ORIGINAL ARTICLE

Evaluation of Hemoglobin, Serum Iron, and Vitamin B12 Levels in Patients with Oral Submucous Fibrosis and their Comparison with Healthy Individuals

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Abstract

Background: Oral submucous fibrosis (OSMF) is a chronic, premalignant condition frequently associated with hematological abnormalities and mucosal degradation secondary to micronutrient deficiencies.

Objective: To evaluate hemoglobin (Hb), serum iron, and vitamin B12 levels among patients with OSMF and compare them with healthy controls.

Methods: This comparative cross-sectional, multi-center research study has been conducted at PNS Rahat Hospital, Karachi; the Dental OPD at Chital Dental and Medical College, Mirpurkhas; and PNS Darmaanjah Hospital, Ormara, Baluchistan. The study evaluated 115 clinically diagnosed OSMF patients and 115 age-matched healthy controls between July 2022 and January 2024. Hemoglobin, serum iron, and vitamin B12 levels were quantified using automated laboratory blood analysis.

Results: A total of 230 participants were evaluated (mean age: 41.52 ± 15.24 years for OSMF; 40.08 ± 14.31 years for controls). OSMF patients exhibited significantly lower mean levels of Hb (12.81 ± 1.35 *vs.* 13.45 ± 1.84, p<0.001), serum iron (67.76 ± 21.54 *vs.* 75.72 ± 23.68, p<0.001), and vitamin B12 (254.38 ± 109.66 *vs.* 345.03 ± 196.06, p<0.001) compared to healthy controls. However, multivariable logistic regression revealed no independent association between these hematological parameters and OSMF status.

Conclusion: Patients with OSMF present with a high prevalence of low Hb, serum iron, and vitamin B12 levels. Rather than serving as independent etiological factors, these micronutrient deficiencies function as concurrent compounding clinical features or secondary nutritional consequences of the disease. Consequently, comprehensive OSMF management protocols should integrate systematic nutritional screening and dietary supplementation alongside aggressive habit-cessation interventions.

Keywords: *Oral submucous fibrosis, serum iron, hemoglobin, vitamin B12, premalignant.*

INTRODUCTION

The chronic, progressive illness known as oral submucous fibrosis (OSMF) affects the oral cavity and can extend up to the pharynx and esophagus. The disease begins with the production of vesicles, which are preceded by a juxtaepithelial inflammatory response and the formation of fibroelastic bands. This causes the buccal mucosa in the area of the labial, buccal, and faucial pillars to stiffen, which can result in trismus [1, 2]. The disease stage determines the clinical appearance of this progressive, chronic illness upon diagnosis.

The most common symptoms are hearing loss, restricted tongue movements, lip, tongue, and palate stiffness that decreases mouth opening, sensitivity to hot meals, and progressive dysphagia [3].

An estimated five million cases of oral submucous fibrosis (OSMF) occur worldwide [4]. South and Southeast Asia have the highest frequency, especially among those who chew areca nuts. Palpable bands on the mucosa of the labial, buccal, and faucial pillars provide the basis for OSMF diagnosis. Malignant transformation rates ranged from 2.6% to 7.6% [5]. Researchers believe that OSMF is a complex illness, and further study is necessary to determine how each element contributes to its progression.

The pathophysiology of OSMF has been suggested to involve nutritional deficiencies, specifically iron and vitamin deficiencies. Iron is necessary for the gross health and integrity of the epithelium of the digestive system, as well as for supporting the proper activity of enzymes. OSMF is also thought to be an Asian form of sideropenic dysphagia, in which a chronic iron deficiency causes mucosal sensitivity to irritants like chilies and areca nut products [3].

Hemoglobin levels, particularly serum iron levels, are considered biochemical indicators for nutritional evaluation [6]. Iron, vitamin B12, and folic acid deficiencies can all compromise the integrity of the oral mucosa. OSMF has been associated with significant hematological abnormalities, including elevated total iron-binding capacity, decreased serum iron, and elevated erythrocyte sedimentation rate. Each of these trace elements is crucial to the integrity of the oral mucosa [7].

