ORIGINAL ARTICLE

ANALYSIS OF SERUM IRON AND HEAMOGLOBIN LEVEL IN PATIENTS WITH ORAL SUBMUCOUS FIBROSIS

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Background: OSMF is chronic underlying disease. The disease is well recognized and had an importance due to high malignant transformation rate. Multiple etiological factors are found responsible in the pathogenesis of the disease course. The effect of areca-nut is mainly causing trismus of the oral cavity. This may compromise the food intake of the patients who are facing this precancerous disease. **Method:** Total one hundred and 28 patients were selected and equally distributed into two groups. It is an observational, cross- sectional study. Sixty four patient with OSMF were selected on the basis of fibrous band formation and mouth opening. The etiological factors such as chewing habit had been denoted in forms. The heamoglobin and iron levels were analyzed from laboratory using automated machines used for blood analysis. **Results:** The OSMF group showed insignificant variation when the two groups were compared. Whereas the sub-groups of OSMF are showing moderately significant correlation for the serum iron and heamoglobin levels. The outcome of the present study emphases on the assessment of heamoglobin and serum iron for OSMF patients. The patients must be prescribed with the micronutrients therapy so that any deficiency which may contribute in the disease course can be intercepted at the beginning. Further the patients must be educated in term of quitting the chewing habit so that his/ her life not get compromised due to OSMF.

Keyword: OSMF; Serum Iron; Heamoglobin

INTRODUCTION

Oral Submucous Fibrosis (OSMF) is a disease with chronic course. OSMF affect any part of oral cavity and extend up to the pharynx and esophagus. Initially the disease present with the vesicle formation, accompanying juxta-epithelial inflammatory reaction shadowed by fibro-elastic bands formation. The band formation leads to stiffening of oral mucosa in labial, buccal and faucial pillars area results in trismus.[1] OSMF is more prevalent in Asian countries, present as sideropenic dysphagia. Correspondingly the disease also known as Plummer Vinson syndrome which had deficiency of iron may lead to mucosal susceptibility to irritant such as areca nut and spices like chili.[2] The chronic iron deficiency in Plummer Vinson syndrome develop dysphagia due to esophageal web formation. It is documented that iron deficiency causes decline in enzymes present in various human body tissues such as myoglobin and cytochrome.[3] It is studied that iron is being utilized during the hydroxylation process of proline and lysine in arecoline treated mucosa secondarily affecting level of iron.[4]

Diagnostic criteria of OSMF are based on presence of palpable bands on labial, buccal and faucial pillars mucosa. OSMF had a higher incidence of malignant transformation rate, ranges from 2.6%–7.6%.[5] In view of researchers OSMF is a multifactorial disease, more research is required to understand the contribution of the various factors in the disease course. The prognostic value of iron in head and neck malignancies such as oral cancer, oesophageal cancer, post-cricoid carcinoma of larynx is documented.[6] Studies shown that micronutrients and traces of elements like iron is receiving much consideration in the detection of precancerous and cancerous lesions of head and neck.[7] Therefore a study is designed to assess is there any alteration of iron and heamoglobin levels occur in OSMF patients and disease free patient. Hence, this study was done to assess the serum iron levels in both groups with areca nut chewing habit and to understand the association of the levels of iron and haemoglobin levels in different stages of the OSMF.

MATERIAL AND METHODS

The study was conducted at Dr. Ishrat-ul-Ebad Khan Institute of Oral Health Sciences during the period of June 2013 and April 2014. The patients were randomly selected who were visiting the dental OPD. Every second OSMF patient was selected for the case group. Whereas cases were selected on the basis of chewing habit. Every second disease free patient were selected for the purpose of this study. Patients who were not willing to participate were excluded from the study. All patients were having a history of chewing in different forms of betel nut whether it is Paan, Gutka, Chalia or other chewing habits such as Naswar, Tobacco, smoking and Huqqa. The information obtained from the patient is recorded on printed forms. The ethical

approval had been given by Institutional Review Board (IRB) of Dow University of Health Sciences.

