SALIVARY EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR IN POTENTIALLY ORAL PRE-MALIGNANT EPITHELIAL LESIONS

Dr.Shafaq Saeed Roghay[1], Dr.Afifa Razi [2],Dr.Hira BAtool[1],Dr.Saima Butt [3], Dr.Zaeem Arif Abbasi[4],Dr.Fizza Abidi [3] ,Prof.Shoaib Khan [5]

Affiliations:

1) Department of Oral Biology, Ziauddin College of Dentistry, Ziauddin University, Karachi, Pakistan.
2) Department of Oral Medicine, Ziauddin College of Dentistry, Ziauddin University, Karachi, Pakistan.
3) Department of Oral Pathology, Ziauddin College of Dentistry, Ziauddin University, Karachi, Pakistan.
4) Department of Oral Pathology, Baqai Dental College, Baqai University, Karachi, Pakistan.
5) Department of Dental Materials, Ziauddin College of Dentistry, Ziauddin University, Karachi, Pakistan.

Corresponding Author:

Dr. Shafaq Saeed Roghay

Address: Department of Oral Biology, Ziauddin College of Dentistry, Ziauddin University, Karachi, Pakistan
Email: shafaq.saeed@zu.edu.pk

Abstract:

Introduction: Basic fibroblast growth factor (bFGF) - a cytokine that is sometimes also referred to as FGF2. It is identified as a strong factor for proliferation and migration of fibroblasts. It also promotes capillary formation and accelerates the tissue regeneration (1, 2). The objective of this research is estimation of levels of bFGF in saliva of healthy controls and in cases with potential oral premalignant epithelial lesions.

Objectives:

- To study the expression of salivary bFGF in patients with potential premalignant oral epithelial lesions.
- To study the levels of salivary bFGF expression with clinical characteristics in cases and controls.

Study design: Case - control

Materials and methods: This study includes 90 controls and 90 cases with potential pre-malignant oral epithelial lesions. Socio - demographic data was recorded for all the study participants using a questionnaire. An intraoral examination was also done and the daily frequency and total extent of tobacco usage (in years) was also recorded. Clinical parameters were evaluated for all the potential pre-malignant oral epithelial lesions and levels of salivary bFGF were investigated using ELISA technique.

Results: The saliva collected from all the cases indicated occurrence of bFGF. On analysis, significant association ($p < 0.0001$) was observed between salivary levels of bFGF in controls and diseased cases.

Conclusion: It can be concluded that elevated levels of bFGF can reflect the irreversible progression in potential pre-malignant oral epithelial lesions.

Keywords: Saliva, Basic Fibroblast Growth factor, Oral Sub mucous Fibrosis, Oral leukoplakia

Introduction

Potentially pre-malignant oral epithelial lesion (PPOEL) is defined as a "morphologically altered tissue in which oral cancer is more likely to occur than its apparently normal counterpart" (3, 4). It encompasses lesions that include erythroplakia, leukoplakia, erythroleukoplakia, oral submucous fibrosis and lichen planus (5, 6). The importance of oral premalignant lesions lies in its risk of transformation to OSCC (7). Cells of the oral cavity are immersed in salivary bio-fluid (8). When oral epithelium transforms, it sheds cells and molecules in to the salivary environment (Ni et al., 2015) which may alter the biochemical composition of salivary fluid and may represent an individual's health status (9).

Basic fibroblast growth factor (bFGF) is a protein-based biomarker, which is also termed as fibroblast growth factor-2(FGF-2) (8, 10). It is mainly formed by fibroblasts, smooth muscle cells, glial cells and endothelial cells and is widely distributed in different body tissues (1, 8, 11). However, its expression is found to be absent or detected at relatively low levels in normal tissues while it tends to be higher in tumor tissues (12).

bFGF promotes the formation of capillaries and accelerates the process of tissue regeneration by inducing active proliferation of fibroblasts and its migration (2, 8, 13). It also causes secretion of various proteolytic enzymes that leads to invasion and metastasis in malignant tumors (12, 14). The results of a study conducted in 2015, reported that increased expression of bFGF and its associated receptors plays an important role in transforming premalignant lesions to OSCC following various stages of epithelial dysplasia (15). Hence, it can be used as a marker for the recognition of potential change or disease progression in PPOELs.

