Original Article

Evaluation of Hematological profile in Oral Submucous Fibrosis: A Cross-sectional Study

Deepa Jatti Patil[1], Manjiri Joshi[2]

[1]Department of Oral Medicine and Radiology, KM Shah Dental College and Hospital, Sumandeep Vidyapeeth Deemed to be University, Piparia,[2]Department of Oral Medicine and Radiology, Manubhai Patel Dental College, Hospital and Oral Research Institute, Vadodara, Gujarat, India

Abstract

Aims: The purpose of this study was to estimate hemoglobin (Hb) level, red cell indices (RCIs), serum iron level and Vitamin B12 level in patients with oral submucous fibrosis (OSMF) and to analyze the association of these parameters in different stages of OSMF.

Setting and Design: This case–control study comprised of 65 individuals, with 40 OSMF patients and 25 controls. The OSMF patients were graded clinically according to the classification by Arakeri *et al*. Fasting blood samples were collected from both groups for hematological evaluation.

Statistical Analysis Used: The mean values and standard deviations were calculated by Chi-square test. Normality of various parameters was evaluated by Kolmogorov–Smirnov test. The independent *t*-test was used to compare more than two means simultaneously. Correlation analysis was done by Karl Pearson's correlation coefficient method.

Results: The OSMF patients were in the age range of 21–67 years, with a mean age of 39.85 ± 10.42 years. The mean value of Hb of the control group was 14.24 ± 1.03 g/dL, whereas that of OSMF group was 11.18 ± 2.06 g/dL ($P < 0.001$). The mean value of the serum iron level of the control group was 119.67 ± 42.42 μg/dL, whereas that of the OSMF group was 45.04 ± 10.41 μg/dL ($P < 0.001$). The mean value of serum Vitamin B12 levels of the control group was 422.98 ± 112.57 μg/dL, whereas that of the OSMF group was 211.78 ± 45.17 μg/dL ($P < 0.001$). The RCIs including packed cell volume, mean corpuscular volume, mean corpuscular Hb (MCH) and MCH concentration were significantly reduced in OSMF cases. Iron deficiency was present in 38 patients among the study group and Vitamin B12 deficiency was present in 22 patients of the study group.

Conclusion: OSMF causes depletion of minerals and trace elements, and its replenishment is required for the healing of tissues and performing daily routine activities.

Keywords: Chemoprevention, iron deficiency, oral submucous fibrosis, Vitamin B12 deficiency

Address for correspondence: Dr. Deepa Jatti Patil, Department of Oral Medicine and Radiology, KM Shah Dental College and Hospital, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara - 391 760, Gujarat, India.
E-mail: iafdeepa@gmail.com

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INTRODUCTION

Since 1950, oral submucous fibrosis (OSMF) has emerged as one of the most prevalent potentially malignant disorders (PMDs), largely among the people of Asian origin. The disease is mainly seen in India, Bangladesh, Sri Lanka, Pakistan, Taiwan, Southern China, Polynesia and Micronesia.[1] About 2.5 million individuals are affected worldwide.[2] The most common etiological factor hypothesized to initiate the condition is areca nut chewing, and nutritional deficiencies, immunologic processes and genetic predisposition are other associated factors.[3,4]

The condition is progressive, and patients present with varied clinical presentation depending on the stage of the disease at the time of diagnosis. The most common presentations include intolerance to spicy food and rigidity of lip, tongue and palate leading to decreased mouth opening; restricted tongue movements; dysphagia and hearing impairment in the advanced stages. The trademark of the disease is submucosal fibrosis involving most parts of the oral cavity, pharynx and upper third of the esophagus.[1]

The role of nutritional deficiencies, principally of iron and vitamins, has been proposed in the etiology of OSMF.[5] Significant research has been carried out to emphasize their role in OSMF. Wahi *et al.*[6] reported a significantly higher prevalence of malnutrition in 104 OSMF patients than in 200 normal control participants. Iron has been studied as a diagnostic and prognostic marker in malignancies such as esophageal cancers (Plummer–Vinson syndrome) and postcricoidal carcinoma. Serum levels of iron were found to be significantly altered in oral cancer and PMDs.[7] Vitamin B12 deficiency can cause moderate-to-severe epithelial dysplasia that resolves after correction of the deficiency. Both the trace elements are required for maintaining the integrity of the oral mucosa.[8,9]

