Original Article

Alpha-L-fucosidase levels in patients with oral submucous fibrosis and controls: A comparative study

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Abstract

Background: Oral submucous fibrosis (OSMF) in recent times has been recognized as a potentially malignant disorder (PMD) with an increased risk of developing oral squamous cell carcinoma with malignant transformation rates that vary from 0.6% to 36%. Alpha-L-fucosidase (AFU) is a lysosomal enzyme that is involved in maintaining the homeostasis of fucose metabolism. In benign and malignant tumors, the cells modulate their surface by increasing fucosylation leading to uncontrolled growth.

Aims and Objectives: This study was designed to estimate the levels of salivary and serum AFU in patients with OSMF and healthy controls and also to evaluate the clinical utility of salivary AFU levels over serum.

Materials and Methods: Saliva and blood samples were collected from twenty participants in both the groups (OSMF and healthy controls). Serum and salivary alpha-L-fucosidase levels were measured by enzyme-linked immunosorbent assay. The data were subjected to appropriate statistical analysis.

Results: We found a significant increase in alpha-L-fucosidase level in OSMF compared with healthy subjects. Pearson's correlation showed salivary alpha-L-fucosidase level to have superior sensitivity in detecting OSMF compared with serum alpha-L-fucosidase.

Conclusion: The outcome of this study suggests that salivary alpha-L-fucosidase can be utilized as a biomarker in early detection of oral precancer and cancer.

Keywords: Alpha-L-fucosidase, oral squamous cell carcinoma, oral submucous fibrosis, salivary biomarker

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INTRODUCTION

Oral cancer is one of the most aggressive malignancies worldwide and is ranked as the sixth leading cause of cancer mortality worldwide and the second leading cause of cancer mortality in India. Thus, in spite of advancements in treatment strategies, the 5-year survival rate for oral cancer patients remains only 50% for the past three decades.[1] The major risk factor for oral squamous cell carcinoma (OSCC) is tobacco use in various forms (smoking, chewing and snuff dipping) and alcohol consumption. Approximately 80,000 new cases are diagnosed annually, mainly attributed to different forms of tobacco consumption.[2] Due to its high propensity for local invasion, distant metastases and a lack of early detection methods when diagnosed, more than two-thirds of cases demonstrate advanced or unresectable forms of the disease.

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The pathogenesis of oral cancer is a complex, multistage and multigenetic process, of which the precise mechanisms are not yet fully understood. Most of the oral cancers are OSCC and it has been reported that there is a transition from potentially malignant disorders (PMDs) (leukoplakia, oral submucous fibrosis, etc.) to malignancy for over a period of time.[3]

Oral submucous fibrosis (OSMF) is a chronic, progressive, disabling, scarring, PMD of the oral mucosa with a malignant transformation rate of 7.6% in rural Indian population.[4-6] This makes it necessary to constantly screen the population where there is high incidence of these lesions due to tobacco chewing/smoking habits. Hence, a quick and cost-effective mass screening biomarker that will differentiate a lesion as malignant or benign with high specificity and sensitivity is needed. For the utmost clinical utility, the biomarker should be measurable in specimens that are collected with minimal discomfort and that foster early and frequent screening of patients, such as saliva specimens.

In recent years, the study of glycomics in cancer is a promising field. Glycosylation is the most ubiquitous form of posttranslational modification of proteins and it is critically important to many of the signaling pathways involved in turning a normal cell into a cancer cell.[5-14] Fucosylation is one of the major type of glycosylation changes in which there are terminal modifications of proteins that mediate vital biological functions.[6-15] Alpha-L-fucosidase (AFU) is a lysosomal enzyme that catalyzes the hydrolytic cleavage of terminal fucose residue, and it is involved in maintaining the homeostasis of fucose metabolism. Hence, monitoring the AFU levels could be a promising approach for the early detection, diagnosis and prognosis of oral precancer and cancer.

Hence, the present study was undertaken to estimate the levels of salivary and serum AFU in patients with OSMF and healthy controls and also to evaluate the clinical utility of salivary AFU levels over serum.

MATERIALS AND METHODS

This study was approved by the Scientific Advisory Committee and Institutional Ethical Committee of SRM University, Chennai, India. According to ethical principles, written and informed consent was obtained from all the study participants before the drawing of blood and collecting of saliva.

Study subjects recruitment

The study subjects comprised 20 cases of OSMF and 20 healthy controls, and they were recruited from the Outpatient Department of SRM Dental College, Chennai. The demographic details and information on previous history were collected [Table 1]. Subjects with infectious diseases during 1 month before saliva sampling, active dental abscesses, systemic illness, collagen vascular diseases and those undergoing any form of treatment were excluded from the study. Pregnant and lactating subjects were also excluded. None of the control participants had oral lesions.

