

Salivary Basic Fibroblast Growth Factor Expression: A Gateway to Early Detection of Oral Submucous Fibrosis

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ABSTRACT

Background: Basic fibroblast growth factor (bFGF) is a cytokine, mainly secreted by fibroblast cells which are implicated as a source of extracellular matrix in fibrotic regions. The objective of this research was to study the expression of salivary bFGF with clinical characteristics in patients with Oral submucous fibrosis and healthy controls.

Methods: This case-control study was conducted in the Dental OPD of Ziauddin University and Abbasi Shaheed Hospital, Karachi in 2019 (0370718SSOM). A total 180 salivary samples, including 90 controls and cases of Oral submucous fibrosis were processed and investigated at Ziauddin University's clinical laboratory in North Nazimabad using ELISA technique. Non-probability consecutive sampling technique was used. Questionnaire was used for recording socio-demographic data, frequency and duration of tobacco for all participants. For all the diagnosed cases of Oral Submucous Fibrosis (OSF), clinical parameters were noted. SPSS v20 was used for analysis with a 95% confidence interval and 80% power; normality was tested via Kolmogorov-Smirnov test, associations between clinical characteristics and biomarker were assessed using one-way ANOVA and pooled t-test, salivary bFGF levels were compared using pooled t-test, and logistic regression was applied to calculate odds ratios with 95% confidence intervals to assess exposure-outcome associations.

Results: The presence of bFGF was detected in the saliva samples taken from all participants. Mean levels of bFGF in controls were 2.59–2.44 whereas in cases the levels were elevated to 8.97–2.96 and showed a significant correlation among cases and controls ($p < 0.001$).

Conclusion: An increase in salivary level of bFGF may indicate an irreversible progression in cases with Oral submucous Fibrosis.

Keywords: Saliva, Basic Fibroblast Growth Factor, Tumorigenesis, Oral Submucous Fibrosis.

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INTRODUCTION

Potentially pre-malignant oral epithelial lesions (PPOEL) is defined as a "morphologically altered tissue in which oral cancer is more likely to occur than its apparently normal counterpart"¹. It has also been referred to as "potentially malignant diseases of the oral cavity," a term recently introduced to encompass both precancerous lesions and conditions that carry a risk of malignant transformation². Depending on severity of the lesion, its duration and type, oral premalignant disorders (OPMDs) have an overall malignant transition rate of about 0.13% and 36.4%^{2,3,4}.

In South East Asian region, one of the most common OPMD is Oral submucous fibrosis (OSF) because of frequent consumption and easy availability of areca nut or its flavoured formulations⁵. This condition is linked to collagen and is typified by an excess of aberrant collagen deposited in the submucosa, which causes tissue fibrosis, hyalinization, and degenerative alterations in the muscles⁶. Patients with OSF complains of burning sensation in the mouth, following a meal of spicy food⁷. In progressive stages of OSF, patient complains of pain, limited mouth opening, dysphagia, taste disorders, restricted tongue mobility, trismus, and altered tone⁸. Cells of the oral cavity are immersed in salivary bio-fluid⁹. When oral epithelium changes, it releases molecules and cells in to salivary fluid which may alter the biochemical composition of salivary fluid and may represent an individual's health status¹⁰.

Basic fibroblast growth factor (bFGF) is a protein-based biomarker, which is mainly formed by fibroblasts, smooth muscle cells, glial and endothelial cells. It is widely distributed in different body tissues¹¹. Literature suggests strong role of bFGF in proliferation, migration, self renewal, tissue repair, regeneration and cellular differentiation^{3,12}. Beyond the physiological functions of the bFGF, its role is critically important in promoting tumorigenesis as well^{1,13}.

One of the studies reported that the increased expression of bFGF and its associated receptors plays a critical role in transforming premalignant lesions to OSCC following various stages of epithelial dysplasia¹³. Hence, it can be used as a marker for the recognition of potential change or disease progression in Oral Submucous Fibrosis. The purpose of this study was to evaluate and compare the salivary levels of bFGF in healthy controls and cases with Oral Submucous fibrosis.