There is paucity of literature regarding the role of Vitamin B12 and iron in OSMF. There is a need to assess whether these trace elements alter the course and progression of OSMF. Considering the multifactorial etiology, more research is required in this direction to develop sensitive, specific and faster tests in the diagnosis and prognosis of OSMF. Therefore, the study was conducted to estimate hematological parameters in OSMF patients and to analyze the association of the levels of serum iron, hemoglobin (Hb) and Vitamin B12 in different stages of OSMF.

SUBJECTS AND METHODS

This cross-sectional study was designed after obtaining permission from the institutional ethical committee (IEC/MPDC_152/OD-11/18). The study population were selected from patients presenting for routine dental treatment with signs and symptoms of OSMF visiting the Department of Oral Medicine and Radiology, Manu Bhai Patel Dental College, Vadodara, Gujarat, India, from October 2018 to June 2019. Age- and gender-matched healthy controls were selected from patients who are free from systemic diseases and reporting for routine hematological investigations. Written informed consent was taken from all the study participants. The sample size was calculated using quantitative data by Ganapathy *et al.*[10]

INCLUSION CRITERIA

The study population was divided into the following two Inclusion criteria-groups:

- Group I – Forty individuals with clinical diagnosis of OSMF above 18 years of age according to the diagnostic criteria by Arakeri Thomas *et al.*[11]
- Group II – Twenty-five age- and gender-matched healthy controls, free of systemic diseases, with no oral lesions and no history of tobacco or areca nut chewing or alcohol intake.

Patients with stage IV OSMF, Oral mucosal lesions other than OSMF observed clinically; previously treated cases of OSMF; patients with oral mucosal lesions, systemic disorders such as diabetes, hypertension and liver/kidney disorders and/or those under treatment for any other systemic diseases were excluded from the study. In the control group, patients with pregnancy, those with a history of tobacco and areca nut chewing or alcohol intake, patients on iron and Vitamin B12 supplements in the last 6 months and patients unwilling to participate in the study were also excluded from the study.

All the patients fulfilling the inclusion criteria were informed about the study in their own mother tongue and only those who agreed to give a written signed voluntary consent were enrolled in the study. All the enrolled participants were interviewed and examined in the dental clinic using clinical examination tools after recording case history. During the interview, details of deleterious habits were also noted. After satisfying the diagnostic criteria of OSMF by Arakeri Thomas *et al.*,[11] the patients were recalled the next day early in the morning. The fasting blood sample was collected. Approximately 5 mL of venous blood was obtained by venipuncture of the

median cubital vein after taking all aseptic precautions.

Estimation of iron, hemoglobin and red cell indices

Estimation of iron was done using Ferrene's method. The red cell indices (RCIs) and Hb level were analyzed by the automated cell counter method. All the test tubes used for analysis were immersed overnight in deionized water and then washed the next day using deionized water. The serum sample used for the estimation was mixed with appropriate proportions of buffer and color reagents supplied in the iron estimation kit in clean, dry test tube as per the manufacturer's instructions. The absorbance of these samples was compared to that of the standard solution provided in the kits at 578 nm in a semi-autoanalyzer (Microlab – 200). Bucheon-si;Gyeonggi-do :South Korea.

Estimation of Vitamin B12 was done by the chemiluminescent microparticle intrinsic factor assay for the quantitative determination of Vitamin B12 in human serum method.[12]

Statistical analysis

Statistical procedure was carried out using the IBM SPSS Statistics for Windows, version XX (IBM Corp., Armonk, N.Y., USA)'. The mean values and standard deviations for all the groups were calculated by Chi-square test. Normality of various parameters in the control and study groups was assessed by Kolmogorov–Smirnov test. Independent *t*-test was used to compare more than two means simultaneously, that is, whether there was a significant difference between the mean values of serum iron, Vitamin B12 and Hb among the two groups. Correlation analysis among various parameters in the control and study groups was done by Karl Pearson's correlation coefficient method. A logistic regression analysis was performed to predict which hematological variable was more significant for OSMF. The level of significance was fixed at $P < 0.05$.

