

# Environmental, Lifestyle Factors and Dietary Patterns affecting Serum Vitamin D Status in Oral Submucous Fibrosis: A Cross-Sectional Study

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## ABSTRACT

### Background:

Oral submucous fibrosis (OSMF) is a chronic, potentially malignant disorder influenced by multiple environmental, nutritional, behavioural and host-related factors. Vitamin D may have a role in inflammatory and fibrotic processes, but its relationship with OSMF and associated environmental determinants remains insufficiently explored.

### Aim:

To compare environmental, dietary, lifestyle and behavioural factors and serum vitamin D status between individuals with and without OSMF, and to assess their association with OSMF.

### Methods:

This cross-sectional comparative study included 136 participants, comprising 68 clinically diagnosed OSMF patients and 68 healthy individuals aged 18–50 years. A standardized questionnaire assessed sunlight exposure, clothing practices, sunscreen use, physical activity, occupation and dietary patterns. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured using chemiluminescent immunoassay. OSMF severity was assessed using the Khanna and Andrade classification. Statistical analysis was performed using SPSS version 26.0.

### Results:

Significant differences were observed between the OSMF and control groups in reported sunlight exposure, full-sleeved clothing, sunscreen use and dietary patterns ( $p < 0.001$ ). More than 60 minutes of daily sunlight exposure was reported by 69.1% of OSMF participants compared with 23.5% of controls. Full-sleeved clothing was less frequently reported among OSMF participants (14.7% vs. 45.6%), while all OSMF participants reported never using sunscreen. Consumption of dairy, fish and eggs was also lower among OSMF participants. Vitamin D deficiency was significantly more prevalent in the OSMF group than in controls (97.1% vs. 70.6%;  $p < 0.001$ ). No significant differences were observed in physical activity or occupational exposure.

### Conclusion:

Individuals with OSMF demonstrated significant differences in environmental and dietary characteristics and had a substantially higher prevalence of vitamin D deficiency compared with healthy individuals. These findings suggest an association between OSMF and factors potentially influencing vitamin D status; however, the cross-sectional design precludes causal inference. Longitudinal studies are warranted to further clarify these relationships.

**Keywords:** Oral submucous fibrosis; Vitamin D deficiency; 25-hydroxyvitamin D; Environmental factors; Dietary patterns; Lifestyle factors.

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### Introduction:

OSMF is a chronic, progressive, potentially malignant disorder characterized by juxta-epithelial inflammation and progressive fibrosis of the oral mucosa, resulting in reduced mouth opening and impairment of oral function.<sup>1</sup>

Nearly 5 million people in India are afflicted by it (0.5% of the population)<sup>7</sup>, with one of its highest prevalence in the state of Maharashtra being 21.9/1000<sup>(8)</sup>. This potentially malignant disorder, has a 7-23% rate of conversion into oral cancer, most common being oral squamous cell carcinoma (OSCC), with a high mortality rate.<sup>9</sup>

Although tobacco and areca-nut<sup>2</sup> exposure remain the principal recognized etiological factors, the development and progression of OSMF are likely influenced by multiple interacting environmental, nutritional, behavioural and host-related factors<sup>6</sup>.

Vitamin D has emerged as a potential modifier of inflammatory and fibrotic processes<sup>3</sup>. Accumulating evidence from cellular and experimental studies suggests that it may also possess anticancer properties, including the suppression of angiogenesis, metastatic progression, and tumor cell invasion<sup>4 5</sup>. However, vitamin D status is itself influenced by several factors, particularly sunlight exposure, clothing practices, sunscreen use, dietary intake, physical activity and lifestyle<sup>6</sup>.

This creates the rationale for a more comprehensive observational study evaluating these factors with vitamin D levels simultaneously. A comprehensive assessment of these determinants may therefore provide greater insight into the epidemiological context in which OSMF develops and progresses.

The present study is therefore designed as a cross-sectional investigation to compare environmental, dietary, lifestyle and behavioural factors between individuals with and without OSMF and to evaluate their association with the presence of OSMF. Serum 25-hydroxyvitamin D [25(OH)D] status will additionally be assessed to explore its association with the clinical severity of OSMF.

### Methodology:

An observational comparative study was conducted to evaluate the association of environmental, dietary, lifestyle and behavioural factors, including serum vitamin D status, with the presence and clinical severity of oral submucous fibrosis. The study began

after being granted clearance by the Institutional Ethics Committee (IEC/GDCHN/05/2024). All the willing participants of the study were thoroughly explained about the study in their vernacular language and their written consent was taken.