Sample collection

Saliva samples were collected between 9 and 11 A.M under nonstimulatory conditions. Participants were asked to refrain from eating, chewing and drinking at least 1 h before collection. Three to five milliliter ml of salivary samples from patients with OSMF was collected before any therapeutic procedure. Following collection, the saliva was immediately centrifuged to remove cell debris, and the supernatants were then stored at −80°C further analysis. Two milliliter of peripheral blood was drawn from all study subjects through standardized phlebotomy procedure and was transferred to centrifuge tubes without and allowed to coagulate for 1 h at room temperature. Serum was then collected by centrifuging the coagulated blood and stored at −80°C until further use. No more than one freeze-thaw cycle was allowed for each sample

Estimation of alpha-L-fucosidase

Concentrations of salivary and serum AFU were quantified by commercially available ELISA Kit (Bioassay Technology). The assay was carried out according to the manufacturer's instructions. Absorbency was 620 nm and measured by microplate reader (Robonik ELISA plate reader). The results were expressed as ng/ml of saliva or serum.

Statistical analysis

The data were analyzed using SPSS software, version 16.0 (SPSS, Chicago, IL, USA). The descriptive

Table 1: Demographics of control and oral submucous fibrosis groups

Characteristics	Control	OSMF
Subjects	18.1±5.6	15.2±3.3
Age range	36.3±25.7	34.3±13.4
Sex		
Male	18	18
Female	2	2
Habits		
Tobacco chewing	-	7
Smoking	-	3
Tobacco chewing and smoking	-	7
Smoking and alcohol	-	2
Alcohol only	-	1

OSMF: Oral submucous fibrosis

statistics such as mean and standard deviations were calculated for the individual groups. Comparison between serum and saliva in both the groups was done using Pearson's correlation test.

RESULTS

The demographic characteristics of the patients are outlined in Table 1. Briefly, age–sex matched 20 control participants, and 20 participants with OSMF were included. The age of control and OSMF groups ranged from 24 to 50 and 22–54 years, and both the groups were predominantly male. All the participants in the OSMF group had tobacco/paan chewing/smoking or alcohol intake habits.

Serum alpha-L-fucosidase level in control and oral submucous fibrosis group

The serum AFU level of control and OSMF groups ranged from 8.9–28.6, and 10.6–74.4 ng/ml, respectively, with mean (standard error [±SE]) 18.1 ± 5.6 and 36.3 ± 25.7 ng/ml, respectively, as shown in Table 2. The mean serum AFU level of the OSMF group was comparatively higher than the control group.

Salivary alpha-L-fucosidase level in control and oral submucous fibrosis group

The salivary AFU level of control and OSMF groups ranged from 10.9 to 25.8, and 16.2–59.4 ng/ml, respectively, with mean (±SE) 15.2 ± 3.3 and 34.3 ± 13.4 ng/ml, respectively, as shown in Table 2. The mean salivary AFU level of the OSMF group was comparatively higher than the control group.

On performing Pearson's correlation, serum AFU and salivary AFU in Group I patients showed a weak correlation that was not statistically ("*P*" >0.01) [Table 3 and Figures 1and 2]. The Pearson's correlation coefficient value was 0.768 for OSMF patients, and the correlation was strong and statistically significant ("*P*" < 0.01) [Table 4 and Figures 3 and 4].

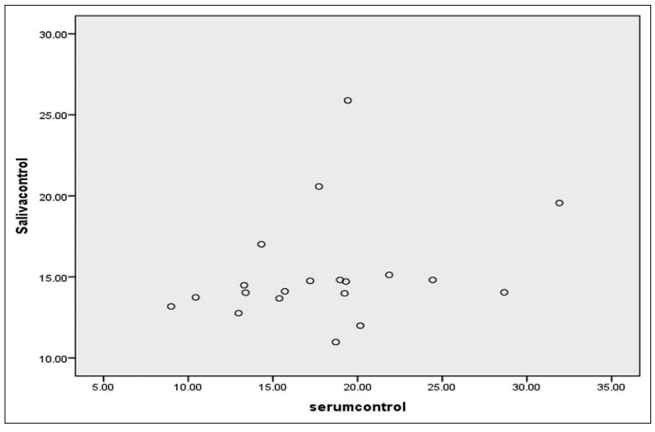

Figure 1: Comparison of serum and salivary alpha-L-fucosidase levels in control group– Pearson's correlation test-scatter plot

DISCUSSION

Oral cancer does not currently satisfy criteria for a screenable disease; however, theory suggests that most cases are preceded by asymptomatic clinical lesions collectively known as oral PMDs (OPMDs). There are several OPMDs that precede the development of OSCC, and the most commonly encountered are erythroplakia, leukoplakia and OSMF (encountered in Southeast Asia).[16]

