng/mL
Deficiency -<10 ng/mL
Severe deficiency - <7 ng/ml.

2.5. Statistical analysis

The STAT-13IC software (Stata Corp LLC, Dublin, USA) was used to statistically analyse the obtained data. The Chi square test was used to perform descriptive statistics. The data was checked for normality by Kolmogorov-Smirnov & Shapiro-Wilk test. One-way Anova test was used to compare the means and statistical difference between the groups. Tukey's post HOC test compared inter group difference between the mean Vitamin D values. Pearson's coefficient was used to find out correlation between serum and salivary Vitamin D levels. The values of $p < 0.005$ was considered as significant.

3. Results

3.1. 1Patients' characteristics

During the aforementioned study period, a total of 135 patients diagnosed with OPMDs and OSCC were considered as suitable for evaluation. After the careful application of inclusion and exclusion criteria, a total of 75 patients met the eligibility requirements for participation in the study. Among the 75 patients, 45 patients diagnosed with OPMDs and OSCC together with 15 healthy individuals, voluntarily agreed to participate in the study. Therefore, the final study population comprised of sixty patients. A high prevalence of tobacco chewing was seen among the study group. Table 1 depicts the mean age and gender of the patients in the study and control group and was not statistically significant. (p-0.071, p- 0.669) Therefore, the study showed no bias regarding age and gender among cases and controls.

3.2. Serum vitamin D

Table 2 depicts the Vitamin D status among cases and controls. Vitamin D insufficiency was seen in 73.3 % patients with OSMF and oral leukoplakia patients & 86.6 % of OSCC patients. Among controls only 13.3 % patients had VD insufficiency. Vitamin D deficiency was seen only in a single case of non-homogenous leukoplakia and 13.3 % of OSCC patients. Two patients among the control group also had Vitamin D insufficiency.

Fig. 1 depicts the normality of data by kolmogorov-smirnov & shapiro-wilk test.

Fig. 2 depicts the mean levels of serum Vitamin D (ngm/ml) among the study and control group. The values were statistically significant between the groups i.e., cases and controls ($p < 0.001$) and not within the groups according to One-way ANOVA test. Tukey's post HOC test showed statistically significant difference between OSMF and controls, Oral leukoplakia and controls and OSCC and controls ($p < 0.001$) (Table 3).

3.3. Salivary vitamin D

Fig. 3 depicts the mean levels of salivary Vitamin D among the study and control group. For salivary Vitamin D both according to One-way Anova and Tukey's Post HOC Test (Table 4), the values were statistically significant between the groups i.e., between OSMF and controls, Oral leukoplakia and controls and OSCC and controls ($p < 0.001$) But the values were not statistically significant within groups of OSMF, Oral Leukoplakia and OSCC both for serum and saliva.

3.4. Serum & salivary vitamin D

A strong link between serum and salivary Vitamin D was shown by Pearson's coefficient, which was 0.737 ($p < 0.001$). (Fig. 4).

4. Discussion

The present cross-sectional study was the first to compare serum and salivary levels of Vitamin D among OPMDs, (OSMF, Oral Leukoplakia) OSCC and controls. A positive correlation (0.732) was found between serum and salivary levels of Vitamin D.

In the present study the mean serum levels of Vitamin D in OSMF, Oral Leukoplakia and OSCC were 20.12 ng/ml, 24.19 ng/ml and 18.15 ng/ml respectively and in controls it was 34.16 ng ml. Similar results were seen with salivary levels of Vitamin D. The present study revealed that individuals with OSMF, oral leukoplakia, and OSCC had lower levels of Vitamin D in their blood and saliva compared to the control group. Both patients with OPMDs and OSCC exhibited Vitamin D insufficiency. However, the decrease in Vitamin D levels was more pronounced in OSCC patients when compared to other groups. The values are statistically significant as both one way ANOVA and Tukey's Post HOC Test were used for multiple comparisons between the four groups. The results of our research align with multiple studies that have documented low levels of Vitamin D in individuals diagnosed with cancer.

Various studies conducted by Orell-Kotikangas et al.,[20] Grimm et al.[13] and Mostafa et al.,[21] observed a greater occurrence of Vitamin D insufficiency among patients with OSCC. According to a study by Udeabor et al.,[22] a positive association was seen between the incidence of OSCC and Vitamin D deficiency, particularly when the Vitamin D level was below 25 ng/ml. The authors, suggested that Vitamin D deficiency could potentially serve as a reliable indicator for OSCC. While most of the studies have co-related low levels of Vitamin D with increased risk of OSCC, two studies by Negri et al.[23] and Dudding et al.[24] showed a weak corelation with Vitamin D and OSCC.

