Original Article

A clinic-pathological research explored the significance of ascorbic acid and iron levels in serum and saliva in premalignant disorder patients at Kanpur, Uttar Pradesh

ABSTRACT

Context: Oral submucous fibrosis (OSMF), or OSMF, is a well-known, potentially premalignant condition of the oral cavity. Monitoring OSMF widespread effects necessitate interventions in at-risk individuals, ideally before the disease becomes aggressive. Ascorbic acid and iron, for instance, are significant micronutrients in the pathogenesis of OSMF.

Aims: This study aimed to investigate the significance of ascorbic acid and iron levels in serum and saliva in patients with premalignant disorder (OSMF) and to correlate variations in ascorbic acid and iron levels with histopathological grading.

Settings and Design: The present study was conducted on 195 patients over a period of 10 months.

Subjects and Methods: These patients were divided into two groups, Group I (n = 88, Control), Group II (n = 107, clinically diagnosed and histopathologically confirmed cases of OSMF). Serum and salivary ascorbic acid were analyzed by the dintrophenyl hydrazine method, whereas serum and salivary iron were analyzed by the dipyridyl method.

Statistical Analysis Used: Paired t-test and Fisher test were used to compare between the mean and to find the level of significance P value.

Results: The serum and salivary ascorbic acid levels consistently decreased with the progression of histopathological grading of OSMF. Serum and salivary iron levels were also decreased in OSMF patients, and it came as significant.

Conclusions: Excess collagen synthesis during OSMF may have been promoted with ascorbic acid and iron. As a reason, serum and salivary monitoring may be significant in detecting and diagnosing OSMF early on.

Keywords: Ascorbic acid, iron, oral submucous fibrosis, saliva, serum

INTRODUCTION

Oral submucous fibrosis (OSMF) was originally described in the early 1950s and is a potentially cancerous disorder that primarily affects Asian people. OSMF is a debilitating and crippling disorder of the oral mucosa whose clinical presentation is based on the stage of the disease at the time of detection.[1] 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 the opening of the mouth and tongue movement.[2,3] Oral submucosal fibrosis particularly affects most of the oral cavity, pharynx, and upper third of the esophagus, is the disease's characteristic. In India, Bangladesh, Sri Lanka, Pakistan, Taiwan, and Southern China, the disease is most common.[4,5]

Access this article online	
Website: www.njms.in	Quick Response Code
DOI: 10.4103/njms.njms_470_21	

Rohan Sachdev, Kriti Garg[1], Vishal Mehrotra[1], Samiksha Shwetam[1], Praveen Singh[1], Akash Srivastava[1]

Department of Public Health, UWA School of Population and Global Health, Nedlands, Australia, [1]Department of Oral Medicine and Radiology, Rama Dental College, Kanpur, Uttar Pradesh, India

Address for correspondence: Dr. Rohan Sachdev, UWA School of Population and Global Health, The University of Western Australia, WA 6009, Nedlands, Australia.
E-mail: drrohansachdev@live.com

Received: 21 September 2021, **Revised:** 21 January 2022, **Accepted:** 12 February 2022, **Published:** 19 March 2024

How to cite this article: Sachdev R, Garg K, Mehrotra V, Shwetam S, Singh P, Srivastava A. A clinic-pathological research explored the significance of ascorbic acid and iron levels in serum and saliva in premalignant disorder patients at Kanpur, Uttar Pradesh. Natl J Maxillofac Surg 2024;15:131-5.

Etiological factors hypothesized to trigger the disease process include areca nut chewing, nutritional deficiencies, immunologic processes, and genetic predisposition.[6,7] The etiology of OSMF has been connected to nutritional deficits, specifically iron and vitamin deficiencies. The overall integrity and health of the epithelia of the digestive tract, as well as its contribution to appropriate enzymatic activities, are all dependent on iron. OSMF is also characterized as an Asian variant of sideropenic dysphagia, a disorder in which chronic iron deficiency causes mucosal irritability to stimulants like chilies and areca nut derivatives.[8] Ascorbic acid has the potential to protect both cytosolic and membrane components of cells from oxidant damage. In the cytosol, ascorbate acts as a primary antioxidant to scavenge free radical species that are generated as by-products of cellular metabolism.[9] Because of this, the purpose of this research was to determine the levels of ascorbic acid and iron in serum and saliva in patients with premalignant disorders such as OSMF and their correlation with different histopathological grades of OSMF among Kanpur residents who visited a private dental college.