OpenEpi Version 3 software was used to calculate the sample size based on a reported OSMF prevalence of 4.6% <sup>(2)</sup> in the Indian population and a confidence level of 95%, using the formula ( $n = [DEFF * Np(1-p) / ((d^2 / Z^2_{1-\alpha*} (N-1) - p^{*(1-p)})]$ ). The minimum sample size required was determined to be 68 per group.

To minimize selection bias, participants in both groups were recruited from the same institutional setting during the same study period and were assessed using identical eligibility criteria, except for the presence or absence of clinically diagnosed OSMF.

A detailed case history of the consenting patient was taken before including them in the study. It included chief complains of the patients, habit history (in terms of time, duration, frequency and pattern of substance abuse), extra oral examination, palpation of lymph nodes, TMJ movements, competency of lips, by assessing difficulty in blowing cheeks or whistling.

Intra Oral examination was conducted in the view of OSMF for: presence or absence of burning sensation, difficulty in mouth opening, presence or absence of fibrous bands with sites, tongue protrusion and depapillation, mucosal blanching, shape of uvula, change in voice, presence or absence of ulceration, irritation to spicy and hot food, vesicles (present or absent). The patients were then further classified on clinical signs and symptoms using JN Khanna and Andre classification (1995).

68 Healthy individuals aged 18–50 years with no clinical evidence of OSMF were recruited within the same institution during the study period who met the eligibility criteria and had no clinical evidence of OSMF.

Patients with chronic systemic illnesses, endocrine disorders, cardiovascular diseases, autoimmune disorders, chronic kidney disease, chronic liver disease, diabetes mellitus were excluded. Pregnant patients and people unwilling to participate in the study were not included in the study as well.

All the participants were explained about the study in their regional language, and a written consent was obtained from the willing individuals. On the basis of the inclusion criteria, OSMF cases were selected and a detailed case history recording demographic details, habit history and clinical history was recorded.

A standardized but same questionnaire was administered to both OSMF participants and individuals in the comparison group to avoid recall bias. It assessed environmental, dietary, behavioural and lifestyle factors potentially associated with OSMF and vitamin D status. Dietary habits were assessed based on consumption of vitamin-D-rich and fortified food sources, including dairy products, eggs and fish, with the frequency of consumption recorded as the number of times per week.

Sunlight exposure was assessed according to the average duration of daily exposure and categorized as <15 minutes, 15–30 minutes, 30–60 minutes, or >60 minutes per day. Occupational exposure was classified as predominantly indoor, outdoor, or mixed. Physical activity was assessed based on whether participants engaged in regular physical activity or not. Clothing practices were assessed by asking participants whether they wore full-sleeved clothing, with responses categorized as yes, no, or maybe. Sunscreen use was assessed according to the frequency of application and categorized as always, occasionally, or never.

The same structured questionnaire was administered to both groups using standardized response categories to facilitate comparison of these environmental, dietary, behavioural and lifestyle factors.

Under strict aseptic precautions, 3 mL of venous blood was collected from the antecubital vein with minimal trauma. Serum vitamin D levels were subsequently assessed using the chemiluminescent immunoassay (CLIA) method, following the manufacturer's instructions.

Participants were categorized based on their serum vitamin D concentrations according to the following criteria<sup>10</sup>:

**Sufficient:** 31–100 ng/mL

**Insufficient:** 21–30 ng/mL

**Deficient:** <20 ng/mL.

#### Statistical Analysis:

Data obtained from both the OSMF patients and healthy group was systematically compiled in Microsoft Excel. Statistical analysis was subsequently performed using the Statistical Package for the Social Sciences (SPSS), version 26.0 (IBM Corp., Armonk, NY, USA).

Descriptive statistics were used to summarize the study data. Continuous variables were expressed as mean  $\pm$  standard deviation, while categorical variables were presented as frequencies and percentages.

#### Results:

A total of 136 participants were included in the study, comprising 68 individuals with OSMF and 68 individuals without OSMF. The mean age of the study population was  $38.26 \pm 9.40$  years. There were 96 (70.6%) males and 40 (29.4%) females. The control group had identical sex distributions, with 48 males and 20 females in each group to avoid confounding ( $p = 1.000$ ).

Among participants with OSMF, Grade 2 disease was the most frequently observed clinical category, comprising 26 (38.2%) participants, followed by Grade 1 in 17 (25.0%), Grade 3 in 16 (23.5%) and Grade 4 in 9 (13.2%). The mean mouth opening was  $1.96 \pm 0.76$  finger breadths. Reduced mouth opening was the most frequently reported clinical complaint.