RESULTS

A total of sixty patients were evaluated for hematological investigations (32 males and 33 females). The study and the control group comprised 40 and 25 individuals, respectively. The OSMF group showed a slight female predominance with 22 females and 18 males. Table 1 shows the gender distribution in the OSMF and control groups. The OSMF patients were in the age range of 21–67 years with a mean age of 39.85 ± 10.42 years. Comparison of the two study groups for age (control and study) by Chi-square test revealed the presence of majority of cases between 31 and 40 years and was statistically significant ($P = 0.0111$) [Figure 1]. The mean age of the control group was 31.00 ± 10.85 years.

Table 1: Distribution of male and females in the control and study groups

Gender	Control group (%)	Study group (%)	Total
Male	14 (56.00)	18 (45.00)	32
Female	11 (44.00)	22 (55.00)	33
Total	25 (100.00)	40 (100.00)	65

$\chi^2=0.7452$, $P=0.3881$

Figure 2 shows the distribution of OSMF patients according to the classification of Arakeri Thomas *et al*.[11] Table 2 shows the comparison of mean values of the hematological parameters in the study and control groups by independent *t*-test, and the values were statistically significant ($P < 0.0001$).

The mean value of Hb of the control group was 14.24 ± 1.03 g/dL, whereas that of the OSMF group was 11.18 ± 2.06 g/dL ($P < 0.001$). The mean value of serum iron level of the control group was 119.67 ± 42.42 μg/dL, whereas that of OSMF group was 45.04 ± 10.41 μg/dL ($P < 0.001$). The mean value of serum Vitamin B12 levels of the control group was 422.98 ± 112.57 μg/dL, whereas that of OSMF group was 211.78 ± 45.17 μg/dL ($P < 0.001$).

Figure 3 depicts the comparison of hematological parameters in the study and control groups. On analyzing the values by Pearson's correlations, it was observed that the values of Hb, packed cell volume, mean corpuscular volume, mean corpuscular Hb (MCH) and MCHC levels had a significant correlation with serum iron levels in the OSMF Stage III (correlation value: 0.6870, 0.6220, 0.6200, 0.4490 and 0.2110, respectively) [Table 3].

Table 4 depicts the logistic regression analysis. Iron deficiency was present in 38 patients among the study group as compared to 3 patients in the control group. Odds

