Original Article

Estimation of hemoglobin, serum iron, total iron-binding capacity and serum ferritin levels in oral submucous fibrosis: A clinicopathological study

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Abstract

Introduction: Oral submucous fibrosis (OSMF) is also considered as an Asian version of sideropenic dysphagia, wherein chronic iron deficiency leads to mucosal susceptibility to irritants, such as chillies and areca nut products. Hemoglobin (Hb) levels, in particular, serum iron levels, are considered as biochemical indicators for nutritional assessment. Serum iron content can thus be a predictor for the progression of the condition, and there appears to be an association between serum iron content and oral carcinogenesis. Therefore, biochemical assessment of oral precancerous conditions may help in early diagnosis and prognosis. Hence, the present study was aimed to evaluate Hb, serum iron, total iron-binding capacity (TIBC) and serum ferritin in OSMF patients.

Materials and Methods: Forty cases of OSMF were staged clinically and graded histopathologically to assess the levels of Hb, serum iron, TIBC and serum ferritin and compared with normal subjects. The results were compared using *t*-test, ANOVA, and Tukeys multiple *post hoc* procedures.

Results: It was observed that the levels of Hb, serum iron and serum ferritin levels gradually reduced in OSMF patients compared with controls and as the clinical stage and histological grade of OSMF progressed with a statistically significant $P < 0.05$. It was observed that the levels of TIBC gradually increased in OSMF patients compared with controls and as the clinical stage and histological grade of OSMF progressed with a statistically significant $P < 0.05$.

Conclusion: The Hb, serum iron and serum ferritin levels in OSMF patients were reduced and TIBC increased as compared with controls and as the clinical stage and histological grade of OSMF advances indicating their role as a reliable biochemical indicator.

Keywords: Iron deficiency, oral submucous fibrosis, serum ferritin, sideropenic dysphagia

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Received: 17.01.2017, **Accepted:** 18.01.2017

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, premalignant condition of the oral mucosa which was first described by Schwartz in 1952.[1] The importance of this disease lies in its inability to open the mouth and the highest malignant transformation rate (7%–13%).[2,3] It is predominantly seen in Southeast Asia and Indian subcontinent with

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_131_15

How to cite this article: Thakur M, Guttikonda VR. Estimation of hemoglobin, serum iron, total iron-binding capacity and serum ferritin levels in oral submucous fibrosis: A clinicopathological study. J Oral Maxillofac Pathol 2017;21:30-5.

few cases reported from South Africa, Greece and the United Kingdom. The prevalence rate of OSMF in India is about 0.2%–0.5%.[4] Although the etiological factor in the causation of OSMF is believed to be multifactorial, areca nut plays an important role in the disease manifestation.[5,6] Nutritional deficiency, primarily of iron and vitamins, is implicated in the etiology of OSMF. OSMF is also considered as an Asian version of sideropenic dysphagia, wherein chronic iron deficiency leads to mucosal susceptibility to irritants, such as chillies and areca nut products. Hemoglobin (Hb) levels, in particular, serum iron levels, are considered as biochemical indicators for nutritional assessment.[7]

Utilization of iron in collagen synthesis by the hydroxylation of proline and lysine leads to decreased serum iron levels in OSMF patients. In most cases, clinical anemia may be a contributing factor.[8] The concentration of serum iron does not fall until the body's iron stores are exhausted. As the stores are depleted, the concentration of transferrin rises while the concentration of ferritin falls.[9] Total iron-binding capacity (TIBC) measures the blood's capacity to bind iron with transferrin.[10]

The aim of our study was to estimate the Hb, serum iron, TIBC and serum ferritin levels in patients with OSMF.

MATERIALS AND METHODS

In the present study, forty patients of OSMF and forty normal subjects (controls) were included in this study. After obtaining the informed consent from the patients, the history and clinical findings of each patient was recorded. These cases were staged clinically according to Kiran Kumar *et al*.[4,11] (Stage-I: Mouth opening >45 mm; Stage-II: Restricted mouth opening 20–44 mm; Stage-III: Mouth opening <20 mm), [Figure 1] and histopathologically were graded according to Utsunomiya H, Tilakratne WM, Oshira K *et al.* [Figure 2].[4,12]

Early stage

A large number of lymphocytes in subepithelial connective tissue zone along with myxoedematous changes.