METHODS

In this case control study, an overall 180 participants were recruited among which 90 were healthy individuals (controls) with or without habit of

Chewable smokeless tobacco(CSLT) and 90 were those who presented with Oral Submucous Fibrosis (cases)with or without habits (CSLT) Salivary samples from all the participants were collected in the Dental OPD of Ziauddin University as well as Abbasi Shaheed Hospital, Karachi from January 2019 to December 2019 (0370718SSOM). These samples of saliva were processed at Ziauddin University's clinical laboratory in North Nazimabad, Karachi. Demographically matched candidates between the age ranges of 18 – 68 years were enrolled for the research.

Patients who had any history of metabolic disorders, malignancy, or patients with compromised immunity, pregnant and nursing mothers, local or systemic inflammatory diseases, and patients with a history of using any form of corticosteroids or another immunosuppressive drug a week before the collection of salivary samples were excluded. The sample size was calculated by using OpenEPI version 20. A non-probability consecutive sampling technique was used. Unstimulated whole saliva samples were collected early in the morning (8:00 a.m. to 11.00 a.m.) by using passive drool technique¹⁴. First, the cases and controls were told to rinse their mouths with water to wash away any debris or food particles and wait for 10 minutes to prevent dilution of the sample. The patients were then asked to remain seated comfortably on a dental chair and collect the unstimulated whole saliva in the oral cavity for a period of 5 minutes. After this, they were asked to expectorate it into plastic funnel tubes that were graded^{15,27}. At least one milliliter of saliva was collected from all the participant. After collection, samples were immediately transferred to an icebox, which were then instantaneously centrifuged for 20 minutes at a rate of 2500 rotations per minute (Gemmy Industrial Corp., USA) at a temperature of 4°C in research lab at Ziauddin University. About 3 ml of supernatant was stored at -80°C in multiple aliquots in research facility.

Quantification and estimation of salivary concentration of bFGF was carried out using "human bFGF enzyme linked immunosorbent assay (ELISA)" kit (glory science company ltd). To perform the indirect ELISA technique, instructions given by the manufacturer were followed and spectrophotometer was used to determine the absorbance (plate reader stat, fax-2100, awareness technology, USA) by interpreting the plates at 50 nano meter. To make sure the outcomes could be repeated, this process was carried out twice. Detection limit of the kit was set at 20 – 6000 nanogram per litre (ng/l).

SPSS version 20 was used for results analysis. The confidence interval was set at 95% and power of

test was set at 80%. Normality of the sample was checked using the Kolmogorov-smirnov test. One way ANOVA and independent pooled t- test was used to check the association between the clinical characteristics of oral pre – malignant lesions and biomarker. Differences in the salivary levels of the marker bFGF were evaluated in pre-malignant oral lesions by applying the Pooled t–test. Logistic regression was applied to calculate the Odds ratio with a confidence interval of 95%. Odds ratio was calculated to establish if there was any association between exposure and outcome. It was calculated by using formula of ad/bc , where 'a' is the number of cases exposed to CSLT, 'b' is the number of controls exposed to CSLT, 'c' is the number of cases not exposed to any of CSLT, and 'd' is the number of controls not exposed to CSLT.

RESULTS

A total of 180 individuals were included in this study among which 90 were healthy controls and 90 were

cases with Oral Submucous Fibrosis. Socio demographic data was gathered using a questionnaire from all the study participants already diagnosed with OSF and healthy individuals visiting the dental OPD. In addition to this, the total duration (in years) and daily frequency (per day) of tobacco consumption were recorded along with intra-oral examination. Following the assessment of the clinical parameters of oral submucous fibrosis, oral hygiene practices were also recorded. Among all the 180 participants, 116 (64.4%) were males and 64 (35.5%) were females with a mean age of 32.08 ± 10.08 years for the cases and controls respectively as shown in **Figure 1**. Mean age of both the cases and controls were 24 years + 10.08.

Figure 1 shows the gender distribution in cases and controls. There was a total of 180 participants, out of which the majority (86.6%) were males and only about 13.4% were females.