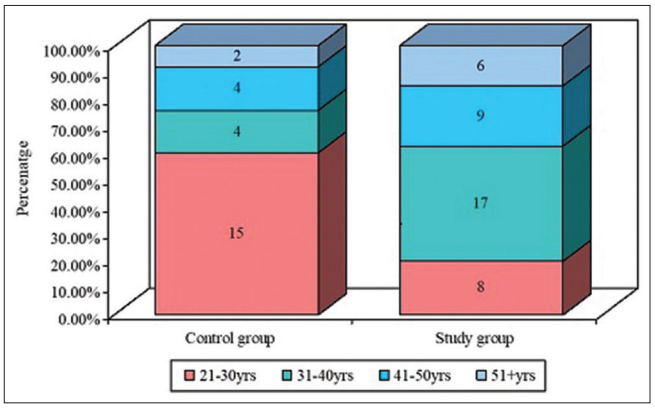

Figure 1: Age distribution among the study and control groups

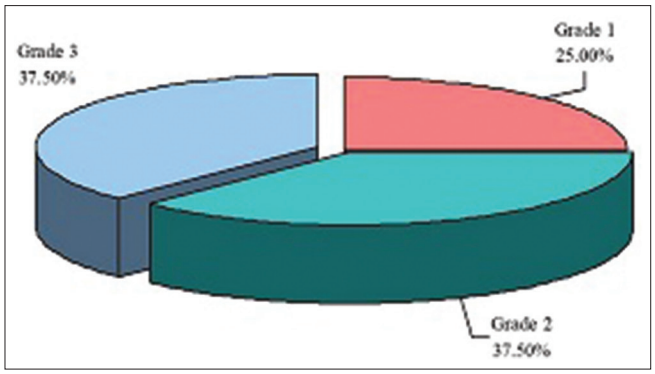

Figure 2: Patient distribution in various grades of oral submucous fibrosis

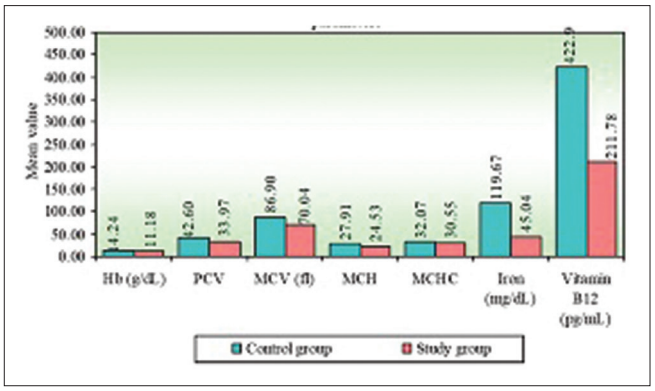

Figure 3: Comparison of hematological parameters in the study and control groups

ratio (OR) was 27.36, predicting the high occurrence of iron deficiency 27.36 times than the control group. Vitamin B12 deficiency was present in 21 patients in the study group as compared to 1 patient in the control group, with the OR of 3.91, predicting the occurrence of Vitamin B12 deficiency 3.91 times than that of the control group.

The mean maximum mouth opening of the forty OSMF patients was 14.1. The soft palate, retromolar area and buccal mucosa were the three sites involved by OSMF in every patient, with extra involvement of labial mucosa in 19 patients (60.3%), floor of the mouth in 5 patients (48.5%) and tongue in 8 patients (39.7%). Of the 40 OSMF patients, 17 had three-site involvement, 18 had four-site involvement, 9 had five-site involvement and 5 had six-site involvement by OSMF.

Table 2: Comparison of control and study groups by independent *t*-test

Parameters	Mean±SD		*t*	*P*
	Control group	Study group		
Hb (g/dL)	14.24±1.30	11.18±2.06	6.6377	<0.001
PCV	42.60±5.36	33.97±6.42	5.6071	<0.001
MCV (fl)	86.90±6.48	70.04±9.08	8.0768	<0.001
MCH	27.91±2.63	24.53±4.12	3.6593	<0.001
MCHC	32.07±1.13	30.55±2.41	2.9658	<0.01
Iron (mg/dL)	119.67±42.42	45.04±12.99	10.4158	<0.001
Vitamin B12 (pg/mL)	422.98±112.57	211.78±45.17	10.6148	<0.001

MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean cell hemoglobin, MCV: Mean corpuscular volume, PCV: Packed cell volume, Hb: Hemoglobin, SD: Standard deviation

DISCUSSION

More and Rao[13] proposed a new definition for OSMF based on extensive clinical research as *a debilitating, progressive, irreversible collagen metabolic disorder induced by chronic chewing of areca nut and its commercial preparations; affecting the oral mucosa and occasionally the pharynx and oesophagus; leading to mucosal stiffness and functional morbidity; and has a potential risk of malignant transformation*. Our study involving forty OSMF patients showed that 74.29% were gutkha users, 5.44% were pan users and only 21.11% were areca nut users, which was in concordance with studies carried out by Ranganathan *et al.* (69%)[14] and a review by Murti *et al.* (98%).[2] The prevalence of increased gutkha chewing over areca nut could be due to the easy availability of attractive, tiny, multicolored gutkha packets.