In the present study Vitamin D insufficiency was seen in patients with OPMDs and OSCC who had the habit of consumption of tobacco as compared to healthy controls. The presence of Vitamin D insufficiency in the present study can be attributed to the consumption of tobacco and its products and initiation of carcinogenesis augmented by Vitamin D deficiency. Vitamin D decreases oxidative stress by acting through the Vitamin D receptor and by raising the expression of super oxide dismutase and glutathione peroxidases and decreasing proinflammatory cytokines.[25] Consumption of tobacco in both the smoking and smokeless forms increases the free radical damage and increase the oxidative stress in our body. Therefore, use of tobacco can indirectly decrease the efficacy of Vitamin D in decreasing oxidative stress and may cause the precancerous and cancerous lesions. Similar to our study, "Orell-Kotikangas et al.".[20] discovered that Vitamin D deficiency was substantially more prevalent among tobacco smokers (57 %) than among non-smokers (12 %).

With encouraging results, researchers have utilised Vitamin D as an anticancer drug to treat hepatocellular, prostate, and colon cancers.[26–28] Various studies observed that the clinical management of cancer patients could be enhanced by using calcitriol as an adjuvant to chemoprevention by targeting adjuvant residual tumour cells.[29] This was

Table 1
Depicting the mean age and gender among cases and controls.

Group	N	Mean (Years)	Std. Deviation	95 % Confidence Interval for Mean		Minimum (Age in years)	Maximum (Age in years)
				LB	UB		
OSMF	15	40.13	11.019	34.03	46.24	29 (years)	65 (years)
Leukoplakia	15	46.67	18.627	36.35	56.98	20 (years)	90 (years)
OSCC	15	53.33	8.764	48.48	58.19	42 (years)	77 (years)
Control	15	42.13	16.898	32.78	51.49	24 (years)	85 (years)
Total	60	45.57	14.942	41.71	49.43	20 (years)	90 (years)
Gender	OSMF	Leukoplakia	OC	Control	Total	Chi-square value = 1.560; p-value = 0.669	
Male	11(73.3 %)	12(80 %)	11(73.3 %)	9(60 %)	43		
Female	4(26.7 %)	3(20 %)	4(26.7 %)	6(40 %)	17		
Total	15	15	15	15	60		

OSMF-Oral submucous fibrosis, OSCC-Oral squamous cell carcinoma UB-upper bound, LB-lower bound.

Table 2
Vitamin D status among cases and controls.

Vitamin D status	25-OH Vitamin D concentration (ng/ml)	OSMF	Leukoplakia	OSCC	Controls	One-way ANOVA
Normal	30–100	4 (26.6 %)	3 (20 %)	1(6.6 %)	13(86.6 %)	<0.001
Insufficiency	29–10	11(73.4 %)	11(73.4 %)	12(80 %)	2(13.4 %)	
Deficiency	<10	–	1(6.6 %) (Non homogenous)	2(13.4 %)	–	
Total-60		15	15	15	15	

OSMF-Oral submucous fibrosis, OSCC-Oral squamous cell carcinoma.

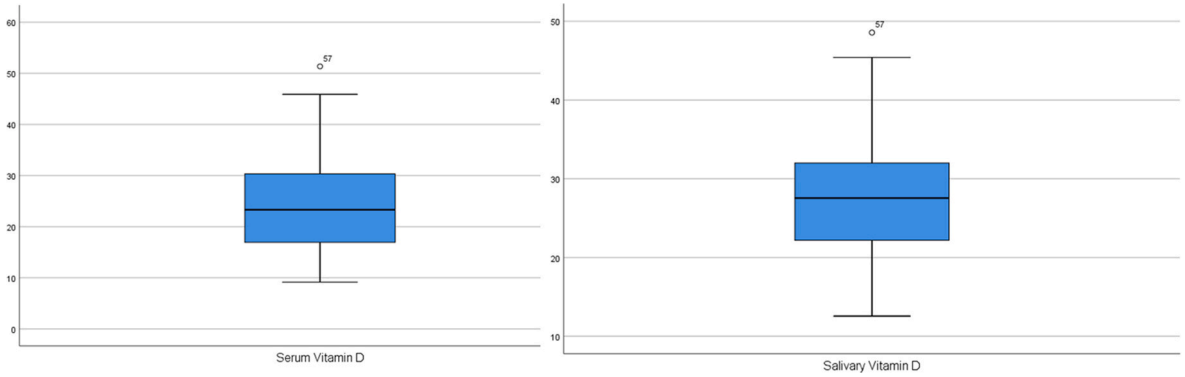

Fig. 1. Box plot showing normal distribution of mean values of serum and salivary levels of Vitamin D.