OSMF is defined as a PMD that primarily affects any part of the oral cavity and sometimes the pharynx. The disease is chronic, insidious and progressive in nature. This generalized condition of the mouth eventually becomes a debilitating disease with mucosal rigidity causing discomfort, burning sensation and limitation of opening of the mouth. OSMF is common amidst the South Asians because of their frequent use of tobacco products. Arecanut, being a chief ingredient in betel quid, plays a pivotal role in disease manifestation in OSMF. The initiation of OSMF is subtle and takes about a decade or two to worsen. Trismus is a common feature, which is due to submucosal fibrosis of diverse areas of oral cavity.[17] Subjects suffering from OSMF show an increased risk for the development of oral cancer. Malignant transformation of OSMF to squamous cell carcinoma has been estimated to be between 2% and 8%.[18]

Table 2: Alpha-L-fucosidase levels (mean±standard error, *n*=20) in control and oral submucous fibrosis groups

Groups	Serum alpha-L-fucosidase	Salivary alpha-L-fucosidase
Control	18.1±5.6	15.2±3.3
OSMF	36.3±25.7	34.3±13.4

OSMF: Oral submucous fibrosis

Table 3: Comparison of serum and salivary alpha-L-fucosidase levels in control group - Pearson's Correlation test

Control group	*n*	Mean±SD	Pearson's correlation significant (two-tailed)	*P*
Serum	20	18.1041±5.65813	0.269*	0.251 (not
Saliva	20	15.2123±3.36069	0.269*	Significant)

*Correlation is significant at the 0.01 level (two-tailed). SD: Standard deviation

Table 4: Comparisons of serum and salivary alpha-L-fucosidase levels in oral submucous fibrosis group - Pearson's correlation test

OSMF group	*n*	Mean±SD	Pearson's correlation significant (two-tailed)	*P*
Serum	20	36.3424±25.74435	0.768**	0.000
Saliva	20	34.3616±13.43698	0.768**	(significant)

**Correlation is significant at the 0.01 level (two-tailed). OSMF: Oral submucous fibrosis, SD: Standard deviation

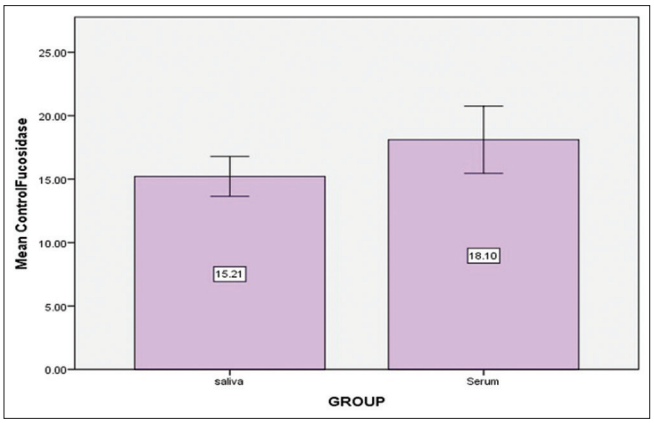

Figure 2: Comparison of mean concentration between saliva and serum in control group

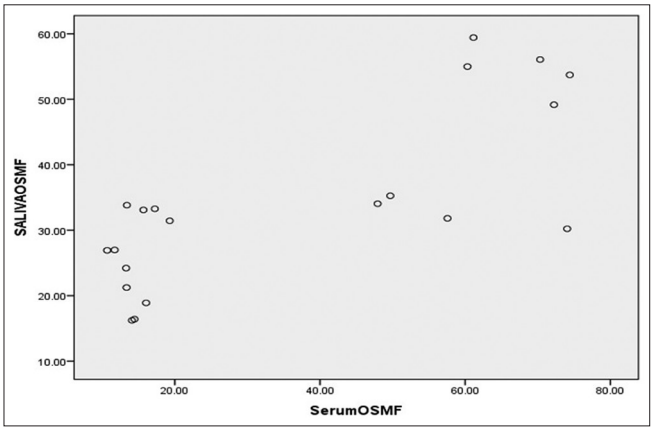

Figure 3: Comparisons of serum and salivary alpha-L-fucosidase levels in oral submucous fibrosis group-Pearson's correlation test-scatter plot