Intermediate stage

granulation changes close to the muscle layer and hyalinization appears in the subepithelial zone where blood vessels are compressed by fibrous bundles.

Advanced stage

inflammatory cell infiltrates hardly seen. A number of blood vessels dramatically small in subepithelial zone. Marked fibrosis, areas with hyaline changes extending from subepithelial to superficial muscle layers. Atrophic, degenerative changes start in muscle fibers.

After histological confirmation, the patients were recalled, and 5 ml of venous blood was collected of which 1 ml was transferred to vacutainer with ethylenediaminetetraacetic acid (anticoagulant) for Hb analysis immediately by cyanoHb method, and 4 ml was allowed to clot and serum separated from the blood samples by centrifugation for 5 min at 3000 rpm. Serum iron, TIBC and serum ferritin levels were analyzed using the Tulip iron and TIBC kit and Bios Microwell ELISA Diagnostic Systems kit and were processed in photocolorimeter.

RESULTS

Out of forty OSMF patients, according to clinical staging (Kiran Kumar *et al*.),[4,11] Stage-I were 12 cases (30%), Stage-II were 18 cases (45%) and Stage-III were ten cases (25%) [Table 1], and according to histopathological grading (Utsunomiya H and *et al*.),[4,12] early stage were 12 cases (30%), intermediate stage were 18 cases (45%) and advanced stage were ten cases (25%) [Table 2].

A control group of forty normal sex- and age-matched subjects were also included in the study. Hb, serum iron, TIBC and serum ferritin values were evaluated in both study group and the control group.

Comparison of mean total Hb% (gm/dl), serum iron (µg/dl), TIBC (µg/dl) and serum ferritin (ng/ml) levels in OSMF patients and control groups by *t*-test: [Table 3].

It was observed that the mean Hb% (12.52), serum iron (70.18) and serum ferritin (187.13) levels were significantly reduced in OSMF patients as compared with controls Hb% (14.94), serum iron (110.78) and serum ferritin

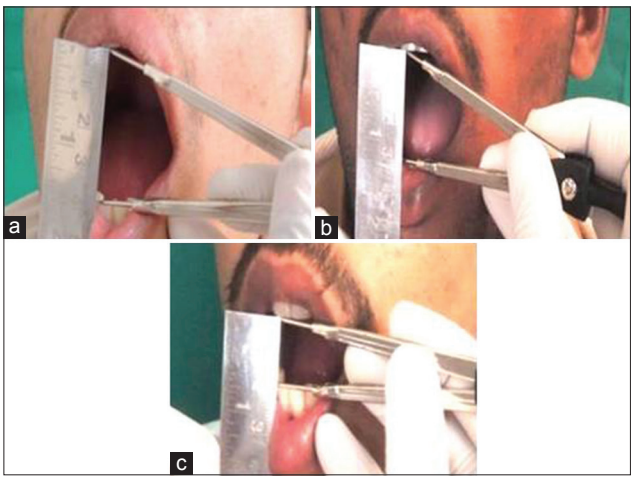

Figure 1: Clinical staging–(a) Stage-I, (b) Stage-II and (c) Stage-III

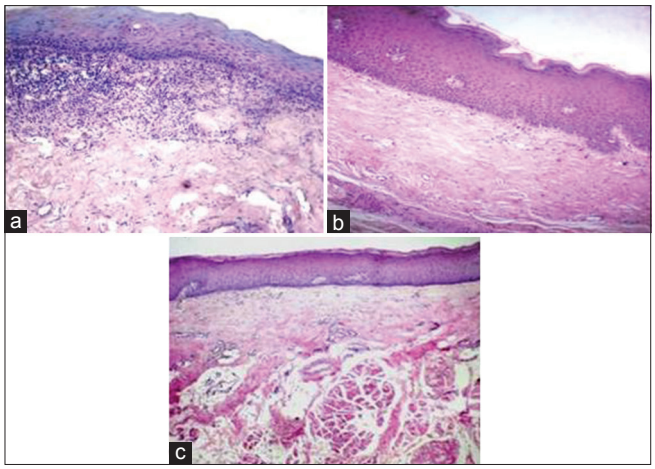

Figure 2: Histological grading - (a) Early stage, (b) intermediate stage, (c) advanced stage