The objective of the study was to evaluate and compare the levels of salivary bFGF in healthy individuals and cases with potential oral premalignant epithelial lesions.

Materials and Methods:

Study Setting:

This study was conducted in the Dental OPD of Ziauddin University as well as Abbasi Shaheed Hospital, Karachi from January 2019 to December 2019. Salivary samples collected from these centers were processed at Clinical laboratory, Ziauddin University, North Nazimabad, Karachi.

Sample size

An Overall of 180 participants were recruited for the study among which 90 were healthy individuals (controls) and 90 were those who presented with a potential pre-malignant lesion (cases).

Selection criteria

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 other immunosuppressive drug a week before the collection of salivary samples were excluded.

Sampling Technique

A non-probability consecutive sampling technique was used.

Data Collection Procedure

Unstimulated Whole saliva samples were collected early in the morning (8:00 a.m. till 11.00 a.m.) by using passive drool technique (16). This method of saliva collection largely reduces the risk of potential errors that may occur following other techniques. In addition, it also allows collecting large amount of saliva in a short period (17). The patients in this study were first instructed to rinse

the oral cavity with water in order 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. 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 revolutions 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 50nano meter. This procedure was carried out twice to check for the reproducibility of the results. Detection limit of the kit was set at 20 – 6000 nanogram per litre (ng/l).

Statistical analysis: Sample size calculation was done using Open Epi where the confidence interval was set at 95% and power of test was set at 80%. A non-probability consecutive type of sampling technique was employed. SPSS version 20 (Statistical Package for Social Sciences) was used for results analysis. Normality of the sample was checked using the Kolmogorov-smirnov test.

One way ANOVA and independent pooled t- test were 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 done to establish if there was any association between exposure and outcome.

Ethical Approval

Ethical approval was taken from the "Ethical review committee of Ziauddin University" (0370718SSOM.)

Results

A total of 180 individuals were included in this study among which 90 were healthy controls and 90 were cases with a potential diagnosis of pre-malignant oral epithelial lesions. Socio demographic data was gathered using a questionnaire from all the study participants. In addition to this, the total duration (in years) and daily frequency (per day) of tobacco consumption was recorded along with intra-oral examination. Oral hygiene practices were also recorded followed by evaluation of clinical parameters of potential pre-malignant oral epithelial lesions. Levels of salivary bFGF were investigated using the ELISA technique.

Among all the participants, 116 were males and 64 were females with a mean age of 32.08 ± 10.08 years for the cases and controls respectively. Out of 90 cases of potential pre-malignant oral epithelial lesions 78 patients (86.6 %) had oral sub mucous fibrosis (OSF), 7 subjects (7.7%) were diagnosed with oral leukoplakia (OL), 3 (3.3%) with Lichen Planus (LP) and 2 patients (2.2%) had Oral Erythroplakia (OE). A total of 58 lesions were pigmented, 18 were erythroplakic and 14 were leukoplakic. 44 (24.4%) patients were using betel nuts frequently while only 24 of them were pan users. 16 were mawa users and a very few were gutka users, gem and combined users of all of these.It was observed that affected individuals majorly consumed betel nut for 12.9 ± 5.099 years. Buccal mucsa was the most common site for these cases (38), followed by buccal mucosa along with tongue and lips because of position of placement CSLT. 40% lesions were single, whereas 11.1% of the lesions were multiple. A smooth surface texture was noted in 40 lesions, whereas 26 cases were found have an ulcerated and rough texture. 19 of 90 cases had regular margins whereas rest of them had an irregular margin. Most of the patients included in this study were diagnosed with OSF with a presenting complaint of limited mouth opening and burning sensation. On intra-oral examination showed the mean restricted mouth opening to be 16-18 mm with the presence of fibrous bands on the buccal mucosa anteriorly and posteriorly both.