OSMF manifests early in gutkha chewers than betel quid chewers due to the presence of higher amount of dry weight tobacco content and absence of betel leaf (has beta carotene which acts as an antioxidant).[15,16] Areca nut induces mechanical abrasion, cytotoxicity and collagen cross-linking of the oral mucosa. The addition of nicotine amplifies the cytotoxicity induced by nicotine, leading to the production of damaged collagen.[17]

The trismus, fibrosis and malignant classification by Arakeri Thomas *et al.*[11] *et al.* was used in the present study as it helps in classifying the information efficiently, recording the data, proper communication, obtaining prognostic information and also to make it easy to understand the disease characteristics, which can be easily applied by the trainees and clinicians. It is a three-tier staging system, differentiating between medical, surgical and malignant disease therapy.

The present study shows decreased levels of serum iron, Hb and Vitamin B12 in OSMF patients as compared to the controls. Similar results were found in other studies conducted worldwide.[6,18-26] Table 5 summarizes the studies conducted by various authors investigating the role of trace elements in OSMF. According to Wang *et al.*,[18] the levels of folic acid and Vitamin B12 had decreased in OSMF patients. Wahi *et al.*[6] also found decreased Vitamin B12 and Vitamin C levels in OSMF group. Apart from these two studies, the present study assessed the prevalence of Vitamin B12 and RCIs in addition to other hematological parameters. The RCIs were not assessed in other studies.

Table 3: Correlation analysis among hematological parameters in the study group by Karl Pearson's correlation coefficient method

Parameters	Summery	Hb (g/dL)	PCV	MCV (fl)	MCH	MCHC	Iron (mg/dL)	Vitamin B12 (pg/mL)
Hb (g/dL)	*r*							
	P							
PCV	*r*	0.8450						
	P	<0.001						
MCV (fl)	*r*	0.7890	0.7880					
	P	<0.001	<0.001					
MCH	*r*	0.6090	0.5480	0.5790				
	P	<0.001	<0.001	<0.001				
MCHC	*r*	0.2630	0.1620	0.0610	0.1200			
	P	0.1010	0.3170	0.7100	0.4620			
Iron (mg/dL)	*r*	0.6870	0.6220	0.6200	0.4490	0.2110		
	P	<0.001	<0.001	<0.001	<0.01	0.1920		
Vitamin B12 (pg/mL)	*r*	0.0060	0.0220	0.0070	−0.0810	−0.0320	0.2800	
	P	0.9710	0.8910	0.9650	0.6200	0.8450	0.0800	

*$P<0.05$. MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean cell hemoglobin, MCV: Mean corpuscular volume, PCV: Packed cell volume, Hb: Hemoglobin, SD: Standard deviation

Table 4: Logistic regression analysis of the study group by haematological parameters

Parameters	Control group	Study group	OR	95% CI for OR		*P*
				Lower	Upper	
Hb (g/dL)						
Deficiency	5	35	0.88	0.13	6.18	0.8990
Normal	20	5				
PCV						
Deficiency	1	18	2.64	0.14	51.52	0.5230
Normal	24	22				
MCV (fl)						
Deficiency	2	32	2.99	0.20	44.68	0.4280
Normal	23	8				
MCH						
Deficiency	6	26	0.19	0.02	1.87	0.1530
Normal	19	14				
MCHC						
Deficiency	5	22	0.27	0.03	2.66	0.2630
Normal	20	18				
Iron (mg/dL)						
Deficiency	3	38	27.36	1.08	692.25	0.0450*
Normal	22	2				
Vitamin B12 (pg/mL)						
Deficiency	1	21	3.91	0.35	44.20	0.2700
Normal	24	19				
Total	25	40				

*$P<0.05$. MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean cell hemoglobin, MCV: Mean corpuscular volume, PCV: Packed cell volume, Hb: Haemoglobin, SD: Standard deviation, CI: Confidence interval, OR: Odds ratio

Ramanathan[9] in his study reported that 10 (77%) of the 13 OSMF patients have iron deficiency anemia (IDA). The present study showed a significantly greater frequency of IDA in 37 OSMF patients (92.5%) than that in healthy control patients. Some studies have shown contrasting results. Wahi *et al.*[6] discovered that 6% of the seventy male OSMF patients and 11% of the 34 female OSMF patients had anemia, but the frequency of anemia in OSMF patients did not differ significantly from that of the controls.