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The literature on the roles of iron and vitamin B12 in OSMF is scarce. It is necessary to determine if these trace components can change this debilitating disorder. More studies are needed in this area to develop sensitive, specific, and rapid assays for OSMF diagnosis and prognosis, given the multifactorial etiology. This study is therefore aimed at determining hemoglobin (Hb), serum iron, and Vitamin B12 levels among patients with OSMF and comparing them with those of healthy individuals.

METHODOLOGY

This comparative cross-sectional, multi-center research study has been conducted at PNS Rahat Hospital, Karachi; the Dental OPD at Chital Dental and Medical College, Mirpurkhas; and PNS Darmaanjah Hospital, Ormara, Baluchistan. Ethical approval was obtained from PNS Rahat Hospital before conducting this study (Ref. ERC/MED/011/2022). Patients from diverse demographic backgrounds with signs and symptoms of OSMF who visited dental OPDs between July 2022 and January 2024 for routine dental care were selected as research participants. Patients without systemic disorders who were presenting for routine hematological testing were matched for age and gender to create healthy controls. All study participants gave their written informed consent.

Sample size was calculated using the Open-Epi calculator, with mean iron among cases = 224.0±30.9 and mean iron among controls = 211.3±37.5 [8], at 80% power and a 95% confidence level; the calculated sample size was 230, with 115 in each group.

The study population consisted of all patients who were willing to participate voluntarily and to perform blood test s on their own. They were divided into two groups based on the following inclusion criteria: Group A comprises 115 age- and gender-matched healthy controls with no systemic illnesses, no oral lesions, and no history of alcohol, tobacco, or areca nut chewing. In contrast, 115 participants in Group B have a clinical diagnosis of OSMF and are older than 18 years, according to the diagnostic criteria of Arakeri *et al.* [9].

Patients with a history of previous therapy for OSMF, systemic illnesses (*e.g.*, diabetes, hypertension, liver/ kidney disorders), or other oral mucosal lesions were excluded. Crucially, pregnant patients and individuals currently taking iron or Vitamin B12 supplements were excluded from the study to prevent confounding the hematological baseline. Equal numbers of patients were taken from each center. Only patients who provided written, signed consent were included in the study, and all patients who met the inclusion criteria were informed in their mother tongue. After obtaining oral histories, all registered individuals underwent clinical examination and interviews at the dental clinic.

After following the necessary aseptic procedures, a venipuncture of the median cubital vein was performed by a trained medical nurse, yielding approximately 5 mL of venous blood. Following sample collection, the patient's name and age were written on the sample label. The specimen was stored in an icebox. After that, the sample was brought to the lab for a serum analysis. Blood samples were analyzed using Sysmex automated analyzers. Quality control was maintained through daily calibration and the use of standardized reagents. For this study, anemia was defined according to WHO criteria as Hb <13 g/dL in males and <12 g/dL in females. Iron deficiency was defined as serum iron < 60 mcg/dL, and Vitamin B12 deficiency was defined as levels < 200 pg/ mL [10].

To analyze the data, IBM SPSS Statistics version 27 was utilized. Frequencies and percentages were computed for qualitative factors. The mean and standard deviation were calculated for quantitative variables after assessing the normality of the distribution with the Shapiro-Wilk test. While the independent t-test was applied for mean comparison, the chi-square/Fisher exact test was used to assess the relationship between qualitative variables. The odds ratio with its 95% confidence interval was calculated using binary logistic regression. Multivariable logistic regression was used for variables that were significant in univariate logistic regression ($p<0.05$). P-values below 0.05 were regarded as statistically significant.

RESULTS

Our study included 230 patients, split into two equal groups of 115 patients each. Patients without oral submucous fibrosis are in Group A, while those with it are in Group B. Group A had 60% males and 40% females, while Group B had 68.7% males and 31.3% females; there was no difference (p=0.169). Group A's mean age was 40.08±14.31 years, whereas group B's was 41.52±15.24 years, which was not significantly different between the two groups (p=0.463). Mean levels of hemoglobin (13.45±1.84 *versus* 12.81±1.35, p=0.003), serum iron (75.72±23.68 *versus* 67.76±21.54, p=0.010), and serum vitamin B12 (345.03±196.06 *versus* 254.38±109.66, p<0.001) were higher in group A than group B. Frequency of anemia (50.4% *versus* 36.5%, p=0.033), iron deficiency (57.4% *versus* 37.4%, p=0.002), and B12 deficiency (47.8% *versus* 33%, p=0.002) were higher in Group B than in Group A. Table **1** presents detailed results for both groups.