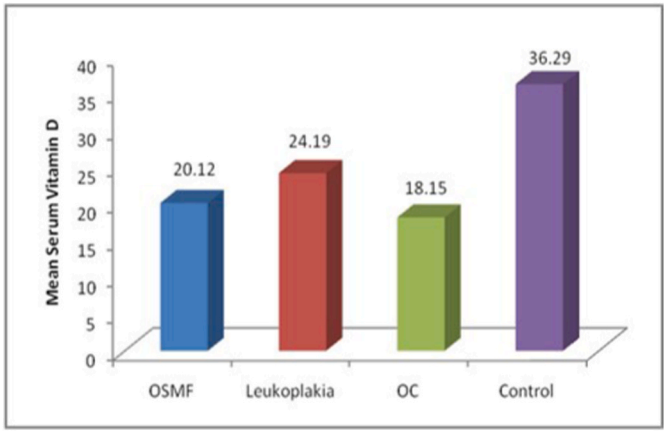

Fig. 2. Mean serum Vitamin D levels among cases and controls.

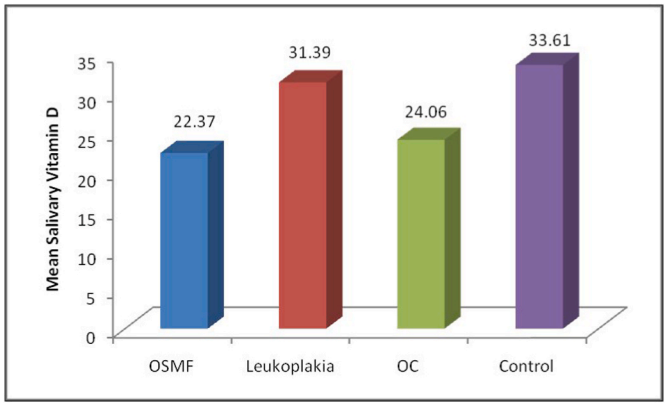

Fig. 3. Mean salivary Vitamin D levels among cases and controls.

Table 3
Tukey's Post HOC Test of serum Vitamin D for multiple comparisons between OPMDs, OSCC and controls.

Group		Mean Difference	*P*-value	95 % Confidence Interval	
				Lower Bound	Upper Bound
OSMF	Leukoplakia	−4.07	0.277	−10.01	1.87
	OSCC	1.98	0.814	−3.96	7.91
	Control	−16.16[a]	<0.001	−22.10	−10.22
Leukoplakia	OSCC	6.05[a]	0.044	0.11	11.98
	Control	−12.09[a]	<0.001	−18.03	−6.15
OSCC	Control	−18.14[a]	<0.001	−24.08	−12.20

OSMF-Oral submucous fibrosis, OSCC-Oral squamous cell carcinoma, OPMD-Oral potentially malignant disorder.
[a] Indicates significance at 5 % level.

Table 4
Tukey's Post HOC Test of salivary Vitamin D for multiple comparisons between OPMDs, OSCC and controls.

Group		Mean Difference	*P*-value	95 % Confidence Interval	
				Lower Bound	Upper Bound
OSMF	Leukoplakia	−9.02[a]	0.001	−15.10	−2.95
	OSCC	−1.69	0.881	−7.77	4.38
	Control	−11.24[a]	<0.001	−17.32	−5.16
Leukoplakia	OSCC	7.33[a]	0.012	1.25	13.40
	Control	−2.22	0.769	−8.29	3.86
OSCC	Control	−9.55[a]	0.001	−15.62	−3.47

OSMF-Oral submucous fibrosis, OSCC-Oral squamous cell carcinoma, OPMD-Oral potentially malignant disorder.
[a] Indicates significance at 5 % level.

corroborated by Anand et al.[12] in which they examined Vitamin D levels and VDR expression in OSCC and oral precancer. They observed that OSCC patients who received Vitamin D supplements after chemotherapy and radiotherapy, had improved quality of life and reduced treatment toxicity. Similarly, Bochen et al.,[30] observed increased cytotoxic activity of patients' natural killer cells following Vitamin D supplementation. Maturana-Ramirez[31] conducted a systematic review that identified a