Table 1: Details regarding the socio demographic and clinical characteristics of potentially pre-malignant oral epithelial lesions

Diagnosis	No. of cases (n = 90)	Age	Gender	Type of tobacco	Duration	Location of lesion
Oral Submucous Fibrosis (OSF)	78 (86.6%)	18 – 30	Male	Betel Nut	1 – 10 years	Buccal Mucosa
Oral leukoplakia (OLP)	7(7.7%)	40 – 50	Female	Betel quid	10 years	Buccal mucosa along with lips
Oral Lichen Planus (OL)	3 (3.3%)	22 25 28	Female	Betel Quid	6 months	Buccal mucosa
Oral Erythroplakia	2 (2.2%)	53 57	Male	Betel quid, gutka, Mainpuri	>10 years	Soft Palate

Table 2: bFGF Levels in controls and cases

	Minimum	Maximum	Mean	Standard deviation	Odds ratio	P-value*
Controls	1.00	11.66	2.59	2.44	2.087	<0.0001*
Cases	2.18	23.41	8.97	2.96		

*P < 0.05 was considered statistically significance

*Binary logistic regression was applied

Graph 2: Standard Curve showing bFGF levels in Controls and Cases
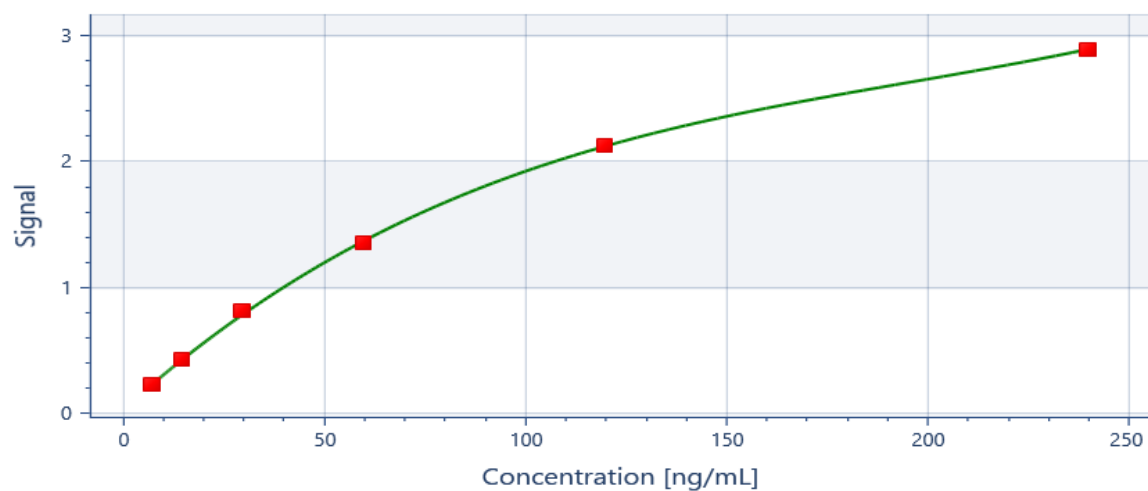
Graph showing the levels of bFGF in which ▇ represent standard

Discussion

The findings of the present study showed statistically significant higher expression of bFGF in PPOELs cases as compared to healthy controls along with a positive correlation between the PPOELs levels and the clinical parameters assessed in this study as reported in table 2. It also showed early signs of carcinogenic damage to oral mucosa, which usually appears as white patches with or without red patches (18). In the present study, out of 90 cases, 78 were diagnosed with OSF belonging to the age range of 18 – 30 years as mentioned in table 1. All the affected individuals were betel nut consumers and presented at an earlier age at the time of diagnosis. It has been reported in literature that OSF is caused by chewing betel quid and *gutka*, which supports the findings of our study (19, 20).

In this study, we have also observed a few cases of leukoplakia (n=7), lichen planus (n=3) and erythroplakia (n=2). Betel quid was the most commonly used habit among patients with oral leukoplakia between the age ranges of 40-50 years as mentioned in table 1. Various studies reported in literature have shown similar age range, which supports the findings of our research (12, 21). A study conducted by Liu et al in 2010 and the results of our study showed female predilection however male preponderance was reported in a study conducted by Lee in 2003.

In this study, 03 patients who presented with oral lichen planus were included belonging to the age range of 18-28 years. These patients also gave history of betel quid chewing as mentioned in table 1. In these patients, reticular lichen planus was observed to be the predominantly found pattern over the buccal mucosa. Literature review shows that these lesions are usually seen at the site of placement of the quid. Regression of such lesions may occur if the patient decreases the consumption of habits or alters the site of placement of

quid. The lesion may also disappear completely if the patients stops using the habit of eating

betel nut (22, 23).

