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Role of Serum Iron and Haemoglobin in Oral Submucous Fibrosis

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Abstract

The aim of the present study was to assess the role of serum iron and Hb among patient with oral submucous fibrosis (OSMF). A hospital-based analytical cross-sectional study was carried out on 61 patients who were clinically and histologically confirmed OSMF to explore relationship between serum iron and haemoglobin level with different grading of oral submucous fibrosis. Data was collected by face-to-face interview using a semi structured questionnaire, at Oral and Maxillofacial Surgery Department, Bangabandhu Sheikh Mujib Medical University (BSMMU) and Dhaka Dental College & Hospital. Obtained data were statistically analyzed by SPSS software version 23 and p-value <0.05 was considered as statistically significant. The result showed that out of 61 patients, the mean age was 47.39 year (range 30 years to 60 years). 41% showed involvement of right sided buccal mucosa, 50.8% had pain in mouth, 70% had burning sensation, 77.05% belonged to moderate cases type of OSMF and 22.95% belonged to mild cases. No severe case was found. Mean serum iron and Hb level were 63.85±12.96 µg/dl and 11.73±1.84 gm/dl respectively. Statistically significant relationship was found between OSMF and sex of the respondent, site of buccal mucosa, educational status, and history of taking betel nut (p=≤0.001, 0.013, ≤0.001 and ≤0.001 respectively). Difference of serum iron and Hb level between mild and moderate group of OSMF was statistically significant (p=≤0.001 and 0.004 respectively). In conclusion, serum iron and Hb level decrease with the increased severity of OSMF.

Keywords: Serum Iron, Haemoglobin, Oral Sub Mucous Fibrosis, Histology, Buccal Mucosa, Burning Sensation

Introduction

Oral Submucous Fibrosis (OSMF) is defined as "An insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with a juxta-epithelial inflammatory reaction followed by a fibroelastic change of the lamina propria with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat [1].

Oral submucous fibrosis (OSMF), first described in the early 1950s, is a potentially malignant disease predominantly seen in people of Asian descent [2]. It is a chronic progressive disorder, and its clinical presentation depends on the stage of the disease at detection. The majority of patients present with an intolerance to spicy food and rigidity of lip, tongue, and palate leading to varying degrees of limitation of opening of the mouth and tongue movement. The hallmark of the disease is submucosal fibrosis that affects most parts of the oral cavity, pharynx, and upper third of the esophagus [3]. Nasal twang due to fibrosis of nasopharynx and hearing impairment due to stenosis of eustachian tube may be observed in advanced stages of the condition [4]. Majority of OSMF patients present with irreversible moderate-to-severe condition. The changes of OSMF are limited to oral tissues and similar to those of scleroderma. It may be associated with oral leukoplakia and other potentially malignant disorders or with malignancy such as squamous cell carcinoma [5]. In the process of collagen synthesis, iron gets utilized, by the hydroxylation of proline and lysine, leading to decreased serum iron level [6]. The presence of palpable bands is a diagnostic criterion for this disease, and the incidence of malignant change in these patients ranges from 2% to 10%. About 2.5 million individuals are affected worldwide, with most cases being in Southern India. It also occurs in migrant chewers of betel quid in

other countries [7].

Etiological factors hypothesized to trigger the disease process include areca nut chewing, nutritional deficiencies, immunologic processes, and genetic predisposition [8]. Nutritional deficiencies, primarily of iron and vitamins, are implicated in the etiology of OSMF. Iron is essential for the overall integrity and health of epithelia of digestive tract and its contribution to normal enzymatic functions. OSMF is also considered as an Asian version of sideropenic dysphagia, where in chronic iron deficiency leads to mucosal susceptibility to irritants, such as chilies and areca nut products [9]. Hemoglobin levels, in particular serum iron levels, are considered as biochemical indicators for nutritional assessment [10]. Deficiency of iron, Vitamin B-12, and folate can affect the integrity of the oralmuzcosa. Significant hematological abnormalities have been reported in OSMF, including an increased blood sedimentation rate, and a decrease in serum iron and an increase in total iron binding capacity [3]. Habit of chewing areca nut is the major etiological factor of OSMF. Its extract acts as a potent stimulator for collagen synthesis in human fibroblasts culture leading to excessive accumulation of collagen, leading to fibrosis. High levels of Copper in areca nuts, a major etiological factor in OSMF plays an initiating role in stimulation of fibrinogenesis by up-regulation of lysyl oxidase and thereby causing inhibition of degradation of collagen and causing its accumulation and thereby causing OSMF. The high serum copper levels may also lead to generate high levels of free radicals by metal-catalyzed Haber-Weiss reaction and this can be one of the reasons for the carcinogenesis in tobacco and areca nut users [11].