A study conducted by Bhardwaj *et al.*[7] on 120 participants showed a progressive decline in serum iron and Hb levels from Stage I OSMF to Stage IV OSMF, which was also observed in the present study. The results obtained in our study are in accordance to the studies conducted by Ganapathy[10] *et al.*, Karthik *et al.*,[23] Lavina *et al.*[25] *et al.* and Rupak *et al.*[26]

Iron is the key element in the human body with important functions in DNA, RNA and collagen and involves in antibody synthesis apart from the regulation of numerous physiological and metabolic processes. It plays a vital role in the development and maintenance of oral mucosa.[27]

IDA due to low serum iron levels manifests clinically as fatigue, achlorhydria, atrophy of epithelium, loss of attention, irritability, dyspnea and lowered memory. The oral manifestations include angular cheilitis, atrophic glossitis, generalized oral mucosal atrophy, candida infections, pallor and stomatitis. Plummer–Vinson syndrome or Paterson–Kelly syndrome or sideropenic dysphagia is a rare premalignant condition characterized by IDA, dysphagia and koilonychia, with women being affected more than men. Dysphagia results from the presence of abnormal esophageal webs which have predisposition toward malignant transformation.[28,29]

The hallmark histopathological features in OSMF are epithelial atrophy, dense corium and increased collagen production. The hypothesis of decreased iron levels in OSMF can be attributed to the following reasons. Cytochrome oxidase is required for the normal maturation of epithelium. In cases of IDA, it causes epithelial atrophy, making the mucosa vulnerable to irritants.[30] OSMF is basically a disorder of collagen metabolic disorder due to overproduction of highly cross-linked insoluble collagen Type I. The hydroxylation reaction of collagen requires ferrous iron and ascorbic acid. Utilization of iron for the

Table 5: Comparison of studies by various authors depicting the role of trace elements in oral submucous fibrosis

Authors	IDA	Hb	RCI	Iron	Ferritin	Folic acid	Vitamin B12	Vitamin C
Present study	92.5%	Decreased	Decreased	Decreased	NA	NA	Decreased	NA
Wahi *et al*.[6]	NA	NA	NA	NA	NA	NA	Decreased	Decreased
Bharadwaj *et al*.[7]	NA	NA	NA	Lowest in OSMF group	NA	NA	NA	NA
Ramanathan[9]	77%	NA	NA	NA	NA	Decreased	NA	NA
Ganapathy *et al*.[10]	NA	Decreased	NA	Decreased	NA	NA	NA	NA
Wang *et al*.[18]	NA	NA	NA	NA	NA	Decreased	Decreased	NA
Tadakmadla[19]	NA	NA	NA	Decreased	NA	NA	NA	NA
Anuradha CD[20]	NA	NA	NA	Decreased	NA	NA	NA	Decreased
Thakur and Guttikonda[22]	NA	Decreased	NA	Decreased	Decreased	NA	NA	NA
Karthik *et al*.[23]	NA	Decreased	NA	Decreased	NA	NA	NA	NA
Lavina *et al*.[25]	NA	Decreased	NA	NA	NA	NA	NA	NA
Rupak *et al*.[26]	NA	Decreased	NA	NA	NA	NA	NA	NA

NA: Not assessed, IDA: Iron deficiency anemia, OSMF: Oral submucous fibrosis, RCI: Red cell index, Hb: Hemoglobin

hydroxylation of proline and lysine, leads to decreased serum iron levels.[31]

Lack of iron in tissues causes improper vascular channel formation, resulting in decreased vascularity. This leads to derangement in the inflammatory reparative response of the lamina propria, resulting in defective healing and scar formation, further augmenting the fibrosis.[27]

