

Estimation of Serum Iron in Oral Submucous Fibrosis Patients and its Comparison with Healthy Controls.

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ABSTRACT: OBJECTIVE: The present study aimed to determine the mean serum iron concentration among subjects with oral submucous fibrosis of different clinical stages and its comparison with healthy controls in our local population here in Karachi Pakistan **SUBJECTS & METHODS:** 89 clinically diagnosed oral submucous fibrosis and 89 healthy controls were selected from maxillofacial surgery departments of Abbasi Shaheed Hospital and Civil Hospital, Karachi. 5 ml of venous blood was collected using aseptic measures in sterile vials for estimation of serum iron. Serum iron was estimated using calorimetric method using semi-auto analyzer. Means and standard deviations were calculated for serum iron levels in study and control subjects. Statistical analysis was done to determine the association of serum iron with oral submucous fibrosis and its predictability was tested alone and in stepwise method with other independent variables. **RESULTS:** Overall age range was 49 year, while OSF patient's age range was 23 years. Male 88.8% and female were 11.2%. Mean serum iron level differs significantly between patients and controls ($p < 0.05$). OSF patient exhibiting lower serum iron concentration (111.66 ± 18.43) in comparison to healthy controls (122.38 ± 17.04). Serum Iron concentration is also decrease as Functional Stage of OSF increases. Serum iron concentration is also found to be significant as the disease progress towards nil mouth opening, multiple fibrous bands anterior + posterior ($p < 0.05$). OSF which found to be only 8.5% but when combined with age it become 31.2% while age found to be best predictable variable for OSF 28%. **CONCLUSIONS:** Serum iron should be part of routine assessment of OSF patients, as a preventive measure for high-risk groups and strict follow up especially in those people which shows decreased serum iron in order to detect early malignant transformation of OSF into oral squamous cell carcinoma. Iron therapy along with other trace element and vitamins should be started as early as possible to correct the deficient state and getting benefits in overall treatment of oral submucous fibrosis with other treatment modalities. This helps in stopping or decreased further progression of the condition

Key Words : Oral submucous fibrosis, Serum iron, Clinical staging, Paan, Betel quid, Ghutka.

INTRODUCTION : Oral Submucous fibrosis is a pre-malignant, chronic progressive disease of upper aerodigestive tract submucosa which starts from oral cavity, soft palate or faucial pillars, and go on to involve other sub-mucosal areas of upper aerodigestive tract. OSF is clinically characterized by decreased mouth opening, blanching and stiffness of the oral mucosa, burning sensation and hypomobility of soft palate and tongue with decrease gustation. These features are due to pathological fibro elastic transformation of lamina propria, along with juxtaepithelial inflammatory reaction and epithelial atrophy, which leads to stiffness of oral mucosa, causing trismus and difficulty in eating or swallowing^{1,2}. Literature endorse the dose dependent relationship of habits^{3,4}, such as areca nut, ghutka, mawa chewing, its frequency and duration, but there is a need for a modality affording early diagnosis, or predicting worsening or potential for transformation into malignant disease⁵, for which trace elements could be investigated in relation to pre-malignant potential^{5,6,8}. Among many trace elements, copper, iron, selenium, and vitamin C have been investigated. These vital element play vital role in maintaining and functioning of body enzymatic reactions, and disparity of these bio-

chemical markers may be a reason for malignant transformation⁷.

Iron has been recognized as an important element for maturation of epithelium and it is well documented that iron deficiency is associated with epithelial abnormalities in addition to malignancies like post cricoid carcinoma and tumors of the pharynx and mouth⁸.

Serum iron content can be a predictor for the advancement of the condition⁹, and could possibly associated with carcinogenesis or transformation into malignant disease. Thus variation in serum iron concentration can be utilize as an indicator for prognosis and also can help in diagnosing those submucous fibrosis patients which have higher risk of transformation into malignant disease⁹.