Considering the multifactorial etiology, more research is required to develop sensitive, specific, and faster tests in the diagnosis and prognosis of these diseases. The diagnostic and prognostic value of iron in malignancies like oesophageal cancers (Plummer Vinson syndrome), oralcarcinoma, post-cricoidal carcinoma, and oesophageal carcinoma is well recognized. Recently trace element like iron is receiving much attention in the detection of oral cancer and precancerous lesions or conditions as it was found to be significantly altered in these conditions [12].

Iron metabolismis important for maintaining the health of oral mucosa, and many disease states, including cancers, are associated with Iron depletion. The concentration of serum iron does not fall until the body's iron stores are exhausted [13]. Serum Iron content can be a predictor for the progression of OSMF. There appears to be an association between serum Iron content and oral carcinogenesis. It has been also postulated that Vitamin C and Iron are interrelated as Vitamin C plays an important role in absorption of Iron from the gut. Vitamin C helps the body to absorb non-heme Iron. Vitamin C enhances Iron absorption by reducing dietary Iron from ferric form to the ferrous form. Thus, Vitamin C deficiency may reduce the availability of intracellular Iron. Iron is also necessary to convert folic acid to its active metabolite, folinic acid [14]. The iron dependent enzyme, cytochrome oxidase, causes atrophy of the epithelium. A deficiency of iron in OSMF reduces the levels of cytochrome oxidase, resulting in atrophy of the epithelium [15].

Deficiency as well as excess body iron, both may cause carcinogenesis. Some researchers emphasize that few habitual etiology have synergistic effect on iron to cause carcinogenesis [16]. Based on the features of OSMF, such as clinical symptoms, maximal mouth opening, and palpable fibrous bands, many researchers have divided it into different clinical stages [7, 10]. Reviewed the first clinical classification of OSMF based on the physical findings of the disease. But this classification did not include the mouth opening of the patients. The first step of preventive measure should be in discontinuation of habit, which can be encouraged through education, counseling and advocacy. Medical treatment is symptomatic and predominantly aimed at improving mouth movements. But each treatment has its own limitations.

Khanna and Andrade (1995) categorized OSMF into different stages considering the clinical features, histological features and mouth opening of the patients [2]. More *et al*. (2012) provided a clinical staging of OSMF considering the symptoms of the disease and presence of the palpable fibrous bands. Rose and Balan (2007) classified OSMF according to interincisal mouth opening into three groups. These groups are: Group A-mild cases: Mouth opening>3 cm, Group B- moderate cases: Mouth opening 1.5-3cm and Group C- severe cases: Mouth opening <1.5 cm.

This study was performed to determine the serum iron level and Hb among OSMF patients and also to comprehend the association of the levels of haemoglobin and serum iron in different stages of OSMF. Thus, the present study was conducted to assess the level of hemoglobin and serum iron among clinically and histopathologically diagnosed patients with oral submucous fibrosis and find out if there any relationship persists between them.

Materials and methods

This Analytical cross-sectional study was conducted at the oral and Maxillofacial Surgery Department, Bangabandhu Sheikh Mujib Medical University (BSMMU) and Dhaka Dental College & Hospital, Dhaka, Bangladesh, between the periods of September 2019 to August 2020.

Study Population

Patient coming to Oral and Maxillofacial Surgery Department, Bangabandhu Sheikh Mujib Medical University (BSMMU) and Dhaka Dental College & Hospital with OSMF. Purposive sampling technique was applied and the inclusion criteria are as follows: Age: ≥18 years, Patient with definitive habit of areca nut/tobacco in any form, Patient with clinical signs and symptoms of OSM, Histopathologically diagnosed case of OSMF.

Study Procedure

Informed consent form

For this study, a well-informed, voluntarily signed written consent was taken in an understandable local language from the study subjects after convincing them that their privacy and confidentiality will be safeguarded. Proper treatment will be provided if there is any injury occurred or complication developed because of this study. However, no monetary compensation will be provided for the loss of working time.