Majority of the literature suggests that OSMF leads to iron deficiency due to impaired dietary habits, burning sensation, vesiculation and ulceration of the oral mucosa, causing difficulty in the consumption of normal diet, leading to poor nutrition. Bhattacharya *et al.* reported an interesting case where IDA primarily resulted in the development of OSMF, which was successfully treated by oral administration of iron supplements and antioxidants.[32]

The key features of OSMF including chronic inflammation and epithelial dysfunction have been observed in individuals and laboratory animals with iron deficiency.[33] Chronic inflammation has been associated with greater risk of cancer in many organs of the body.[34] It has also been noted that serum ferritin levels are elevated and serum iron concentrations are decreased with tumor progression in head-and-neck carcinomas and thus heme can be used as a follow-up tool for patients along with nutritional assessment.[35]

Vitamin B12 is produced as hydroxocobalamin within bacteria, and its conversion to methylcobalamin and 5'-deoxyadenosylcobalamin, enzymatically active cofactor forms, occurs within the body. Cyanocobalamin, the fourth vitamer of Vitamin B12, can be metabolized in the body to an active coenzyme form and used in food supplements.[36] The most well-known manifestation of cobalt deficiency in oral cavity is pernicious anemia which is characterized by glossitis, burning sensation, beefy red tongue present in the form of patches or completely red tongue which is also referred to as Hunters' or Moeller's glossitis and shallow ulcers.[37]

In the present study, Vitamin B12 deficiency was seen in 52.5% of the study group. In a South African study, Seedat[38] reported that serum Vitamin B12 and folate levels of OSMF patients were within normal limits. According to Rajendran *et al.*,[5] a subclinical Vitamin B complex deficiency has been suspected in cases of OSMF with vesiculation and ulcerations of the oral cavity. The deficiency could be precipitated by the effect of defective nutrition due to impaired food intake in advanced cases and may be the effect, rather than the cause of the disease. They also reported that Vitamin and iron deficiency together with malnourished state of the host leads to derangement in the inflammatory reparative response of the lamina propria with resultant defective healing and scarification, which ultimately lead to OSMF.[5]

In the present study, most of the patients consumed gutkha which is a combination of areca nut and tobacco. One of the harmful effects of tobacco is depletion in the levels of micronutrients. Low Vitamin B12 levels may not be the sole initiating factor, but may act synergistically with carcinogens and genetic and environmental factors, increasing the rate of malignant transformation. It plays an important role in protection against cancer due to its role in DNA synthesis and repairing damaged DNA. The deficiencies may not be carcinogenic but increase the susceptibility to action by other carcinogens.[39,40]

The prevalence of OSMF in this part of Gujarat is considerably high owing to the increased consumption of areca nut and tobacco products across all age groups irrespective of gender. However, there are very few studies being carried out to investigate the role of the hematological indices. Therefore, this study attempted to evaluate the hematological profile in OSMF patients as it can aid in the treatment and improving the prognosis of this chronic

debilitating disorder. A significant finding in the present study is the presence of IDA (92.5%) and Vitamin B12 deficiency (52.5%) in the study group as compared to the control group. The hematological parameters are deterrent factors in maintaining the integrity of oral mucosa and in the progression of OSMF. The limitation of the study is the small sample size, and future studies should be carried out on larger number of patients.

CONCLUSION

OSMF is associated with a high malignant transformation rate (7%–13%). Low serum iron levels have been linked with head-and-neck cancers, which may further aggravate the condition. Therefore, it is imperative to detect and treat the low serum iron and Vitamin B12 levels in OSMF patients. In addition to the conventional treatment modalities in OSMF, iron and vitamin supplementation must be followed as a routine treatment protocol. Future clinical trials should be targeted toward the systemic use of iron and vitamins in OSMF patients. Nutritional supplementation with iron and Vitamin B12 is a form of chemoprevention, halting the disease progression, thereby preventing malignant transformation.

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Conflicts of interest

There are no conflicts of interest.

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