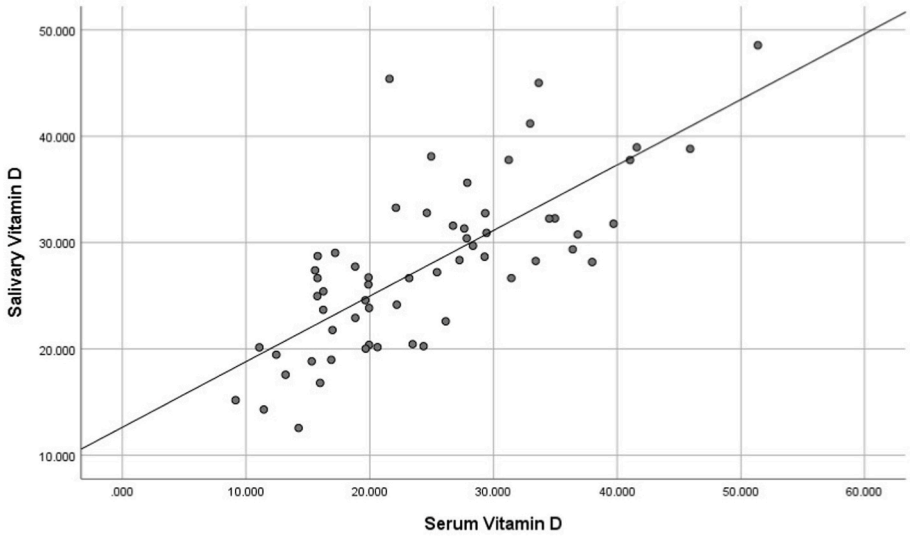

Fig. 4. Pearsons's correlation between salivary and serum Vitamin D.

correlation between hypoVitaminoses D and reduced survival rates in patients with oral squamous cell carcinoma (OSCC). Additionally, the review revealed a higher occurrence of post-operative recurrence and an elevated risk of adverse reactions to chemotherapy in individuals with hypovitaminoses D. Due to its affordability and minimal toxicity, the utilisation of Vitamin D supplementation presents a compelling avenue that warrants additional investigation.[12]

One more important observation in our study was good correlation of 0.737 between serum and salivary levels of Vitamin D. Apart from the present study decreased levels of salivary Vitamin D levels were studied by Bahramian et al.[15] among OSCC patients. Similarly, a cross-sectional study was carried out in Indonesia[32] among 56 healthy males and females aged between 18 and 60 years to assess the correlation between serum and salivary levels of Vitamin D. The results of their investigation revealed a moderate correlation between salivary levels and serum Vitamin D levels. The results of these studies and the present study emphasise the role of saliva as a non-invasive tool to determine Vitamin D levels in all group of patients.

The present study revealed that gender and age in all the four groups had no effect on Vitamin D levels. This is consistent with the findings of Apuhan et al.[33] One study found that older adults are at higher risk for Vitamin D deficiency.[34] This may be due to a reduced ability of the skin to produce enough vitamin D and a reduced ability to absorb vitamin D from food.[35]

This study represents the first attempt to evaluate the level of Vitamin D insufficiency among individuals diagnosed with OSMF, Oral leukoplakia, and OSCC. The chemiluminescence test was utilised to determine the Vitamin D levels in both serum and saliva samples. Multiple investigations have provided confirmation that the utilisation of this standardised approach successfully portrays the presence of Vitamin D deficiency without amplifying its prevalence.[36] There was a good correlation observed between serum and saliva samples when evaluating Vitamin D levels.

One potential limitation of our investigation is the relatively limited sample size. The findings lack generalizability to the broader population; yet, they can serve as a foundational reference point for future studies of a similar nature due to the multiple statistical analysis used to interpret the results. The rigorous criteria employed to exclude patients with Vitamin D deficiency can affirm that our findings have not been exaggerated. This study does not include cases of idiopathic OPMDs, OPMDs/OSCC caused by trauma or viral factors, and the results cannot be extrapolated to these patient populations. Another limitation of our study is that we did not measure the duration of exposure to sunlight, as well as the physical activity levels, body mass index, lifestyle, and food habits of the patients. Incorporating these factors into future studies would enable the assessment of the impact of Vitamin D on the development and progression of OPMDs and OSCC.

5. Future prospects

It is important to conduct a well-designed prospective cohort study to better assess the part played by Vitamin D and its receptor in the aetiology of OPMD and OSCC. Future randomised clinical trials can be conducted with Vitamin D and its analogues supplementation as a focused epigenetic therapy for both OPMDs and OSCC.

6. Conclusion

Patients in this study who had OPMDs and OSCC were found to have Vitamin D insufficiency and deficiency. It is recommended that the assessment of Vitamin D levels should become an integral part of the routine studies performed on these patients.

Disclosure

None.

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7. Contribution details

Dr. Ishita Samanta: Concepts, Design, Clinical studies, Manuscript preparation, Manuscript review.

Dr. Deepa Jatti Patil: Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor.

Dr. Chandramani B: Definition of intellectual content, Manuscript editing, Manuscript review.

Declaration of competing interest

Nil.

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