It has been postulated that altered serum iron levels were observed among OSMF patients. Thus the present study aimed to determine and compare the serum iron concentration among subjects with oral submucous fibrosis of different clinical stages and healthy controls in our local population here in Karachi Pakistan. **SUBJECTS AND METHODS :** This study was conducted in two department i.e. Department of Oral and Maxillofacial Surgery, Abbasi Shaheed Hospital,

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Karachi and Civil Hospital Karachi from January 2009 to December 2014. This study include 89 clinically diagnosed oral submucous fibrosis and 89 healthy controls. Ethical clearance was taken from ethical committee of institute and written informed consent was obtained from all included subjects and controls All those patients were excluded from study, which were concomitantly suffering from other chronic systemic diseases. Oral submucous fibrosis patients were diagnosed on basis of clinical presentation of disease while control group subjects were taken from outpatient department of hospital which do not had signs and symptom of oral submucous fibrosis and were not indulged rish factor habits and were willing to give informed consent, 5 ml of venous blood was collected using aseptic measures from median cubital vein and sent to laboratory in sterile vials for estimation of serum iron. The blood is allowed to clot at room temperature for 1 to 2 hours and then serum was separated by centrifuging at 3000 rpm for 10 minutes. Serum iron will be estimated using calorimetric method using semi-auto analyzer.

The staging of the OSMF was done according to the criteria of functional grading. Clinical staging was done depending on topography and extent of distribution of bands. Stage 1 subjects had faucial bands while those in stage 2 presented faucial and buccal bands and among individuals in stage 3, faucial, buccal and labial bands were involved. Data was entered and analyzed using Statistical package for Social Sciences (SPSS 19.0, Chicago., IL). Means and standard deviations were calculated for serum iron levels in study and control subjects. Independent samples 't' test was used to compare the mean values of serum iron between the study subjects and control group while one way analysis of variance (ANOVA) was used to assess the significance in difference between the mean iron levels based on the staging of OSMF. Step wise linear regression analysis was executed to assess the influence of various independent variables (age, gender, and iron levels) on the dependent variable (OSF or control group) RESULTS : The overall age range was 49 years with youngest patient was 18 years and oldest one was 67 years of age. Maximum numbers of patients were belonging to 20-30 years age group. Gender distribution was Male 88.8% (79) and female were 11.2% (10)(Fig-2). Mean serum iron level differs significantly between patients and controls ($p < 0.05$). OSF patient exhibiting lower serum iron concentration (111.66 ± 18.43) in comparison to healthy controls (122.38 ± 17.04)(Fig-3). Serum Iron concentration is also decrease as functional stage of OSF increases (Table 1&2). Serum iron concentration in patient who had redness of oral mucosa is only found to be significant as compared to other variables in study 102.71 ± 14.85 ($p < 0.05$), while patients with burning sensation had mean 112.90 ± 21.38 . Serum iron concentration is also found to be significant as the disease progress towards nil mouth opening, multiple fibrous bands anterior + posterior ($p < 0.05$) Stepwise linear regression model analysis performed to determine the predictability of serum iron in determining OSF which found to be only 8.5% but when combined with age it become 31.2% while age found to be best predictable variable for OSF (Table-5).

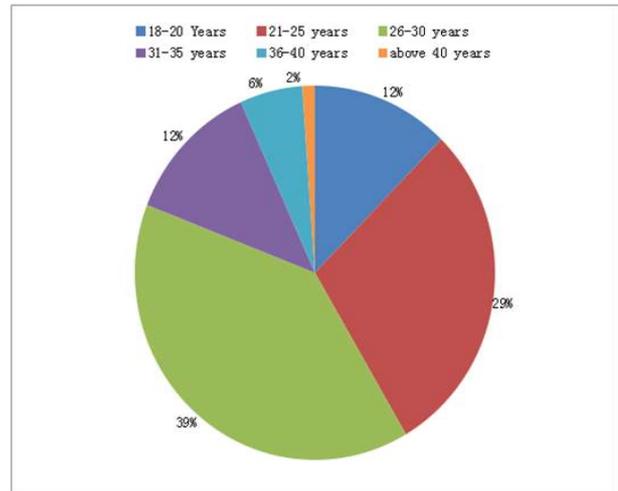


Figure-1 : Stratification of oral submucous fibrosis patients with respect to age.