SUBJECTS AND METHODS

The present study was conducted in the Dental (outpatient department) of a private dental college in Kanpur City over a period of 10 months from August 2020 to July 2021. An approval from the Institutional Ethical Committee (RDC/ESrT/2020/2970) was taken before commencing the study. This is a case control study where 195 patients were selected based on the simple randomized sampling method reporting to the Department of Oral Medicine and Radiology, with age range of 20 and 40 years and were further divided into two groups of 107 clinical-histopathologically confirmed cases of OSMF and 88 healthy controls.

Group I consisted of control group with patients; these patients were free of any deleterious habits such as areca nut or one of its commercial preparations, chewing and were not suffering from any systemic diseases.

Group II consisted patients with a habit of chewing areca nut or one of its commercial preparations, with the presence of burning sensation, inability to consume spices, stiffness of buccal mucosa, vesicle formation, ulceration, and blanching of oral mucosa, and histopathologically confirmed OSMF patients with different grades were included in the OSMF group.

The exclusion criteria included those patients treated for OSMF in any manner, and habit of chewing only tobacco and patients with a habit of chewing areca nut or one of its commercial preparations but without OSMF, patients with systemic diseases, patients under aspirin and antioxidants, pregnant women, and postmenopausal females were excluded from the study.

A preinformed consent form was filled by all the patients included in the study and was advised to routine blood investigation and was followed by areca nut or one of its commercial preparations, cessation counseling before and during the study in our institution. A questionnaire was used to collect the data regarding demographic factors, medical history, the form, in which the patient is consuming areca nut or one of its commercial preparations, frequency of consumption, and duration of the habit. The diagnostic criteria for OSMF included-the presence of burning sensation, restricted mouth opening, mucosal blanching, restricted tongue protrusion, and the presence of palpable fibrous bands. Interincisal distance was measured to measure the mouth opening of the OSMF patients. The clinical diagnosis of OSMF was made by using the criteria as mentioned according to clinical and functional staging of More *et al*.[10] The histopathological grading followed in the study Kiran Kumar *et al*.[11]

- Grade I-loose, thin and thick fibers
- Grade II-loose or thick fibers with partial hyalinization
- Grade III-complete hyalinization.

Taking aseptic precautions, blood samples (approximately 5 ml) were collected in appropriate sterile vials by venous arm puncture after overnight fasting; blood was allowed to clot at room temperature for 1–2 h. Plasma and serum were separated by centrifugation at 3000 rpm for 10 min to get a clear serum sample. The serum thus obtained was pipetted using a micropipette and transferred into sterile plastic storage vial and was stored at-200 C in a dark container until assay. Five milliliters of unstimulated saliva was obtained by the spit method after following a standard precollection protocol.[12]

Biocheimal analysis

Biochemical estimations were carried out for all the samples. Estimation of ascorbic acid done by 2–4 dinitrophenylhydrazine method. The Principle of this method is that Dehydroascorbic acid was coupled with 2, 4 dinitrophenylhydrazine and the resulting derivative is treated with sulphuric acid to produce a newly observed color which is measured at 545 nm.[13] Iron estimation was done by Ferrozine method and the principle of transferrin bound iron breaks into free ferric ions in an acidic medium. These ferric ions react with Hydroxylamine Hydrochloride

reduced into ferrous ions which react with Ferrozine to form a violet colored complex measured at 560 nm. The difference before and after the addition of ferrozine is proportional to iron concentration reaction in the specimen.[14]

Statistical analysis

The software used for the statistical analysis was Statistical Package for Social Sciences (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 19.0. Chicago, Illinois, USA: SPSS Inc.) Paired t-test and Fischer test were used to compare between the mean and to find the level of significance (*P* value), where $P < 0.05$ was considered to be significant.