The sample size of the study is total 164 patients equally distributed in OSMF patients and OSMF disease free individuals. The sample size was validated by using Open-epi sample size calculator. The patients were provided with an informed consent and study information. The patient identity was kept anonymous to maintain the privacy. A separate mask, gloves and examination instruments were used on each patient. OSMF patients were diagnosed according to the criteria given by Haider *at al.*[7] After the examination and diagnosis of the patient, blood sample was collected from the right or left ante-cubital fossa of the upper limb. Sterile phlebotomy technique was used. After sample collection the sample was labeled with patient name and age. The sample was kept in ice box. The sample was then transported to the Dow diagnostic and research laboratory for serum analysis. OSMF patients with band formation on labial, buccal and pharyngeal mucosa was included. Patients with any visible sign of non-healing ulcers or malignancies were excluded. Patients with any previous supplement intake were not included in the study. Pregnant females and children younger than 12 years were not included in the study due to heamatological changes. Patients with pericoronitis which is responsible for reduced mouth opening were excluded. After history and sample collection the patients who require any other dental treatment is directed to the concerned department under guidance. Laboratory results of blood reports were communicated to the patients through message which patient collected on their next follow-up visit to the OPD. Part of the study was funded by Dow University of health Sciences. The samples were centrifuged to obtain serum for analysis. Serum iron was analyzed on '902 automatic analyzer' by Roche HITACHI. For heamoglobin serum level, the vacutainers for blood sample collection contain 5.4mg of ethylenediaminetetraacetic acid (EDTA) which is an anticoagulant. Heamoglobin was assayed by using 'Celtac F' automated analyzer by NIHON KOHDEN.

The data was collected and analyzed using statistical package for social sciences 24 (SPSS 24). The study participants were assessed for mean and standard deviation of heamoglobin and iron between OSMF and disease free patients using descriptive statistics. The functional staging group which marks the mouth opening of the patients were assessed by using One-Way Anova test. Clinical staging which denoted the fibrous band formation among the OSMF patients and the patients without the disease were also assessed by using One-Way Anova test. Further the *p*-value was calculated along with One-Way Anova. The spearman correlation test was performed to assess that whether iron and heamoglobin affected in OSMF cases which are further subgroups in to clinical staging and functional staging. The Mann Whitney test was applied due to decrease number of individuals in subgroups of OSMF patients. The null hypothesis of the study is that there is a relation between the alterations in serum iron level of the OSMF patients.

RESULTS

The age range reported were between >12–68 years. The male to female ratio for all participants was 1.8: 1 (N male= 83, female =45). For OSMF cases the number of male patients were 42 whereas female patient were 22 years. The age group which was more affected was between 20–40 years in OSMF patients (Fig-1). The major ethnic group reported was Urdu speaking patients (n=57). The second and the third reported ethnic groups for both cases and controls were Punjabi (n=27) and Sindhi (n=16) respectively. Other ethnic groups included Balochi (n=11), Hindko (n=9), Memon (n=4) and Pathan (n=3). (Fig-2)

The chewing habit had been observed in both OSMF groups and control group which were disease free. The majority of the patients were reported for areca nut chewing (n=64), second most reported chewing habit was Gutka (n=55). Others chewing habits which were reported through the study were smoking (n=51), Paan (n=49), Tobacco (n=35), Naswar (n=13) and Huqqa (n=3). (Figure-3) The chief complain of the OSMF patients was limited mouth opening. <10 mm of mouth opening was observed in 3.9% (N=5) of OSMF patients. The mouth opening in disease free participants was greater than 40mm. These patients came to OPD for routine Dental checkup and bleeding gums. No band formation was observed in disease free individuals. The OSMF patients 22.7% (N=29) showed fibrous bands formation on labial and buccal mucosa along with involvement of the faucial pillars. (Table-1)

The mean heamoglobin for both groups were 12.816 (S.D.=2.67) whereas mean for iron was 76.312 (S.D.=61.337). The OSMF patients showed slightly decrease level of heamoglobin and iron as compare to OSMF disease free individuals. Mann-Whitney *t*-test was applied to assess the rank median difference of the heamoglobin and iron between the two groups. The results showed no significant difference between the two groups. (*p*-value =0.096, 0.430). The level of serum iron and heamoglobin was assessed among the three groups of different mouth opening and the disease free patients mouth opening. The result was analyzed by using One Way ANOVA, since the test group of OSMF contains three subgroups and it was compared with the OSMF disease free individuals. The heamoglobin and iron levels showed *p*-value of 0.263 and 0.596