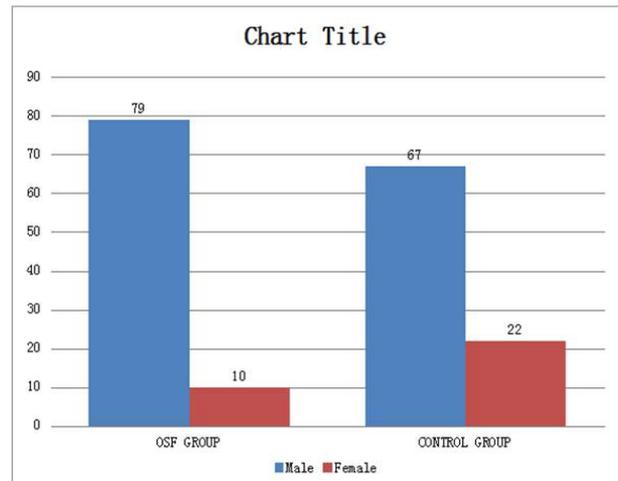


Figure 2 : Gender distribution in study and control groups.

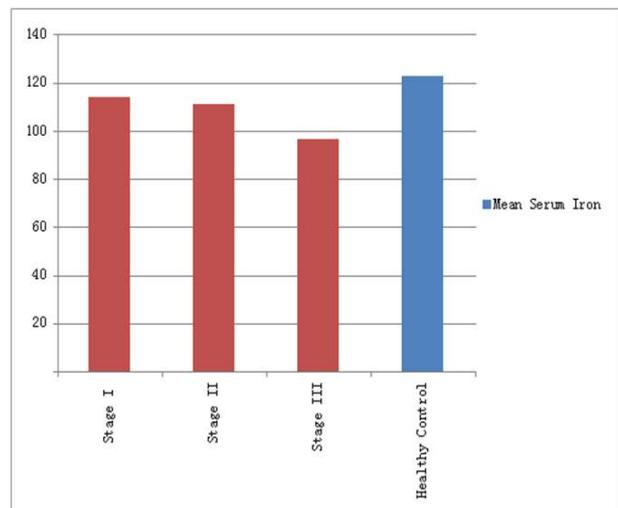


Figure-3 : Estimation of mean Serum Iron concentration with respect to functional staging.

Variables	Case or Control	N	Mean	Std. Deviation	Significance
Serum Iron	Control	89	122.5281	17.33172	Less than 0.001
	Case	89	111.6629	18.43782	

Table-1 : Cross tabulation of Serum Iron with OSF and Healthy Control Groups.

Variables	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Min.	Max
				Lower Bound	Upper Bound		
Stage A Mouth opening more than 20 mm	56	113.87	18.02	109.05	118.69	76	160
Stage B Mouth opening Between 11-19 mm	26	110.92	19.17	103.17	118.66	77	146
Stage C Mouth opening Less than 10 mm	7	96.71	13.06	84.63	108.79	87	119
Total	89	111.66	18.43	107.77	115.54	76	160

Table-2 : Cross tabulation of Serum Iron with respect to functional staging of Oral Submucous Fibrosis.