RESULTS

The present study comprised of 195 patients who were categorized into two groups; Group I-88 controls (without a habit of chewing areca nut or one of its commercial preparations), Group II-107 OSMF (with a habit of chewing areca nut or one of its commercial preparations) and was aimed at the estimation of levels of ascorbic acid and iron in serum and saliva in patient's in these two groups. In the current study, the OSMF patients were graded histopathologically under Grade I, II, and III, respectively after histopathological examination.

Table 1 represents the levels of serum and saliva of ascorbic acid and iron levels in cases and controls. Mean serum and salivary ascorbic acid levels were significantly decreased in cases, when compared with controls ($P < 0.001$). However, serum and salivary iron levels showed a significant difference in case and controls ($P = 0.0155$ and 0.0032, respectively).

Table 2 represents intercomparison of ascorbic acid and iron levels among histopathological grades of OSMF and controls. Both serum and salivary ascorbic levels consistently decreased as the histopathological grading progressed; there was a very highly significant difference among the three groups. Serum and salivary iron levels decreased as the grades progressed, when compared to the control subjects and this was significant ($P \leq 0.0001$).

Tables 3 and 4 represent correlation between serum and salivary ascorbic acid and iron levels. Serum and salivary levels showed significant correlation among cases for ascorbic acid ($r = 0.638$ and $P = 0.0001$) and for iron levels ($r = 0.864$ and $P = 0.0001$) when compared with controls.

Table 1: Comparison between ascorbic acid and iron levels in oral submucous fibrosis patients and control

Parameters	*n*	Mean	SD	*t*#	*P*
Serum Asc patients	107	1.496	0.2964	5.211	0.0001
Controls	88	1.739	0.3548		
Saliva Asc patients	107	0.584	0.2974	6.959	0.0001
Controls	88	0.873	0.2775		
Serum Fe patients	107	129.867	24.986	2.441	0.0155
Controls	88	139.86	32.157		
Saliva Fe patients	107	104.38	24.687	2.984	0.0032
Controls	88	116.38	31.458		

#*t*: Paired *t*-test. SD: Standard deviation, Asc: Ascorbic acid, Fe: Iron

Table 2: Inter comparison of iron and ascorbic acid levels among histopathological grades of oral submucous fibrosis patients and controls

Parameters	*n*	Mean	SD	*F**	*P*
Serum Asc patients					
Grade 1	34	1.2863	0.5785	8.473	<0.0001
Grade 2	37	1.1854	0.2469		
Grade 3	36	1.3678	0.3654		
Controls	85	1.5684	0.3751		
Saliva Asc patients					
Grade 1	34	0.5736	0.2478	7.2601	<0.0001
Grade 2	37	0.6437	0.2999		
Grade 3	36	0.7325	0.2861		
Controls	85	0.8865	0.2738		
Serum Fe patients					
Grade 1	34	134.87	22.569		
Grade 2	37	127.69	25.638		
Grade 3	36	119.64	30.258		
Controls	85	144.28	37.968		
Saliva Fe patients					
Grade 1	34	108.69	28.694		
Grade 2	37	103.58	30.589		
Grade 3	36	101.58	27.684		
Controls	85	129.36	29.365		

**F*: Fischer' s test. SD: Standard deviation, Asc: Ascorbic acid, Fe: Iron

Table 3: Correlation between serum and salivary ascorbic acid levels

Parameters	Serum/salaiva Asc
Cases	
r	0.638
P	0.0001
n	107
Controls	
r	0.539
P	0.0001
n	85

Asc: Ascorbic acid

DISCUSSION

OSMF is a chronic, insidious oral mucosal condition that occurs predominantly among Indians and occasionally in other Asians.[1] In the Indian continent alone, the statistics for OSMF is about 5 million people (0.5%) of the population.[15] OSMF is caused by abnormal collagen deposition in the connective tissues and affects mouth functions.[16,17] The rapid

Table 4: Correlation between serum and salivary iron levels

Parameters	Serum/salaiva Fe
Oral submucous fibrosis patients	
r	0.864
P	0.0001
n	107
Controls	
r	0.736
P	0.0001
n	85