Table 1: Demographics and clinical characteristics of study participants.

Variables	Study Groups n (%)		p-value
	Group A	Group B	
Gender			
Male	69(60)	79(68.7)	0.169
Female	46(40)	36(31.3)	
Age (Years)	40.08±14.31	41.52±15.24	0.463

Variables	Study Groups n (%)		p-value
	Group A	Group B	
Age Group			
≤30 years	31(27)	30(26.1)	0.916
31-45 years	46(40)	44(38.3)	
>45 years	38(33)	41(35.7)	
Hemoglobin (mg/dl)	13.45±1.84	12.81±1.35	0.003*
Anemia			
Yes	42(36.5)	58(50.4)	0.033*
No	73(63.5)	57(49.6)	
Serum Iron (mcg/ld)	75.72±23.68	67.76±21.54	0.010*
Iron Deficiency			
Yes	43(37.4)	66(57.4)	0.002*
No	72(62.8)	49(42.6)	
Serum Vitamin B12 Levels (pg/mL)	345.03±196.06	254.38±109.66	<0.001*
B12 Deficiency			
Yes	38(33)	55(47.8)	0.022*
No	77(67)	60(52.2)	

Chi-square test was applied, ¥ Independent t-test was applied, *Significant at 0.05 levels.

In univariate analysis, males had a higher likelihood of sub mucosal fibrosis than females, but this did not reach statistical significance (p=0.169). In contrast to non-anemic individuals, anemic patients had a higher likelihood of sub mucosal fibrosis (p=0.034). Compared with patients without iron deficiency, those with iron deficiency had a higher likelihood of developing submucosal fibrosis (p=0.003). Compared with patients without vitamin B12 deficiency, those with vitamin B12 deficiency had a higher likelihood of developing submucosal fibrosis (p=0.023). In the adjusted model, none of the three hematological variables were statistically significant (**Table 2**).

Table 2: Univariate and multivariable association of patients' features with submucous fibrosis.

Variables	Univariate Model		Multivariable Mode	
	OR (95% CI)	p-value	aOR (95% CI)	p-value
Gender				
Male	1.463 (0.850-2.517)	0.169	-	-
Female	1.000		-	-
Age Group				
≤30 years	0.897 (0.460-1.750)	0.750	-	-
31-45 years	0.887 (0.484-1.623)	0.696	-	-
>45 years	1.000		-	-
Anemia				
Yes	1.769 (1.044-2.996)	0.034*	1.064 (0.531-2.132)	0.862
No	1.000		1.000	
Iron Deficiency				
Yes	2.255 (1.330-3.826)	0.003*	1.896 (0.926-3.885)	0.080
No	1.000		1.000	
B12 Deficiency				
Yes	1.857 (1.089-3.167)	0.023*	1.414 (0.787-2.538)	0.246
No	1.000		1.000	

CI: Confidence interval, OR: Odds ratio, aOR: Adjusted Odds ratio, *Significant at 0.05 level.

DISCUSSION

More and Rao [11] proposed a new description of OSMF based on a large body of clinical data: a severe, progressive, irreversible collagen metabolic disease caused by continuous consumption of areca nut. The oral mucosa and, in certain cases, the throat and esophagus may be impacted by commercial areca nut formulations. They may also result in functional morbidity and mucosal stiffness. It mostly affects South East Asians, particularly those from India and Pakistan, and entails a risk of malignant change. The prevalence of OSMF patients in Pakistan is 4.1%, and malignancies develop in 3% to 19% of these cases. The condition's rapid spread is attributed to the growing acceptance of this habit among young people in Pakistan, driven by easy access, lower price points, and marketing initiatives, as well as the popularity of commercially produced areca nut preparations (pan masala).

The current investigation found that the blood iron, hemoglobin, and vitamin B12 levels in OSMF patients were lower than in controls. Other foreign studies have observed similar results [12-14].

In our study, OSMF patients had a higher male-to-female ratio. The male preponderance observed in our analysis was consistent with findings reported by Urooj *et al.* [8] and Rangnathan *et al.* [15]. Another study conducted in a similar demography showed similar male predominance [16]. The easy access to chewing gum is likely the reason for this male preponderance pattern.