In our study, only 02 patients with erythroplakia were included. Both of these patients were males and between the ages of 50 - 60 years. Yang et al and Mathew et al., have reported similar findings in their study regarding the age and gender of the patients (24, 25).Patients with erythroplakia had the habit of eating pan, gutka and Mainpuri for last 10 to 20 years. The lesions were most commonly found on soft palate as mentioned in table 1.

In the present study, the role of salivary bFGF was investigated. It was presumed that the salivary fibroblast growth factor is overexpressed in PPOELs, which indicates increased potential of malignant transformation in such lesions (26, 27). ELISA technique was used to measure the baseline level of bFGF in healthy individuals, which was then used to compare the levels of bFGF in patients with PPOELs. The result of comparison showed a positive correlation between the values of bFGF in healthy and diseased patients. The salivary levels of bFGF also showed a rising trend and significant variation between the healthy individuals and patients having PPOELs (P<0.0001). In our study, linear increase in salivary bFGF levels was observed between healthy and diseased individuals as shown in graph 2. Man Liu in 2017, suggested the involvement of bFGF in the formation, growth and invasion of different types of tumors which includes breast cancer, prostate cancer and lung cancer etc. It was also observed in their study that the bFGF levels was low in individuals who were healthy while it was overexpressed in the affected tissues (12). Their results support the findings of our study and suggests that any alteration in the salivary bFGF level indicates irreversible progression of PPOELs 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 so it was carried out at only one point in time and the number of samples were also limited. In addition, we were unable to measure the disease progression in PPOELs because the patients were not followed up in our study.

Conclusion

Salivary levels of bFGF were found to be elevated in PPOELs hence bFGF can be used as a prognostic as well as a putative diagnostic marker for the malignant transformation of potential pre malignant oral epithelial lesion.

Acknowledgements

Conflict of interest

There was no conflict of interest to declare

Grant support & financial disclosures

References

1.   Hase T, Kawashiri S, Tanaka A, Nozaki S, Noguchi N, Kato K, et al. Correlation of basic fibroblast growth factor expression with the invasion and the prognosis of oral squamous cell carcinoma. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2006;35(3):136-9.

2.   Hiwatashi N, Hirano S, Mizuta M, Kobayashi T, Kawai Y, Kanemaru SI, et al. The efficacy of a novel collagen-gelatin scaffold with basic fibroblast growth factor for the treatment of vocal fold scar. Journal of tissue engineering and regenerative medicine. 2017;11(5):1598-609.

3.   Arlen D Meyers M, MBA. Premalignant Conditions of the Oral Cavity emedicine.medscape.com 2018

4.   Porter S, Gueiros LA, Leão JC, Fedele S. Risk factors and etiopathogenesis of potentially premalignant oral epithelial lesions. Oral surgery, oral medicine, oral pathology and oral radiology. 2018;125(6):603-11.

5.   Awadallah M, Idle M, Patel K, Kademani D. Management update of potentially premalignant oral epithelial lesions. Oral surgery, oral medicine, oral pathology and oral radiology. 2018;125(6):628-36.

6.   Parakh MK, Ulaganambi S, Ashifa N, Premkumar R, Jain AL. Oral potentially malignant disorders: clinical diagnosis and current screening aids: a narrative review. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP). 2020;29(1):65-72.

7.   N.Nikolaos G PM, G. Maria , Catherine F. DEP, DMD, PhD, FDS, RCSEd,d, Edwards P. Molecular markers associated with development and progression of potentially premalignant oral epithelial lesions: Current knowledge and future implications. ORAL AND MAXILLOFACIAL PATHOLOGY. 2018;125(6):650-69.

8.   Gupta A, Tripathi A, Patil R, Kumar V, Khanna V, Singh V. Estimation of salivary and serum basic fibroblast growth factor in treated and untreated patients with oral squamous cell carcinoma. Journal of oral biology and craniofacial research. 2019;9(1):19-23.

9.   Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DT. Salivary biomarkers: toward future clinical and diagnostic utilities. Clinical microbiology reviews. 2013;26(4):781-91.