**Table 1: Environmental and lifestyle factors associated with OSMF**

| Environmental/<br>lifestyle factor | Catego<br>ry | OS<br>MF<br>grou<br>p, n<br>(%) | Cont<br>rol<br>grou<br>p, n<br>(%) | $\chi^2$   | p-<br>valu<br>e |
|------------------------------------|--------------|---------------------------------|------------------------------------|------------|-----------------|
| Daily sunlight<br>exposure         | <15<br>min   | 13<br>(19.<br>1)                | 20<br>(29.4<br>)                   | 31.4<br>05 | <0.0<br>01      |
|                                    | 15 min       | 1<br>(1.5)                      | 2<br>(2.9)                         |            |                 |
|                                    | 15–30<br>min | 5<br>(7.4)                      | 20<br>(29.4<br>)                   |            |                 |
|                                    | 30–60<br>min | 2<br>(2.9)                      | 10<br>(14.7<br>)                   |            |                 |
|                                    | >60<br>min   | 47<br>(69.<br>1)                | 16<br>(23.5<br>)                   |            |                 |
| Full-sleeved<br>clothing           | Yes          | 10<br>(14.<br>7)                | 31<br>(45.6<br>)                   | 16.4<br>27 | <0.0<br>01      |
|                                    | No           | 58<br>(85.<br>3)                | 37<br>(54.4<br>)                   |            |                 |
| Sunscreen use                      | Never        | 68<br>(100<br>.0)               | 46<br>(67.6<br>)                   | 26.2<br>46 | <0.0<br>01      |

| Environmental/<br>lifestyle factor | Catego<br>ry   | OS<br>MF<br>grou<br>p, n<br>(%) | Cont<br>rol<br>grou<br>p, n<br>(%) | $\chi^2$  | p-<br>valu<br>e |
|------------------------------------|----------------|---------------------------------|------------------------------------|-----------|-----------------|
|                                    | Occasi<br>onal | —                               | —                                  |           |                 |
|                                    | Regula<br>r    | —                               | —                                  |           |                 |
| Physical<br>activity               | Regula<br>r    | 31<br>(45.<br>6)                | 39<br>(57.4<br>)                   | 1.88<br>4 | 0.17<br>0       |
|                                    | None           | 37<br>(54.<br>4)                | 29<br>(42.6<br>)                   |           |                 |
| Occupation                         | Indoor         | 28<br>(41.<br>2)                | 29<br>(42.6<br>)                   | 3.26<br>4 | 0.19<br>6       |
|                                    | Mixed          | 22<br>(32.<br>4)                | 29<br>(42.6<br>)                   |           |                 |
|                                    | Outdo<br>or    | 18<br>(26.<br>5)                | 10<br>(14.7<br>)                   |           |                 |

#### Environmental and lifestyle factors

Significant differences were observed between the OSMF and control groups with respect to reported sunlight exposure, full-sleeved clothing practices and sunscreen use.

For Sunlight exposure:

Daily sunlight exposure differed significantly between groups ( $p < 0.001$ ), with >60 minutes reported by 69.1% of OSMF participants versus 23.5% of healthy participants. (Table 1)

For clothing:

Full-sleeved clothing was reported by 10 (14.7%) participants with OSMF compared with 31 (45.6%) healthy participants ( $p < 0.001$ ). (Table 1)

For Sunscreen:

All participants with OSMF reported never using sunscreen, compared with 46 (67.6%) healthy participants. The distribution of sunscreen use differed significantly between groups ( $p < 0.001$ ). (Table 1)

Occupation type:

occupational classification as indoor, mixed or outdoor did not differ significantly between the groups ( $p = 0.196$ ). Indoor occupations were

reported by 28 (41.2%) OSMF participants and 29 (42.6%) healthy participants, mixed occupations by 22 (32.4%) and 29 (42.6%), and outdoor occupations by 18 (26.5%) and 10 (14.7%), respectively. (Table 1)

Physical activity:

No statistically significant difference was observed in physical activity between the OSMF and control groups ( $p = 0.170$ ). Regular physical activity was reported by 31 (45.6%) participants with OSMF and 39 (57.4%) healthy participants, whereas 37 (54.4%) participants with OSMF and 29 (42.6%) healthy participants reported no regular physical activity. (Table 1)

Dietary factors

Dietary characteristics also differed significantly between the two groups. The distribution of reported food sources differed significantly ( $p < 0.001$ ). Consumption of dairy, fish and eggs was reported by 20 (29.4%) participants with OSMF compared with 38 (55.9%) healthy participants.