Table 1: Distribution of oral submucous fibrosis patients by clinical staging

Clinical staging	Number of patients	Percentage of patients
Stage-I	12	30.00
Stage-II	18	45.00
Stage-III	10	25.00
Total	40	100.00

Table 2: Distribution of oral submucous fibrosis patients by histological grading

Histological grading	Number of patients	Percentage of samples
Early stage	12	30.00
Intermediate stage	18	45.00
Advanced stage	10	25.00
Total	40	100.00

Table 3: Comparison of hemoglobin % (g/dl), serum iron (μg/dl), total iron-binding capacity (μg/dl) and serum ferritin (ng/ml) levels in oral submucous fibrosis patients and control groups by *t*-test

	OSMF		Controls		*P*
	Mean	SD	Mean	SD	
Hemoglobin	12.52	1.28	14.94	0.86	0.00001*
Serum iron	70.18	19.01	110.78	21.79	0.00001*
TIBC	415.50	46.52	336.50	37.11	0.00001*
Serum ferritin	187.13	48.02	281.38	58.77	0.00001*

*$P<0.05$. TIBC: Total iron-binding capacity, SD: Standard deviation, OSMF: Oral submucous fibrosis

(281.38) levels with a statistically significant $P = 0.00001$, and the TIBC (415.50) levels in OSMF patients were significantly increased compared to controls TIBC (336.50) with a statistically significant $P = 0.00001$.

The comparison of Hb% (gm/dl), serum iron (μg/dl), TIBC (μg/dl) and serum ferritin (ng/ml) levels in various clinical stages of OSMF patients [Table 4].

The mean Hb%, serum iron and serum ferritin levels showed a progressive decrease as the clinical stage of OSMF advances except the mean TIBC levels showed a progressive increase as the clinical stage of OSMF advances. This difference was statistically significant with a $P = 0.00001$ using one-way ANOVA test. In a pairwise comparison of clinical staging done by Tukeys multiple *post hoc* procedures, statistically significant results were obtained between Stage-I and Stage-II, Stage-I and Stage-III, Stage-II and Stage-III respectively with a statistically significant $*P < 0.05$.

The comparison of Hb% (gm/dl), serum iron (μg/dl), TIBC (μg/dl) and serum ferritin (ng/ml) levels in different histopathological grades of OSMF patients [Table 5].

The mean Hb%, serum iron and serum ferritin levels showed a progressive decrease as the histologic grade of OSMF advances except the mean TIBC levels showed a progressive increase as the histologic grade of OSMF advances. This difference was statistically significant with a $P = 0.00001$ using one-way ANOVA test. In a pairwise comparison of histological grading done by Tukeys multiple *post hoc* procedures, statistically significant results were obtained between early versus intermediate stage, early versus advanced stage and intermediate versus advanced stage respectively with a statistically significant $*P < 0.05$.

DISCUSSION

The overall prevalence of OSMF in India is about 0.5% with a range of 0.2%–1.2% in different regions of the country.[13] Recent epidemiological data indicate that the number of cases of OSMF has risen rapidly in India due to an upsurge in the popularity of commercially prepared areca nut preparations and an increased uptake of this habit by young people.[14]

The etiology of OSMF is multifactorial but areca nut chewing is the main causative agent.[4] An equally important second aspect which needs to be considered is the preconditioning of the oral mucosa by a prolonged, chronic deficiency of iron and/or Vitamin B-complex, anemia and a genetic predisposition to the disease.[15]

The pathogenesis of OSMF was at first linked with the continuous and prolonged action of mild irritants on the oral mucosa, like tobacco and areca nut. Pungent and spicy foods, alcohol and iron deficiency have also been suggested.[16] OSMF is basically a collagen disorder. Hydroxyproline is an amino acid found in collagen, and the hydroxylation requires iron. The decrease in iron levels may be due to the utilization of iron in fibrosis.[17,18] Iron-dependent enzyme cytochrome oxidase and hydroxylation of proline to hydroxyproline are considered to be the major factors responsible for the

Table 4: Comparison of hemoglobin % (g/dl), serum iron (μg/dl), total iron-binding capacity (μg/dl) and serum ferritin (ng/ml) levels in oral submucous fibrosis patients with respect to clinical staging by one-way ANOVA