Estimation of Iron and Haemoglobin Values

All the patients fulfilling the above criteria were informed about the study in their own mother tongue and only those who agree and give a written signed voluntary consent will be enrolled in the study. All the enrolled subjects were then be interviewed and examined. After satisfying the diagnostic

criteria patients were subjected to the serum iron and haemoglobin estimation investigations. Approximately 5ml of venous blood was obtained by venipuncture of the median cubital vein after taking all aseptic precautions. Approximately 2ml of blood obtained was transferred to an Ethylene di amine tetra acetate (EDTA) coated clot activator bulb for haemoglobin estimation. Approximately 3ml of blood was allowed to clot for one hour in the plain bulb and will then be centrifuged at 3000 rpm for 10 minutes to provide serum. This serum was preserved in a frozen state and analyzed for serum iron estimation within one hour of its collection.

Estimation of Iron and Haemoglobin Values

Estimation of iron was done using Ferrozine method and haemoglobin by Sahli's method. All the test-tubes were kept 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 were compared to that of the standard solution provided in the kits at 571nm in a Atellica™ CH analyzer. For Sahli's method, approximately 0.2N HCl was put into the graduated test tube to mark 2gm. Blood sample of 0.02 ml was then transferred into the solution with the help of a pipette and mixed quickly. The tube was placed into the tube holder of the Sahli's haemometer.

Data analysis

Computer based statistical analysis was carried out with appropriate technique and systems. Data was processed and analyzed by SPSS 23 (Statistical program for Social Sciences). The summarized data was presented in the form of tables and graphs. The inferential statistics was carried out to see any association between independent and dependent variables. For test of significance, Chi-square test was done to see the relation between grading of OSMF and socio demographic characteristics. Independent sample t test was performed to see the mean difference of Hb and Serum iron in different grade of OSMF. A p-value of less than .05 was considered to be statistically significant.

Results

Table 1: Relationship between OSMF and socio demographical and clinical features

Variables	OSMF		Total	P value
Age (In Year)	Group A	Group B		
<40	4(30.8%)	9(69.2%)	13	
41-50	5(17.2%)	24(82.8%)	29	0.573
>50	5(26.3%)	14(73.7%)	19	
Gender				
Male	12(54.55%)	10(45.45%)	22	≤0.001
Female	2(5.13%)	37(94.87%)	39	
Educational status				
Illiterate	7(22.14%)	22(77.86%)	29	
Primary	4(26.67%)	11(73.33%)	15	
Secondary	2(22.22%)	7(77.78%)	9	≤0.001
Higher Secondary	1(12.5%)	7(87.5%)	8	
Involved Site				
Right	9(36%)	16(64%)	25	
Left	5(27.8%)	13(72.2%)	18	
Both	1(5.56%)	17(94.44%)	18	0.013
Pain				
Present	1(3.2%)	30(96.8%)	31	
Absent	13(43.3%)	17(56.7%)	30	≤0.001
Burning sensation				
Present	12(27.9%)	31(72.1%)	43	
Absent	2(11.1%)	16(88.9%)	18	
History of taking betel nut				
Yes	8(14.8%)	46(85.2%)	54	
No	6(85.7%)	1(14.3%)	7	≤0.001
Total	14(22.95%)	47(77.05%)	61	

Table 1 shows relationship between age of the respondent and OSMF. Statistically significant relationship was not found between age of the respondent and OSMF (p=0.573). Table 2 shows relationship between sex of the respondent and OSMF. Statistically significant relationship was found between sex of the respondent and OSMF (p≤0.001). Table 3 shows relationship between educational status of the respondent and OSMF. Statistically significant relationship was found between educational status of the respondent and OSMF (p≤0.001). Table 4 shows relationship between involved site of buccal mucosa and OSMF. Statistically significant relationship was found between site of buccal mucosa and OSMF (p=0.013). Table 5 shows relationship between presence of pain and OSMF. Statistically significant relationship was found between presence of pain and OSMF (p<0.001). Table 6 shows relationship between presence of burning sensation and OSMF. Statistically significant relationship was not found between presence of burning sensation and OSMF (p=0.197). Table 7 shows relationship between history of taking betel nut and OSMF. Statistically significant relationship was found between history of taking betel nut and OSMF (p=≤0.001).