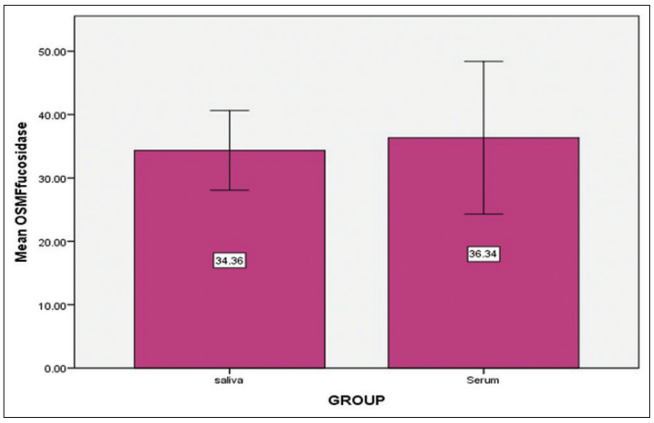

Figure 4: Comparison of mean concentration between saliva and serum in oral submucous fibrosis group

AFU is a lysosomal enzyme present in all mammalian cells; it is involved in the catabolism of the fucose-containing glycoconjugates.[19] AFU is a 6-carbon deoxyhexose that is commonly incorporated into human glycoproteins and glycolipids. It is found at the terminal or preterminal positions of many cell-surface oligosaccharide ligands that mediate cell recognition and adhesion signaling pathways. Events such as early embryologic development and blood group recognition and pathologic processes including inflammation, infectious disease recognition and neoplastic progression are mediated by this enzyme

Keeping in track with the fucosidase, levels could be a unique method to monitor potentially malignant oral disorders and malignancies. Since OSMF being a potentially malignant oral disorder, the role of AFU as a marker in the early diagnosis as well as the malignant transformation can be identified.

The present study included a total of 40 patients, out of which 20 were controls (Group I) and 20 cases of OSMF (Group II) and both serum and salivary AFU concentrations were estimated in both the groups.

In this study, the concentration of AFU in serum between both the groups was tabulated with mean value of 18.10 ng/ml in control group and 36.34 ng/ml in OSMF group). The concentration of AFU in the serum was higher in OSMF group when compared to the control group.

Similarly, the concentrations of AFU in saliva between both the groups were tabulated with mean value of 15.21 ng/ml in control group and 34.36 ng/ml in OSMF group, respectively. The concentration of AFU in the saliva was higher in OSMF group when compared to the control group.

The correlation between serum and salivary mean concentration of AFU within the control group was weak and was not statistically significant when analyzed by Pearson's correlation (Pearson's correlation coefficient value was 0.269, "*P*" > 0.01). In OSMF group, the Pearson's correlation coefficient value was 0.768, and the correlation was strong and statistically significant ("*P*" < 0.01).

The result of this study was in accord with the study done by Bhairavi N. Vajaria *et al*., who showed that serum and salivary AFU activity was higher in oral precancerous condition and oral cancer when compared to controls and stating that salivary levels of AFU may be used for observing the changes related to oral cancer progression.[20]

The result of this study was also in accord with the study done by Shah *et al*., who showed that oral precancer group (OSMF and leukoplakia) had a significant increase in fucosylation of serum proteins. Furthermore, serum AFU levels were markedly higher in patients with oral precancer when compared to controls. The results of the study showed that serum fucosylation was a valuable tool in assessing the changes in patients with oral precancer and oral cancer.[21]

In a study done by Roopesh *et al*., the levels of L-fucose were found to be significantly increased in various stages of precancer in comparison with the normal subjects. The study hypothesized that these glycoconjugates are released into circulation as the tumor increases in size and indicated the importance of L-fucose as a biomarker and its potential use for early diagnosis of cancer and precancer.[22]

In another study by Rai *et al*., the levels of serum fucose in OSCC and leukoplakia patients were significantly elevated which was in contrast to controls. In conclusion, the study suggested that serum fucose levels were highly reliable in patients with OSCC.[23]

Thus, in the current study, the comparison of serum and salivary AFU levels in control group was found to have mild variations and was not statistically significant, whereas the comparison of serum and salivary AFU levels in OSMF group was found to have strong statistical significance demonstrating that salivary levels of AFU could be used as a reliable biomarker for early diagnosis of precancer and cancer. However, further studies are required with large number of samples to assess the levels of salivary AFU in different grades of precancer and OSCC.

There are certain limitations in the present study. The sample size is relatively small to represent the population. A larger sample size is thus required to further validate the study. Furthermore, correlating AFU level with clinical staging and histopathological grading of OSMF and OSCC could aid in better understanding of its role in oral cancer progression.

CONCLUSION

The concentration of AFU was elevated both in the serum as well as the salivary samples in OSMF patients when compared with the control population. The concentration of AFU in saliva showed a progressive and steady increase from control to OSMF indicating that saliva could be used as an effective noninvasive and cost-effective diagnostic biomarker.

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Conflicts of interest
There are no conflicts of interest.

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