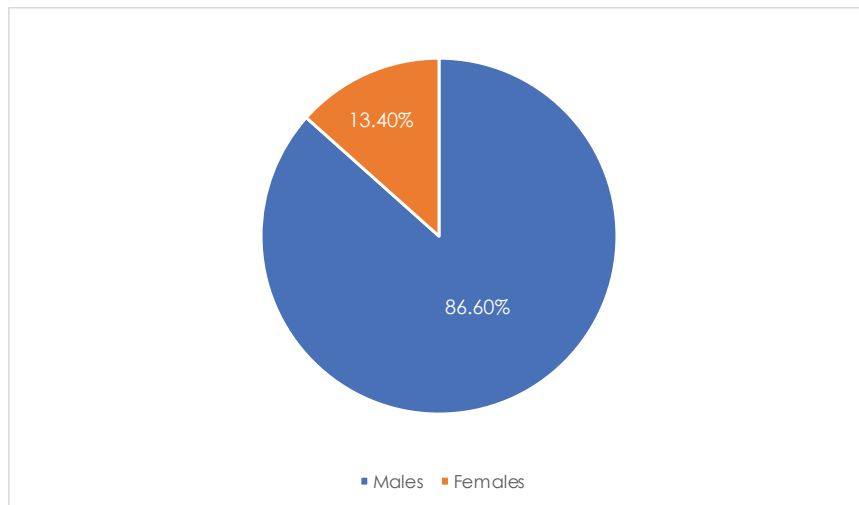


Figure 1: Distribution of Gender in Patients with Oral Submucous Fibrosis

All cases included in this study presented to the outpatient department (OPD) with a complaint of restricted mouth opening with a burning sensation. An intraoral analysis revealed that the anterior and posterior surfaces of buccal mucosa had fibrous bands, and most of the cases with OSF had restricted mouth openings of 16–18 mm. Approximately 80 % of the lesions were pigmented. Lesions were most commonly found on the buccal mucosa. Due to the location of chewable smokeless tobacco (CSLT), the lesions were also observed on the tongue and lips. 40% of the lesions were unilateral, whereas 11.1% of the lesions were bilateral. A smooth surface texture was noted in 50% of lesions, whereas 28.8% of cases were found to have an ulcerated and rough texture. Regular margins were found in 19 (21.1%) of 90 cases, whereas the rest of them, 71 (78%), had an irregular margin. Regarding habits, most of them were consuming betel nuts, as mentioned in **Figure 2**.

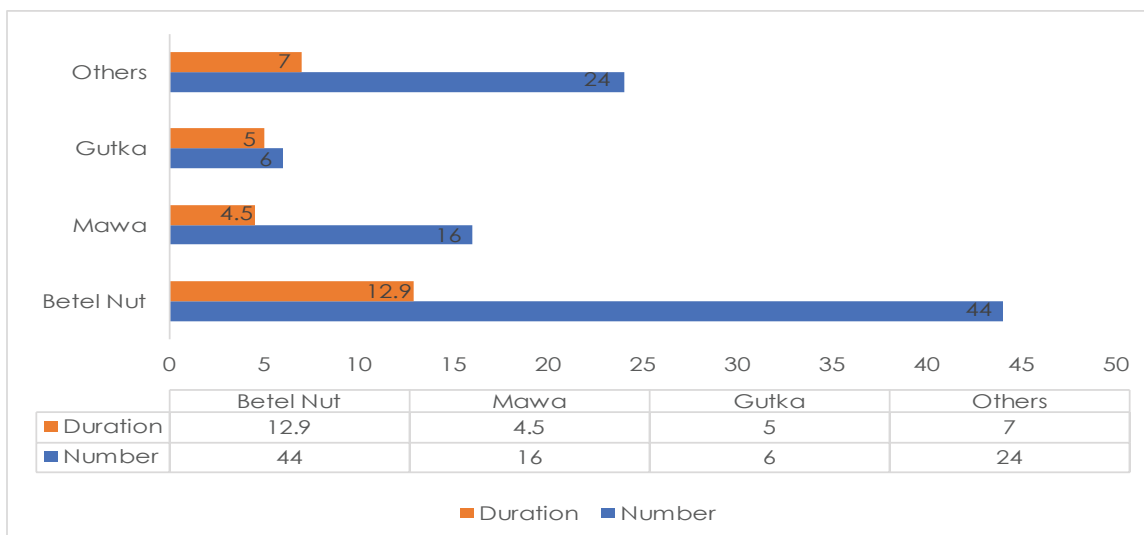


Figure 2: Duration (in years) and Total Number of Individuals Consuming Chewable Smokeless Tobacco (CSLT)

Table 1: Socio-demographic and Clinical Characteristics of Oral Submucous Fibrosis (OSF)

No. of Cases (90)	Age	Gender	Type of Tobacco	Duration	Location of Lesion
87	18-30	Male	Betel Nut	1-10 years	Buccal Mucosa
3	25 – 35	Female	Betel Nut	5- 10 years	Lips and Buccal Mucosa

Among all the diagnosed cases of OSF, male predominance (97%) was observed who were consuming Betel Nut for the past 10 years. Most commonly, the lesions were found on the Buccal mucosa, as mentioned in Table 1. On the contrary, only 3% (3) females reported to dental OPD with a lesion on the lip involving the buccal mucosa, as mentioned in Table 1.