Clinical staging	Hb%		Serum Iron		TIBC		Serum ferritin		*P*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Stage-I	13.83	0.59	90.50	6.91	375.83	22.34	236.67	29.02	0.00001*
Stage-II	12.60	0.66	70.61	10.35	418.33	42.46	181.11	34.96	0.00001*
Stage-III	10.80	0.51	45.00	7.07	458.00	35.21	138.50	26.15	0.00001*
Pairwise comparison of clinical staging by Turkeys multiple *post hoc* procedures									
Stage-I versus Stage-II	*P*=0.0001*		*P*=0.0001*		*P*=0.0081*		*P*=0.0002*		
Stage-I versus Stage-III	*P*=0.0001*		*P*=0.0001*		*P*=0.0001*		*P*=0.0001*		
Stage-II versus Stage-III	*P*=0.0001*		*P*=0.0001*		*P*=0.0209*		*P*=0.0040*		

*$P<0.05$. TIBC: Total iron-binding capacity, SD: Standard deviation, Hb: Hemoglobin

Table 5: Comparison of hemoglobin % (g/dl), serum iron (μg/dl), total iron-binding capacity (μg/dl) and serum ferritin (ng/ml) levels in oral submucous fibrosis patients with respect to histological grading by one-way ANOVA

Histological grading	Hb%		Serum iron		TIBC		Serum ferritin		*P*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Early stage	13.83	0.59	90.50	6.91	375.83	22.34	236.67	29.02	0.00001*
Intermediate stage	12.60	0.66	70.61	10.35	418.33	42.46	181.11	34.96	0.00001*
Advanced stage	10.80	0.51	45.00	7.07	458.00	35.21	138.50	26.15	0.00001*
Pair wise comparison of histological grading by Turkeys multiple post hoc procedures									
Early stage versus intermediate stage	*P*=0.0001*		*P*=0.0001*		*P*=0.0081*		*P*=0.0002*		
Early stage versus advanced stage	*P*=0.0001*		*P*=0.0001*		*P*=0.0001*		*P*=0.0001*		
Intermediate stage versus advanced stage	*P*=0.0001*		*P*=0.0001*		*P*=0.0209*		*P*=0.0040*		

*$P<0.05$. TIBC: Total iron-binding capacity, SD: Standard deviation, Hb: Hemoglobin

decrease in iron levels in OSMF patients. Determining iron status should be a part of biochemical assessment, which may be of proactive intervention for high-risk groups. As the disease progresses, the serum iron levels also decrease. Serum iron content can thus be a predictor for the progression of the condition, and there appears to be an association between serum iron content and oral carcinogenesis. Therefore, biochemical assessment of oral precancerous conditions may help in early diagnosis and prognosis.[19] Hence, the present study was aimed to evaluate Hb, serum iron, TIBC and serum ferritin in OSMF patients.

In the present study, forty clinically diagnosed and histopathologically confirmed OSMF patients and forty age- and sex-matched normal subjects without any oral pernicious habits acted as controls. As compared to controls, OSMF patients showed a significant reduction in the levels of Hb, serum iron and serum ferritin and a significant increase in the levels of TIBC which might be a consequence of a disease that is mediated by utilization of body iron stores. All the values showed a statistically significant difference [$P < 0.05$, Table 3].

In the present study, Hb, serum iron, TIBC and serum ferritin in OSMF patients were compared with respect to clinical staging and histological grading. After statistical evaluation, it was found that a statistically significant reduction in the levels of Hb, serum iron and serum ferritin were found as the clinical stage and histological grade of the OSMF advances, and a statistically significant increase in the levels of TIBC was found as the clinical stage and histological grade of OSMF advances [$P < 0.05$, Tables 4 and 5].

The present study results [$P = 0.0001$, Table 3] coincided with the studies carried out by Dhakray *et al.*[10] wherein the mean of total serum iron was found to be significantly reduced in OSMF patients when compared with controlled group. The present study results [$P = 0.00001$, Table 3] coincided with the study results of Rajendran *et al.*[20] where the results showed a significant decrease in Hb and serum iron in patients with OSMF and TIBC showed a significant change in OSMF patients.

In a study conducted by Karthik *et al.*[21] OSMF patients showed significantly lower levels of both Hb and serum iron when compared with the healthy controls as the clinical stage of OSMF advances ($P < 0.0001$). The study has similar results as present study [$P = 0.00001$, Table 3]. The study conducted by Rupak *et al.*[22] showed a significant decrease in Hb and serum iron levels in patients with OSMF as compared to the healthy control group ($P < 0.001$), coincided with the results of the present study [$P = 0.00001$, Table 3].