Variables	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Min.	Max
				Lower Bound	Upper Bound		
Clinical Stage 1 Faucial Bands	12	115.83	18.18	104.27	127.38	82	147
Clinical Stage 2 Buccal + Faucial Bands	38	114.84	18.11	108.88	120.79	76	160
Clinical Stage 3 Labial + Buccal + Faucial Bands	39	107.28	18.35	101.33	113.23	77	146
Total	89	111.66	18.43	107.77	115.54	76	160

Table-3 : Cross tabulation of Serum Iron with respect to Clinical staging of Oral Submucous Fibrosis.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.292a	.085	.080	.48092

a. Predictors: (Constant), Serum iron

Table-4 : Step wise multiple linear regression analysis with Iron levels as independent variable and groups (patients and controls) as dependent variable.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.529a	.280	.276	.42660
2	.559b	.312	.304	.41820

a. Predictors: (Constant), Age

b. Predictors: (Constant), Age, Serum iron

Table-5 : Step wise multiple linear regression analysis with age, gender, and Iron levels as independent variables and groups (patients and controls) as dependent variable.

DISCUSSION : Oral submucous fibrosis prevalence is increasing in Pakistan especially in southern areas due to increased consumption of areca nut, paan chewing as compared to northern areas of Pakistan¹⁰. In Indo-Pak region statistics for OSMF is more than five million individuals¹¹. Several reasons are proposed for rapid rise in disease statistics; among them attractiveness of commercially prepared pan/chalia in Pakistan and an high usage of pan/chalia by younger people due to easy availability, low price and marketing strategies are the possible explanations. The mean age of the Oral submucous fibrosis group in our study was 26.29 years, which is consistent with findings of 26.85 years of Karthik et al⁹. and slightly lower 29.04 years of Katharia et al¹². and 30 years of Maher et al¹³. A male predominance was seen in the subject group with Male 88.8% (n=79) and female were 11.2% (n=10). Serum iron is found

significantly lower in relation to control group i.e. (111.66±18.43) and (122.38±17.04) respectively (p<0.05 - independent t-test) which is consistent with the finding of Tadakamadla J et al. which shows 114.78 ± 23.47 (subjects) and 130.86 ± 31.01 (controls)¹⁴ Predictability of serum iron in progression of oral submucous fibrosis is 8.5% while other variable combination bring with association to 31.2% which is consistent with study by Tadakamadla J et al. i.e. 41.9%. Literature review reveals that pathogenesis of oral submucous fibrosis is a vicious cycle which begins with burning sensation, vesicle formation and later on minor ulceration which render patient to difficult chewing and swallowing (dysphagia), it resulted in decrease daily intake of food and poor nutrition. Decreased Iron concentration in serum (also other traces element) may result in initiation of anemia and altered cell-mediated immunity, which sequentially promote pre-existing submucous fibrosis in lamina propria¹⁵. Decreased factors further promote pathological fibrosis and shows inverse relation with serum iron concentration¹⁶ Hallmark of pathological Fibrosis dictates that OSF is chiefly a condition of collagen metabolism, in which lysyl oxidase upregulation enhances collagen cross-linking which results in development and progression of the condition¹⁷⁻¹⁹. Hydroxyproline in the hydroxylated form in hydroxylation reaction during collagen synthesis requires ferrous iron in addition to other essential elements²⁰⁻³.

LIMITATIONS : Limitations of this study are that we have studied single variable that is serum iron in OSF patients and its comparison was done with healthy people which were not absolutely matched. Other variable should also be investigated in determining the diagnostic and prognostic predictability. The findings of the present study could not be generalized to all the OSMF patients because of the regional and cultural variation that occurs within and between the countries. Further extensive studies with randomized control trials should be done to recognize the overall association between OSF and serum iron variation and also stagewise variation in OSF, which can be used as an indicator of malignant transformation in near future.

CONCLUSIONS : The current study gives emphasis on assessment of serum iron among patients with oral submucous fibrosis. Serum iron should be part of routine assessment of OSF patients, as a preventive measure for high-risk groups and strict follow up especially in those people which shows decreased serum iron in order to detect early malignant transformation of OSF into Oral squamous Cell Carcinoma. It is suggested that iron therapy along with other trace element and vitamins should be started as early as possible to correct the deficient state and getting benefits in overall treatment of oral submucous fibrosis with other treatment modalities. This helps in stopping or decreased further progression of the condition.

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