Table 2: bFGF Levels in Controls and Cases

Groups	Minimum	Maximum	Mean	Standard Deviation	Odds ratio	P-value*
Controls	1.00	11.66	2.59	2.44	2.087	<0.001*
Cases	2.18	23.41	8.97	2.96		

*P < 0.05 was considered statistically significance. *Binary logistic regression was applied

Table 2 shows salivary levels of bFGF in cases and controls. The mean levels of bFGF in the saliva of controls were 2.59 ± 2.44 . Whereas mean levels in cases were observed to be 8.97 ± 2.96 , showing a positive correlation and significant variation ($P < 0.001$) between the values of bFGF in healthy and diseased patients as mentioned in Table 2.

DISCUSSION

In the present study, the salivary levels of bFGF were investigated. It was found that elevated levels of bFGF in patients with Oral submucous fibrosis who had been consuming betel nuts for the past 10 years. Several local and Indian studies have also shown similar findings confirming our results^{15,16,26}. In the current study, most of the affected individuals were males. As reported by other studies as well, the possible reason for this male predominance and addiction could be the easy approach to the marketplaces, lack of awareness, use of

areca-nut-based products by coworkers (peer pressure), or in the neighbourhood^{17,18}. In our study we have also observed that most of the individuals consuming betel nut, were younger at the time of diagnosis. Researchers considered involvement of youth in consumption of betel nut as quite alarming and suggested that this is because of easy accessibility, marketing initiatives, and impressive pricing modifications¹⁹. Researchers have also suggested that "Chewing areca nut is the main cause of oral submucous fibrosis that can progress to oral cancer", which indicates increased potential of

malignant transformation in OSF cases consuming betel nut²⁰.

In our study, we have observed a significant rise in the levels of bFGF in OSF patients consuming betel nut. One of the studies has also reported that elevated levels of bFGF can lead to tissue homeostasis, formation, growth, invasion, and metastasis of cancer²¹. Few other studies have also reported the potential role of bFGF in metastasis of lung cancer, bladder cancer, and breast cancer and advocated that bFGF attenuates cisplatin resistance²², leading to poor prognosis in patients²³. Researchers suggested that the bFGF produces inflammatory signals that link visceral adiposity and tumorigenesis²⁴. It was also observed in one of the studies that receptors of bFGF are a promising target for treating head and neck cancers due to alterations in its pathways²⁵. Researchers also concluded that bFGF plays significant roles and controls signaling pathways (MAPK, PI3K, and mTOR) that participate in the hallmarks of cancer²⁴. All these results support the findings of our study and suggest that any alteration in the salivary bFGF level indicates irreversible progression of potential pre-malignant disorders into malignancy.

The two important limitations of our study were related to the study design and sample size. As it was a case-control study, it was carried out at only one point in time, and the number of samples was also limited.

CONCLUSION

Salivary levels of bFGF were found to be elevated in cases with Oral Submucous Fibrosis hence bFGF can be used as a prognostic as well as a putative diagnostic marker for the malignant transformation of Oral Submucous Fibrosis.

LIST OF ABBREVIATIONS

PPOELS: Potentially premalignant Oral epithelial lesions

OSF: Oral Submucous Fibrosis

OPMD: Oral pre-malignant disorder

bFGF: Basic Fibroblast Growth Factor

CSLT: Chewable smokeless tobacco

CONFLICT OF INTEREST

None

FUNDING

None

ETHICAL APPROVAL

The study received ethical approval from the Ethical Review Committee of Ziauddin University, under reference number (0370718SSOM).

AUTHORS' CONTRIBUTIONS

AR, SB, and HB conceptualized the study, while **HB, FA, AR, and SB** handled data curation. **SSR and HB** conducted the formal analysis, and **SSR and SB** developed the methodology. Project administration was managed by **HB, FA, and SSQ**. **SSR** drafted the original manuscript, with **SSR, AR, SB, HB, FA, and SSQ** contributing to review and editing. All authors reviewed and approved the final manuscript.

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