respectively. Therefore, the null hypothesis of the study is rejected. (Table-2) The OSMF group and disease groups were assessed by considering functional status of the study participants. Three subgroups of OSMF and group of disease free individual showing heamoglobin and iron levels as mentioned in Table-2. The *p*-value for the heamoglobin and iron levels showed 0.081 and 0.155 respectively. The spearman correlation was applied to correlate the heamoglobin and iron among OSMF patients. It was found that there was moderate correlation (R=0.53). Whereas the correlation was analyzed among cases was observed as R=0.26. This showed small to moderately correlation observed among cases (Table-3).
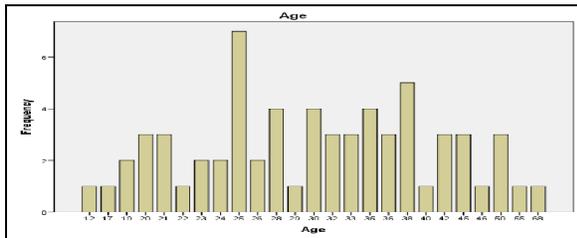

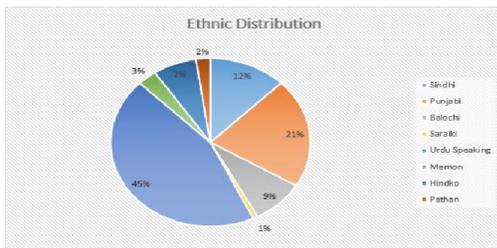**Figure-1: Age distribution among OSMF patients.**

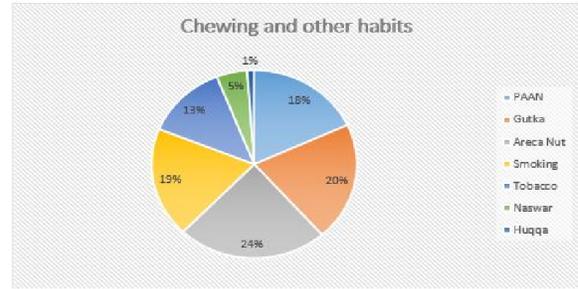**Figure-2: Ethnic background of the study participants.**

Figure-3 Chewing Habit practiced by OSMF patients and Disease-free individuals.

Table-1: Demographics Characteristics

Characteristics	N=128 (%)
Age (Mean±SD) in years	31.98±10.33
Hb (Mean±SD) in g/dl	12.81±2.67
Serum Iron (Mean±SD) in μmol/L	76.31±61.33
Gender	
Male	83 (64.8%)
Female	45 (35.2%)
Group	
Case	64 (50.0%)
Control	64 (50.0%)
Mouth Opening	
≤10 mm	5 (3.9%)
11-19 mm	20 (15.6%)
≥20 mm	39 (30.5%)
≥40 mm	64 (50.0%)
Clinical Staging	
None	64 (50.0%)
Faucial Bands	14 (10.9%)
Faucial and Buccal Bands	21 (16.4%)
Faucial, Buccal and Labial Bands	29 (22.7%)

Table-2: Mean Comparison of Hb & Serum Iron & Hb among Groups & Staging

Group	N	Hb (Mean ± SD)	Hb (95% C.I)	Iron (Mean ± SD)	Iron (95% C.I)
Group					
Case	64	12.38±3.13	(11.60, 13.16)	224.0±30.9	(209.1, 238.9)
Control	64	13.24±2.03	(12.73, 13.75)	211.3±37.5	(186.1, 236.5)
Abs. Mean Diff (P-value)		0.096		0.430	
Functional Staging					
≤ 10 mm	05	13.20±2.03	(10.65, 15.74)	60.60±17.18	(39.26, 81.93)
11-19 mm	20	12.50±2.39	(11.38, 13.61)	61.25±32.71	(45.94, 76.55)
≥ 20 mm	39	12.22±3.59	(11.05, 13.38)	80.46±71.23	(57.37, 103.55)
≥ 40 mm	64	13.24±2.03	(12.73, 13.75)	79.71±63.78	(63.78, 95.65)
P-value		0.263		0.598	
Clinical Staginf					
Faucial Bands	14	11.25±5.25	(8.21, 14.28)	104.21±107.73	(42.00, 166.41)
Faucial and Buccal Bands	21	12.84±2.06	(11.90, 13.78)	59.85±27.92	(47.14, 72.56)
Faucial, Buccal and Labial Bands	29	12.60±2.33	(11.71, 13.48)	67.24±35.50	(53.73, 80.74)
None	64	12.81±2.67	(12.73, 13.75)	79.71±63.78	(63.78, 95.65)
P-value		0.081		0.155	