Fe: Iron

spread of the condition is attributed to a rise in the popularity of commercially produced areca nut preparations (pan masala) in India, as well as an increase in the acceptance of this habit by young people as a result of simple availability, successful pricing modifications, and marketing strategies.[9] OSMF has high malignant transformation rate triggered by areca nut chewing, nutritional deficiencies, immunologic processes, and genetic predisposition. It causes significant hematological abnormalities, resulting in anemia and a decrease in serum iron levels.[18,19] The initial type of intervention was a biochemical analysis of blood, serum, saliva, and tissues. Such research has aided in locating characteristics that influence the progression of the disease, modifying its behavior, and detecting its malignant transformation potential.[20,21] In the current study, we found a significant decrease in the levels of ascorbic acid in both serum and saliva. This could be attributed to its role in collagen synthesis, which was similar to work done by Bhalerao *et al*. and Shetty *et al*. in the year 2012.[21,22] Anuradha and Devi found elevated tissue collagen levels and depleted ascorbic acid and iron reserves in OSMF patients, which was similar to the present study.[23] OSMF is basically a disorder of collagen metabolism where ascorbic acid gets utilized in the conversion of proline into hydroxyproline; this hydroxylation reaction requires ferrous Iron and ascorbic acid.[21] As a result, ascorbic acid could be a significant indicator for the development of OSMF. Leggott *et al*. in their study have stated that there is a lack of correlation between serum and salivary ascorbic acid levels, which was opposite to the results of the current our study, where serum and salivary ascorbic acid levels showed a good correlation.[24]

Bhalerao *et al*. and Shetty *et al*. observed a reduction in iron levels in OSMF cases, although it was statistically not significant, which was found to be opposite to the results of the present study.[21,22] In the present study, the level of serum iron was significantly decreased in OSMF patients when compared to controls. This can be due to cytochrome oxidase, an iron dependent enzyme, is required for the normal maturation of the epithelium.[21,25] In iron deficiency state, levels of cytochrome oxidase are low, consequently leading to epithelial atrophy. The oral mucosa becomes susceptible to soluble irritants when the epithelium turns atrophic. In addition, a deficiency of iron in tissues causes inappropriate vascular channel development, which leads to impaired vascularity. This disrupts the lamina propria's inflammatory reparative response, resulting in impaired wound healing and scarification.[25] Thus, the cumulative effect of these initiating and promoting factors leads to further fibrosis, which is a hallmark of OSMF. Fibrosis dictates that OSMF is basically a disorder of collagen metabolism.[26] Hydroxyproline is an amino acid found only in collagen, which is incorporated in the hydroxylated form. This hydroxylation reaction requires ferrous iron and ascorbic acid. Utilization of iron, for the hydroxylation of proline and lysine, leads to decreased serum iron level.[26,27] In OSMF patients, there is an increase in the production of highly cross-linked insoluble collagen Type I, loss of more soluble procollagen Type III and collagen Type VI. The cross-linking of collagen due to the upregulation of lysyl oxidase plays a crucial role in the development and progression of the condition.[21] This indicated that ascorbic acid and iron levels may have been used for excessive collagen production and cross-linking occurring in OSMF. Hence, serum and salivary iron and ascorbic acid levels could be an important indicator for the progression of OSMF.

Further studies are required on large sample size which will include the uniform distribution of OSMF patients according to the grading. Moreover, serum and salivary ascorbic acid and iron are required to be correlated with molecular markers such as Matrix metalloproteinases and tissue inhibitors of metalloproteinases.

CONCLUSION

All patients of OSMF in the present research had decreased mean serum ascorbic acid and iron levels, as well as decreased mean salivary ascorbic acid and iron levels, confirming that ascorbic acid and iron play a role in the pathogenesis and progression of OSMF. Saliva could be utilized as a noninvasive diagnostic method to evaluate ascorbic acid and serum iron in OSMF patients, according to the present study. The determination of ascorbic acid and iron in OSMF patients could therefore benefit in management and the establishment of therapies based on trace element activity and preventing the development of malignant transformation.