According to the review by Rajendran[23] there is a decrease in serum iron and percentage saturation of transferrin and a significant reduction in total serum iron, concluding

iron-deficiency anemia appearing to be one of the causes of OSMF. In the study done by Khanna and Karjodkar[8] statistically significant reduction in the serum iron levels in precancer and cancer groups compared to normal was observed. According to the study performed by Apeksha and Sathawane[24] reduced levels of iron in descending order from oral leukoplakia to OSMF and oral squamous cell carcinoma was observed ($P < 0.01$). In the study performed by Tadakamadla *et al.*[25] the mean copper and iron level differed significantly between the OSMF patients and controls, with patients exhibiting higher copper and lower iron levels in contrast to controls who presented lower copper ($P < 0.005$) and higher iron levels ($P < 0.01$). The results also showed that there was decrease in serum iron concentration as clinical stage increased ($P < 0.0001$). In the study conducted by Shetty *et al.*[26] the serum and salivary iron levels decreased in OSMF patients with the progression of histopathological grading, but this was not significant. Serum and salivary levels showed significant correlation among cases ($r = 0.315$ and $P = 0.011$), but not among controls. The study by Kode and Karjodkar[27] revealed a significant decrease in serum iron in OSMF patients to healthy individuals ($P = 0.004$). A significant decrease in serum iron in OSMF patients was observed in the study conducted by Hosthor *et al.*[28] ($P < 0.001$) coinciding with the results of the present study.

An extensive search of English literature revealed that the present study was the first study done to evaluate the serum ferritin levels in OSMF patients compared to controls and also the first study done to evaluate Hb, serum iron, TIBC and serum ferritin in OSMF patients with respect to clinical staging and histological grading. Our study results exhibited a significant decrease in serum ferritin in OSMF patients compared to controls and with respect to clinical staging and histological grading [Tables 3-5].

The results of the three parameters Hb, serum iron and TIBC in the present study are in agreement with few above-mentioned studies done on OSMF patients. The values of serum ferritin in the present study were reduced significantly in OSMF patients compared to controls [Table 3]. A statistically significant difference was found in the values of serum ferritin as the clinical stage advances [Table 4], and as histological grade advances [Table 5]. Further studies are required to evaluate the levels of serum ferritin in patients with OSMF.

CONCLUSION

The Hb, serum iron and serum ferritin levels in OSMF patients were reduced, and TIBC increased as compared with controls suggesting that a lower levels of Hb, serum iron and serum ferritin and an increase in TIBC is a useful indicator for initial changes occurring in potentially malignant disorders like OSMF. The Hb, serum iron and serum ferritin levels in OSMF patients were reduced, and TIBC increased as the clinical stage and histological grade of OSMF advances indicating their role as a reliable biochemical indicator. However, studies in larger samples need to be conducted for more conclusive results.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Schwartz J. Atrophia idiopathica mucosa oris. Presented at the 11th International Dental Congress; London, UK. 1952
2. Gupta MK, Mhaske S, Ragavendra R, Imtiyaz. Oral submucous fibrosis – Current concepts in etiopathogenesis. Peoples J Sci Res 2008;1:39-44.
3. Savita JK, Girish HC, Murgod S, Kumar H. Oral submucous fibrosis – A review (part 2). J Health Sci Res 2011;2:37-48.
4. More CB, Gupta S, Joshi J, Varma SN. Classification system for oral submucous fibrosis. J Indian Acad Oral Med Radiol 2012;24:24-9.
5. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis – A collagen metabolic disorder. J Oral Pathol Med 2005;34:321-8.
6. Pandya S, Chaudhary AK, Singh M, Singh M, Mehrotra R. Correlation of histopathological diagnosis with habits and clinical findings in oral submucous fibrosis. Head Neck Oncol 2009;1:10.
7. Ganapathy KS, Shubha G, Bharati B, Sushmini B, Sujatha D. Role of iron deficiency in oral submucous fibrosis: An initiating or accelerating factor. J Indian Acad Oral Med and Radiol 2011;23:25-8.
8. Khanna SS, Karjodkar FR. Circulating immune complexes and trace elements (Copper, Iron and Selenium) as markers in oral precancer and cancer: A randomised, controlled clinical trial. Head Face Med 2006;2:33.
9. Frank F, Bryan R. Inetrpretation of diagnostic tests for iron deficiency, diagnostic difficulties related to limitations of individual tests. Aust Prescr1997;20:1-5.
10. Dhakray V, Prateek K, Manoj M, Metu J, Bipin Y. Risk markers of OSMF, serum albumin, hemoglobin and iron binding capacity? A review of literature. J Microbiol 2012;10:1-6.
11. Kiran Kumar K, Saraswathi TR, Ranganathan K, Uma Devi M, Elizabeth J. Oral submucous fibrosis: A clinico-histopathological study in Chennai. Indian J Dent Res. 2007; 18:106-11.
12. Utsunomiya H, Tilakaratne WM, Oshiro K, Maruyama S, Suzuki M, Ida-Yonemochi H, *et al.* Extracellular matrix remodeling in oral submucous fibrosis: Its stage-specific modes revealed by immunohistochemistry and *in situ* hybridization. J Oral Pathol Med. 2005;34:498-507.
13. Raina C, Raizada RM, Chaturvedi VN, Harinath BC, Puttewar MP, Kennedy AK. Clinical profile and serum beta-carotene levels in oral submucous fibrosis. Indian J Otolaryngol Head Neck Surg 2005;57:191-5.
14. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 2006;42:561-8.
15. Rajendran R. Oral submucous fibrosis: Etiology, pathogenesis, and future research. Bull World Health Organ 1994;72:985-96.
16. Pillai R, Balaram P, Reddiar KS. Pathogenesis of oral submucous fibrosis. Relationship to risk factors associated with oral cancer. Cancer