Table-3: Correlation between Iron & Hb

Group	Correlation (*p*-value)
Case	0.53 (<0.001**)
Control	0.26 (0.035*)
*Sig at 5%; **Sig at 1%	

DISCUSSION

Iron had been one of extensively studied trace element in relation to OSMF. The study participants both with OSMF and disease free individuals had some sort of exposure to the main etiological factor i.e., areca nut and other chewing habits. The most prevalent age groups were observed between 20–40 years. This presentation of age is somewhat similar to previous studies as observed by another study which shows prevalence in second and third decade of life.[9]

The male to female ratio is higher in OSMF patients with 2 male and 1 female. Our study findings for male predominance was consistent with Rangnathan *at al.*[9] This presentation of male predominance was due to easy availability of the chewing products for male individuals whereas the female individuals may find it difficult to access the chewing products. This could be the reason for higher male to female ratio.

Karachi had a population which belongs to multiple ethnic background. Majority of the participants reported were Urdu speaking (n=45). Other ethnic groups reported were Sindhi, Punjabi, Baloch, Saraiki, Hindko, Memon and Pathan. (Figure-2) This huge variation in ethnicity reported in a previous study done in Karachi on OSMF patients.[10] OSMF characteristic clinical feature is formation of fibrous bands. These fibrous bands present in the form of bands in anterior labial mucosa, bands in buccal mucosa and extending in faucial pillars. These bands are palpable on digital examination. This progressive band formation in oral cavity affects the food consumption of the patients. The patients complain about difficulty in mouth opening and chewing food. In our study, the heamoglobin and iron levels had been assessed according to the severity of fibrous band formation.[11]

Studies had documented that the deficiency of trace elements due to nutritional exhaustion is responsible for anemia and changes in immunologic states responsible for pathologic response at the ultra-structures level of mucous membranes. As a result, the pathologic lesion established further put at risk by inadequate food intake due to fibrosis of oral mucosa and reduced mouth opening, thus initiating a vicious cycle.[12,13] In our study the mouth opening and fibrous band formation was assessed in both groups with chewing habits. It is found that there is no significant change observed among the two groups with different mouth opening. This presentation is contradictory to the studies performed in the last decade.[14-16] The mean output of the heamoglobin and iron level among OSMF patients was slightly less as compare to the control group. This variation of serum values was insignificant when statistically compared.

In present study we had analyzed the iron and heamoglobin level among the OSMF group. The similar results were observed among the previous studies which analyzed the serum iron and hemoglobin levels among the stages of OSMF.[13-15] The study result denoted that there were no significant alterations between the two groups. This could be due to presence of chewing habit in both groups. The only significant difference was observed within the sub-groups of OSMF.

The study is limited to the OSMF group and compared with the group who were disease free and did not develop any disease. The number of participants in OSMF group according to stages were less therefore the number of patients could be increased in each sub-group. The patients with iron deficiency anemia group was not compared for the correlation of iron and heamoglobin levels. The study participant was matched for the chewing habit and not for the age and sex which requires more time and budget.

CONCLUSION

The current study highlights the importance of serum iron and heamoglobin analyzed for patients with Oral Submucous Fibrosis and diseases-free individuals. Analyzing iron status is a part of biochemical evaluation of a patient to find the progress of the disease. This evaluation is more helpful in patients in which the disease is at advanced level. It is recommended that the biochemical analysis of oral premalignant conditions more helpful in disease prognosis when it is at the advanced stage. The serum levels were noticeably deranged at the advanced level of the Oral Submucous Fibrosis. The treatment must include supplemental iron therapy in diseased patients along with well-adjusted diet and exercise of the jaw. This supports to cease the further advancement of the disease.

AUTHORS' CONTRIBUTION

AU: Study design, data collection, Manuscript, Statistical Analysis, (half of the study is self funded). AA: Study design, Discussion, Conclusion. AA: Study design, Discussion, Conclusion. SIA: Study design, data collection, Discussion, Conclusion. MZ: Study design, data collection, Statistical Analysis

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