The reported frequency of fish and/or dairy consumption also differed significantly between groups ( $p < 0.001$ ). Consumption three to four times per week was reported by 3 (4.4%) participants with OSMF compared with 21 (30.9%) healthy participants.

Serum Vitamin D

Vitamin-D deficiency was significantly more prevalent among participants with OSMF than healthy participants (97.1% vs 70.6%;  $p < 0.001$ ). (Table 2)

**Table 2: Comparison of frequencies (Vit D with cases and healthy participants)**

| VIT-D | Grp   |                      | Total | Chi-Square<br>value | P value of Chi-<br>Square test |
|-------|-------|----------------------|-------|---------------------|--------------------------------|
|       | Cases | Healthy participants |       |                     |                                |
| Def   | 66    | 48                   | 114   | 17.569              | 0.000**                        |
| Suf   | 2     | 20                   | 22    |                     |                                |
| Total | 68    | 68                   | 136   |                     |                                |

There was a statistically highly significant difference seen for the frequencies between the groups ( $p < 0.01$ ) with higher frequency for Def (deficient) with cases while higher frequency of sufficient (Suf) with healthy participants

#### Discussion:

The present cross-sectional study evaluated environmental, dietary, behavioural and lifestyle characteristics, together with serum vitamin D status, among individuals with and without oral submucous fibrosis. There was significant difference observed between the two groups in reported sunlight exposure, clothing practices, sunscreen use and dietary patterns.

No statistically significant differences were observed in physical activity or occupational exposure between the groups. Regular physical activity was reported by 45.6% of OSMF participants and 57.4% of healthy participants ( $p =$

0.170). Similarly, occupational categories did not differ significantly between groups.

Daily sunlight exposure differed significantly between groups ( $p < 0.001$ ), with more than 60 minutes of exposure reported by 69.1% of OSMF participants compared with 23.5% of healthy participants. However, sunlight duration alone may not reflect effective vitamin-D-producing exposure, which is influenced by factors such as exposed skin, clothing, sunscreen use, time of day and season. In this context, clothing practices and sunscreen use also differed significantly between groups, with full-sleeved clothing less frequently reported and sunscreen use absent among all OSMF participants. Dietary patterns also differed significantly between groups, including food sources and frequency of fish and dairy consumption. Healthy participants more frequently reported consumption of dairy, fish and eggs and higher-frequency fish and/or dairy intake. However, as dietary intake was assessed by food type and frequency rather than quantified vitamin-D intake, these findings should be interpreted as differences in dietary patterns rather than direct differences in vitamin-D consumption.

Serum 25(OH)D concentrations were descriptively lower among OSMF participants than healthy participants (median: 17.0 vs. 20.8 ng/mL), providing a biological context for the observed environmental and dietary differences. However, the cross-sectional design does not permit inference of causality.

As established by Sinor et al.<sup>11</sup> areca-nut exposure remains the principal established etiological factor, but the multifactorial nature of OSMF raises the possibility that nutritional and environmental characteristics may modify the biological context in which the disease develops.

Nutritional factors have previously been proposed as possible contributors to OSMF, although their role remains less firmly established than that of areca-nut exposure. Muti et al.<sup>12</sup> has implicated nutritional deficiencies and malnutrition in the pathogenesis of OSMF, but epidemiological evidence remains inconsistent. The present study extends this area by examining specific dietary patterns and selected food sources relevant to vitamin-D status.

Gondivkar et al.<sup>13</sup> examined socioeconomic status, lifestyle factors and oral premalignant lesions, including OSMF, in a very large population. Higher socioeconomic status and education were associated with lower odds of oral premalignant lesions.

The findings indicate that OSMF may be associated with a broader pattern of environmental and dietary characteristics rather than a single isolated factor. However, comparatively little attention has been given to environmental behaviours that influence vitamin-D status, particularly sunlight exposure, clothing practices and sunscreen use, in individuals with OSMF.

## Conclusion

OSMF participants demonstrated significantly different environmental and dietary patterns compared with healthy participants, including differences in sunlight exposure, clothing, sunscreen use and consumption of vitamin-D-rich food sources. Vitamin-D deficiency was also significantly more prevalent among OSMF participants. These findings suggest a potential relationship between OSMF and environmental and nutritional determinants of vitamin-D status; however, the cross-sectional design precludes causal inference. Larger longitudinal studies are warranted to clarify these associations.

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