Table 2: Relationship between OSMF and serum iron, Hb level

Variables	Mean Value		Standard deviation		P value
Serum iron	Group A	Group B	Group A	Group B	
	74.07	60.81	9.96	12.24	≤0.001
Hb level	12.95	11.37	1.67	1.74	0.004

Table 2 shows the relationship between OSMF and serum iron level of the respondent. Mean score of serum iron for Group A was greater than Group B. For Group A and Group B mean serum iron level was 74.07±9.96µg/dl and 60.81±12.24 µg/dl respectively. Independent sample t test showed the difference of serum iron level between two groups of OSMF was statistically significant (p=≤0.001).
Table 9 shows relationship between OSMF and Hb level of the respondent. Mean score of Hb for Group A was greater than Group B. For Group A and Group B mean Hb level was 12.95±1.67gm/dl and 11.37±1.74 gm/dl respectively. Independent sample t test showed the difference of Hb level between two groups of OSMF was statistically significant (p=0.004).

Discussion

A hospital-based analytical cross-sectional study was carried out on 61 patients who were clinically and histologically confirmed OSMF to assess the role of serum iron and Hb among OSMF patients. Data was collected by face-to-face interview using a semi structured questionnaire, at Oral and Maxillofacial Surgery Department, Bangabandhu Sheikh Mujib Medical University (BSMMU) and Dhaka Dental College & Hospital.

The mean age of the respondent was 47.39 year and majority respondent belonged to 41–50-year age group. In a study conducted by Pindborg *et al* [17]. it was found that maximum members belonged to 40–49-year age group which was similar to our study. In our study 47.5% were illiterate, all respondent showed involvement of buccal mucosa, fibrous band was present among 100% respondent, 70% respondent had burning sensation and 50% respondent complained about pain. In our study majority respondent was Female (64%). Rest 36% respondent was male. But male predominance result was found by Sinor *et al*. [18], Pindborg *et al*. [17] (1984), Ahmad *et al*. [1] and Hazarey *et al*. [19]

Pindborg *et al*. [17] and Ahmed *et al*. [1] showed majority respondent complained about burning sensation and pain which was similar to our study. Majority respondent (77.05%) belonged to group B and rest respondents belonged to group A. This grading was done according to interincisal mouth opening. Severity of OSMF increased with decrease in mouth opening. Different studies like Kumar *et al*. [8] and Hosein *et al*. [20] showed majority respondent belonged to more severe stage/grade. It is well-documented that as the severity of OSMF increases there is a progressive inability to open the mouth associated with varying degrees of restriction in tongue movements. Statistically significant relationship was found between sex of the respondent, site of involvement of buccal mucosa and grading of OSMF. Ahmad *et al*. [1] and Hazarey *et al*. [19] also showed similar sort of result.

Majority respondent (88.5%) had the history of taking betel nut. It was also found that severity of OSMF increased with intake of betel nut. Statistically significant relationship was found between betel nut uses and grading of OSMF ((p=≤0.001). Hosein *et al*. [20], Karthik *et al*. [3] also showed statistically significant relationship between betel nut use and grading of OSMF. These studies showed grading/severity of OSMF was more prevalent among betel nut user.

Mean value of Hb in our study was 11.73±1.84 gm/dl and this value was lower than normal range for both male and female. Bhardwaj *et al*. [12] and Karthik *et al*. [3] showed Hb level below normal range in their study and it was in accordance with our study. The mean value of serum iron was 63.85±12.96 µg/dl. Yadav *et al*. [21] showed mean serum iron value for OSMF patient was 66.57µg/dl which was similar to our study. In our study, serum iron and Hb was found lower in quantity in Group B than Group A. The difference between Group A and Group B both in case serum iron and Hb was found statistically significant. The result obtained in our study was in accordance with Ganapathy *et al*. [13], Karthik *et al*. [3] and Bhardwaj *et al*. [12]

Conclusion

The present study mainly focused on the role of serum iron and haemoglobin in different stages of OSMF. OSMF was more found among bettle nut user. It also showed that there is a progressive decrease in serum iron and haemoglobin levels from Group A to Group B stage of OSMF. A significant relationship was found in serum iron and haemoglobin value in various subgroups of OSMF group. Therefore, as the level of serum iron and haemoglobin level depletes in the OSMF group, the severity of disease increases. Serum iron level is considered as biochemical indicators for nutritional assessment and their low value can indicate the progress from oral precancer to oral cancer stage. So, serum iron and Hb level can be used in assessing the prognosis of disease.

Ethical issue

This study was performed after taking ethical clearance from IRB, Bangabandhu Sheikh Mujib Medical University.

Conflict of interest

Authors declare no conflict of interest.

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