10.   Farooq M, Khan AW, Kim MS, Choi S. The role of fibroblast growth factor (FGF) signaling in tissue repair and regeneration. Cells. 2021;10(11):3242.

11.   Zittermann SI, Issekutz AC. Basic fibroblast growth factor (bFGF, FGF-2) potentiates leukocyte recruitment to inflammation by enhancing endothelial adhesion molecule expression. The American journal of pathology. 2006;168(3):835-46.

12.   Liu M, Xing L-Q. Basic fibroblast growth factor as a potential biomarker for diagnosing malignant tumor metastasis in women. Oncology letters. 2017;14(2):1561-7.

13.   Hase T, Kawashiri S, Tanaka A, Nozaki S, Noguchi N, Kato K, et al. Fibroblast Growth Factor-2 Accelerates Invasion of Oral Squamous Cell Carcinoma. Oral Science International. 2006;3(1):1-9.

14.   Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. Cellular and Molecular Life Sciences. 2020;77(9):1745-70.

15.   Nayak S, Goel MM, Makker A, Bhatia V, Chandra S, Kumar S, et al. Fibroblast Growth Factor (FGF-2) and Its Receptors FGFR-2 and FGFR-3 May Be Putative Biomarkers of Malignant Transformation of Potentially Malignant Oral Lesions into Oral Squamous Cell Carcinoma. PLoS One. 2015;10(10):e0138801.

16.   Yakob M, Fuentes L, Wang MB, Abemayor E, Wong DT. Salivary biomarkers for detection of oral squamous cell carcinoma - current state and recent advances. Current oral health reports. 2014;1(2):133-41.

17.   Bhattarai KR, Kim H-R, Chae H-J. Compliance with saliva collection protocol in healthy volunteers: strategies for managing risk and errors. International journal of medical sciences. 2018;15(8):823.

18.   Rao AK, Parameswar P, Majumdar S, Uppala D, Kotina S, Vennamaneni NH. Evaluation of Genetic Polymorphisms in Glutathione S-Transferase Theta1, Glutathione S-Transferase Mu1, and Glutathione S-Transferase Mu3 in Oral Leukoplakia and Oral Squamous Cell Carcinoma with Deleterious Habits using Polymerase Chain Reaction. International journal of applied & basic medical research. 2017;7(3):181-5.

19.   Niaz K, Maqbool F, Khan F, Bahadar H, Ismail Hassan F, Abdollahi M. Smokeless tobacco (paan and gutkha) consumption, prevalence, and contribution to oral cancer. Epidemiology and health. 2017;39:e2017009.

20.   Raffat MA, Hadi NI, Hosein M, Zubairi AM, Ikram S, Akram Z. Differential expression of salivary S100A7 in oral submucous fibrosis. The Saudi dental journal. 2019;31(1):39-44.

21.   Lee CH, Ko YC, Huang HL, Chao YY, Tsai CC, Shieh TY, et al. The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. British Journal of Cancer. 2003;88(3):366-72.

22.   Singh JSS. Evaluation of prevalence of oral lichen planus in a known population: A cross-sectional study International Journal of Applied Dental Sciences. 2018;4(1):136-13.

23.   Anand R, Dhingra C, Prasad S, Menon I. Betel nut chewing and its deleterious effects on oral cavity. Journal of cancer research and therapeutics. 2014;10(3):499-505.

24.   Yang S-W, Lee Y-S, Chang L-C, Hwang C-C, Luo C-M, Chen T-A. Clinical characteristics of narrow-band imaging of oral erythroplakia and its correlation with pathology. BMC cancer. 2015;15:406-.

25.   Mathew AL, Daniel MP, Cherian SA. Prevalence of oral precancer and cancer in south kerala population. Journal of Oral Diagnosis. 2017;2(1):1-4.

26.   Kosaka PM, Pini V, Ruz JJ, da Silva RA, González MU, Ramos D, et al. Detection of cancer biomarkers in serum using a hybrid mechanical and optoplasmonic nanosensor. Nature nanotechnology. 2014;9(12):1047-53.

27.   Jia Q, Meng Z, Xu K, He X, Tan J, Zhang G, et al. Serum midkine as a surrogate biomarker for metastatic prediction in differentiated thyroid cancer patients with positive thyroglobulin antibody. Scientific reports. 2017;7:43516.