1992;69:2011-20.
17. Ramachandran S, Rajeshwari GA, Vijayabala GS, Krithika C. Oral submucous fibrosis: Realities of etiology. Arch Oral Res 2012;8:153-60.
18. Saba S, Laxmikanth C, Shenai KP, Veena KM, Prasanna KR. Pathogenesis of oral submucous fibrosis. J Can Res Ther 2012;8:199-203.
19. Sanjiv KB, Swati L, Deeksha P. Biochemical changes in OSMF. J Adv Med Dent Sci 2013;1:101-5.
20. Rajendran R, Vasudevan DM, Vijayakumar T. Serum levels of iron and proteins in oral submucous fibrosis (OSMF). Ann Dent 1990;49:23-5, 45.
21. Karthik H, Nair P, Gharote HP, Agarwal K, Ramamurthy Bhat G, Kalyanpur Rajaram D. Role of hemoglobin and serum iron in oral submucous fibrosis: A clinical study. ScientificWorldJournal 2012;2012:254013.
22. Rupak S, Giju GB, Sheba P, Kiran KK. Oral submucous fibrosis and iron deficiency anemia relationship revisited-results from an Indian study. E J Dent 2012;2:159-65.
23. Rajendran R. Oral submucous fibrosis. J Oral Maxillofac Pathol 2003;7:1-4.
24. Apeksha RB, Sathawane RS. Estimation and comparative evaluation of serum iron, copper, zinc and copper/zinc ratio in Oral leukoplakia, submucous fibrosis and squamous cell carcinoma. J Indian Acad Oral Med Radiol 2010;22:73-6.
25. Tadakamadla J, Kumar S, Mamatha GP. Evaluation of serum copper and iron levels among oral submucous fibrosis patients. Med Oral Patol Oral Cir Bucal 2011;16:e870-3.
26. Shetty SR, Babu S, Kumari S, Shetty P, Vijay R, Karikal A. Evaluation of micronutrient status in serum and saliva of oral submucous fibrosis patients: A clinicopathological study. Indian J Med Paediatr Oncol 2012;33:224-6.
27. Kode MA, Karjodkar FR. Estimation of the serum and the salivary trace elements in OSMF patients. J Clin Diagn Res 2013;7:1215-8.
28. Hosthor SS, Mahesh P, Priya SA, Sharada P, Jyotsna M, Chitra S. Quantitative analysis of serum levels of trace elements in patients with oral submucous fibrosis and oral squamous cell carcinoma: A randomized cross-sectional study. J Oral Maxillofac Pathol 2014;18:46-51.