In the present study, we analyzed iron and hemoglobin levels in the OSMF group and found that patients with OSMF had low serum levels of hemoglobin, iron, and vitamin B12. Another study [17] showed similar results, but these results are contradictory to a similar study conducted in Karachi [8].

In his study, Ramanathan found that iron deficiency anemia (IDA) affects 77% of individuals with OSMF [17]. Compared with healthy controls, our study found that 50.4% of OSMF patients had IDA. Our study's findings are consistent with those of research by Patil *et al.* [7], Ganapathy *et al.* [12], Karthik *et al.* [14], Lamlakar and Parashram [18], Taneja *et al.* [19], and Rupak *et al.* [20]. Research by various authors examining the hematology profile in OSMF is presented in Table **3**.

Table 3: Comparison of studies by various authors depicting the role of trace elements in oral submucous fibrosis.

Authors	IDA	Hb	Iron	Vitamin B12
Present study	50.4%	Decreased	Decreased	Decreased
Wahi *et al.* [21]	NA	NA	NA	Decreased
Bharadwaj *et al.* [13]	NA	NA	Lowest in OSMF group	NA

Authors	IDA	Hb	Iron	Vitamin B12
Ramanathan [17]	77%	NA	NA	NA
Lamlakar and Parashram [18]	NA	Decreased	Decreased	Decreased
Ganapathy *et al.* [12]	NA	Decreased	Decreased	NA
Wang *et al.* [22]	NA	NA	NA	Decreased
Tadakmadla [23]	NA	NA	Decreased	NA
Anuradha [24]	NA	NA	Decreased	NA
Thakur and Guttikonda [25]	NA	Decreased	Decreased	NA
Karthik *et al.* [14]	NA	Decreased	Decreased	NA
Taneja *et al.* [19]	NA	Decreased	NA	NA
Rupak *et al.* [20]	NA	Decreased	NA	NA

NA: Not assessed, IDA: Iron deficiency anemia, OSMF: Oral submucous fibrosis, Hb: Hemoglobin

Many studies indicate that OSMF causes iron insufficiency through altered eating patterns, burning sensations, and oral mucosal vesiculation and ulceration, which make it difficult to consume a typical diet and result in inadequate nutrition. In an intriguing example described by Bhattacharya *et al.* [26], IDA predominantly led to the development of OSMF, which was effectively treated with oral antioxidants and iron supplements. Hegde *et al.* [27] also highlight the importance of evaluating hemoglobin and serum iron levels in patients with Oral Submucous Fibrosis and suggest that initiating iron therapy at the time of diagnosis may help prevent further disease progression.

Our study has a few limitations due to its cross-sectional design, which precludes establishing a temporal relationship between micronutrient levels and OSMF progression. Furthermore, certain confounders, such as exact duration or frequency of areca nut use, socioeconomic status, and detailed dietary habits, were not adjusted for in the regression model. We recommend further prospective studies to determine if nutritional intervention can alter the clinical course of the disease.

CONCLUSION

This study confirms a high prevalence of anemia and concurrent iron and vitamin B12 deficiencies among patients diagnosed with OSMF. While multivariable analysis indicates that these deficiencies are not independent statistical predictors of the disease, their frequent co-existence highlights the importance of routine nutritional screening. Corrective nutritional supplementation should be used as a supportive therapeutic measure to optimize mucosal healing, alongside the primary goal of stopping areca nut use.

ETHICS APPROVAL

The study was approved by the ethical committee of PNS Rahat Hospital, Karachi (Ref. ERC/MED/011/2022). All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and the Declaration of Helsinki.

CONSENT OF PUBLICATION

Informed consent was obtained from all patients.

AVAILABILITY OF DATA

The data are not publicly available due to privacy or ethical restrictions, but can be provided upon request to the corresponding author.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Declared none.

AUTHORS' CONTRIBUTION

Nadeem H: Conception and design of the study and revision of the manuscript for important intellectual content, conducted data collection and supervised the statistical analysis, conducted the literature search, interpreted the results, and drafted and finalized the manuscript.

Khan MN: Data collection, statistical analysis, literature search, interpretation of the results, and manuscript drafting.

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