Acknowledgement

Authors would like to acknowledge Oral Pathology and

Biochemical Department for helping with sample evaluation and all patients for kind participation in the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Khan S, Sinha A, Kumar S, Iqbal H. Oral submucous fibrosis: Current concepts on aetiology and management – A review. J Indian Acad Oral Med Radiol 2018;30:407-11.
2. Misra SP, Misra V, Dwivedi M, Gupta SC. Oesophageal subepithelial fibrosis: An extension of oral submucosal fibrosis. Postgrad Med J 1998;74:733-6.
3. Thakur DV, Jassal DS, Kumar DA, Sharma DP, Sahi DS. A short review on OSMF: Oral Sub mucous Fibrosis. J Curr Med Res Opin 2020;619-24.
4. 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.
5. Gupta MK, Mhaske S. Oral submucous fibrosis: Current concept in aetiopathogenesis. People J Sci Rec 2008;1:39-44.
6. Cox SC, Walker DM. Oral submucous fibrosis. A review. Aust Dent J 1996;41:294-9.
7. Goel S, Ahmed J, Singh MP, Nahar P. Oral submucous fibrosis: A clinico-histopathological comparative study in population of southern Rajasthan. J Carcinogene Mutagene 2010;1:108.
8. Rajendran R, Vijayakumar T, Vasudevan DM. An alternative pathogenetic pathway for Oral Submucous Fibrosis (OSMF). Med Hypotheses 1989;30:35-7.
9. Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Pindborg JJ, Mehta FS. Etiology of oral submucous fibrosis with special reference to the role of areca nut chewing. J Oral Pathol Med 1995;24:145-52.
10. More CB, Gupta S, Joshi J, Varma SN. Classification system for oral submucous fibrosis. J Indian Acad Oral Med Radiol 2012;24:24-9.
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;18:106-112.
12. Navazesh M. Saliva as a diagnostic fluid. Ann N Y Acad Sci 1993;694:72-7.
13. Mesquita CS, Oliveira R, Bento F, Geraldo D, Rodrigues JV, Marcos JC. Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Anal Biochem 2014;458:69-71.
14. Tzivanidou HA. Spectrophotometric determination of serum iron with a new reagent. Microchemical J 1980;25:373-79.
15. Angadi PV, Rao SS. Areca nut in pathogenesis of oral submucous fibrosis: Revisited. Oral Maxillofac Surg 2011;15:1-9.
16. Shen YW, Shih YH, Fuh LJ, Shieh TM. Oral submucous fibrosis: A review on biomarkers, pathogenic mechanisms, and treatments. Int J Mol Sci 2020;21:7231.
17. Shih YH, Wang TH, Shieh TM, Tseng YH. Oral submucous fibrosis: A review on etiopathogenesis, diagnosis, and therapy. Int J Mol Sci 2019;20:2940.
18. Bhardwaj D, Dinkar AD, Satoskar SK, Desai SR. Serum iron and haemoglobin estimation in oral submucous fibrosis and iron deficiency anaemia: A diagnostic approach. J Clin Diagn Res 2016;10:C54-8.
19. Roe JH, Kuether CA. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivative of dehydroascorbic' acid. AMA 1943;147:399-407.
20. Stookey LL. Ferrozine – A new spectrophotometric reagent for iron. Anal Chem 1970;42:779-81.
21. Bhalerao SM, Lohe VK, Bhowate RR. Estimation of iron and vitamin C levels in serum and saliva: A clinical and biochemical study in oral submucous fibrosis patients. Ann Int Med Den Res 2018;4:DE43-53.
22. 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.
23. Anuradha CD, Devi CS. Serum protein, ascorbic acid & iron & tissue collagen in oral submucous fibrosis – A preliminary study. Indian J Med Res 1993;98:147-51.
24. Leggott PJ, Robertson PB, Rothman DL, Murray PA, Jacob RA. Response of lingual ascorbic acid test and salivary ascorbate levels to changes in ascorbic acid intake. J Dent Res 1986;65:131-4.
25. Wang YY, Tail YH, Wang WC, Chen CY, Kao YH, Chen YK, *et al*. Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders. BMC Oral Health 2014;14:99.
26. Guruprasad R, Nair PP, Singh M, Singh M, Singh M, Jain A. Serum vitamin c and iron levels in oral submucous fibrosis. Indian J Dent 2014;5:81-5.
27. Ganapathy KS, Gurudath S, Balikai B, Ballal S. Role of iron deficiency in oral submucous fibrosis: An initiating or accelerating factor. Indian Acad Oral Med Radiol 2011;23:25-8.