



## Original Article

## FGFR1 Gene Expression and its Relationship with Sociodemographic and Clinicopathological Indices in Oral Squamous Cell Carcinoma

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### Abstract

**Introduction:** A better prognosis has resulted from the use of molecular techniques in cancer diagnosis. The FGFR family of genes is one of the major gene families involved in the carcinogenic pathways of different malignancies. Tumorigenesis, uncontrolled cell proliferation, and a number of pathologic diseases are linked to improper FGFR activation. The family of genes known as FGFRs encodes cell surface membrane receptors with tyrosine kinases. The development of oral squamous cell carcinoma (OSCC) may be significantly influenced by fibroblast growth factor receptor 1 (FGFR1). Analyzing these elements' expression patterns may provide fresh perspectives on illness management strategies including genetic mediated target therapy.

**Objectives:** In order to improve care and prevent future difficulties from oral squamous cell carcinoma (OSCC), the current study sought to assess the expression of FGFR1 genes in OSCC tumors as a result of the discovery of biomarkers and early diagnosis.

**Methods:** In order to assess the expression of FGFR1 genes in 16 OSCC samples, 16 normal specimens from the same sample, free from cancer margin and 4 control samples from other patient free from cancer, the Oral and Maxillofacial Surgery Department at Dental Unit of Rajshahi Medical College Hospital was collected. Trizol and the appropriate kits were used for RNA extraction and cDNA synthesis. Real-time PCR was used to assess the FGFR1 gene's expression following the construction of a specific primer set in order to find and validate molecular biomarkers. The data is analyzed using the ANOVA and independent t-test. Statistics were shown to be significant when  $P < 0.05$ .

**Results:** According to the findings, there are notable variations in the expression of the FGFR1 gene between tumor and normal or control tissues ( $P < 0.001$ ). Patients over 60 are more likely to have OSCC, and the majority of them smoke. Retromolar trigon 8 is the most frequent location, and the majority of them are in stages III and IV. The FGFR1 gene expression does not significantly differ according to a patient's age, gender, religion and behavior, including whether they smoke, drink, or chew paan. It also does not differ depending on the tumor's location, stages and grade.

**Conclusions:** The FGFR1 gene was often expressed differently in cancerous and control tissues, confirming the gene's involvement in OSCC. The expression of FGFR1 is not correlated with factors such as gender, age, history of smoking, alcohol intake, chewing paan, tumor site, degree of differentiation, or TNM stage. The current investigation demonstrated the critical function of the investigated gene in the diagnosis of OSCC. To verify this, though, more research is required.

**Key words:** Oral Squamous Cell, Gene expression, Real-time PCR, Biomarker.

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### 1. Introduction

Across the globe, oral cancer ranks sixth in frequency and is more common in South Asia than any other region.<sup>1</sup> Among the most significant

etiologial factors for oral cancer are alcohol and tobacco use, and HPV infection has recently been linked to the disease.<sup>2</sup> Poor oral hygiene, nutritional deficiency, and certain chronic

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infections caused by bacteria, viruses, or fungi are additional risk factors.<sup>3,4</sup> Squamous cell carcinomas, which affect the mouth and lips and account for 90% of oral cancer cases, killed 146,000 people in 2015.<sup>4</sup> 1.55% of patients with oral cavity squamous cell carcinoma have altered FGFR1 gene.<sup>5</sup> According to epidemiological studies, oncogenes which are triggered by DNA mutations are the primary risk factors for oral cancer.<sup>6</sup>

Fibroblast growth factor receptor 1 (FGFR1) is a transmembrane polypeptide tyrosine kinase that produces distinct isoforms as a result of alternative splicing. It is a member of the fibroblast growth factor (FGF) receptor family. In this experiment, the specific ligand FGF dimerizes the expression of FGFR1, which then undergoes autophosphorylation to initiate a cascade that activates downstream channel conduction and contributes to various processes such as embryonic development, cell differentiation, nerve regeneration, wound healing, and others. Disease can be brought on by abnormal signaling, which is also intimately linked to multiple tumorigenesis. Rarely was FGFR1 investigated in relation to oral cancers, but it was extensively studied in relation to lung cancer,<sup>7</sup> bladder cancer,<sup>8</sup> breast cancer,<sup>9</sup> and interstitial sarcoma.<sup>10</sup> In order to shed light on the pathophysiology of OSCC and to develop novel concepts for gene and molecular targeted therapy, this study looked at the expression of FGFR1 and its clinical significance.

Nevertheless, an examination of the literature pieces showed that, as of now, our nation has not seen any reports regarding the involvement of the FGFR1 gene in oral squamous cell carcinoma (OSCC).

## 2. Objectives

In order to understand the role of the gene in the diagnosis of oral squamous cell carcinoma and its relationship with sociodemographic and clinicopathological parameters, the current study set out to investigate changes in the expression of the FGFR1 gene in squamous cell carcinoma to establish FGFR1 as a biomarker.

## 3. Materials and Methods

### Common Clinical Information

From September 2020 to January 2022, patients undergoing surgery in the Oral and Maxillofacial Surgery Department of the Dental Unit at Rajshahi Medical College Hospital provided 16 cases of OSCC tissue and 16 cases of adjacent normal mucosa tissue. Eight cases involved the retromolar trigon region; four of them from alveolar ridge area, two of them on lateral border of tongue, and two of buccal mucosa; in all cases, the adjacent normal tissues were more than 0.5 centimeters away from the tumor margin. Four additional patient control tissues from the extraction of an impacted third molar were prepared independently. Table 1 displays the overall clinicopathological and sociodemographic features. Using the eighth edition of the oral squamous cell carcinoma staging criteria, tumor-node-metastasis (TNM) staging was applied. The Rajshahi University Ethics Committee approved the experiment (MemoNo: 249(35)/ 320/ IAMEBBC/IBSc), and each patient signed an informed consent form.

### Qualities for inclusion

(I) The pathology department at Rajshahi Medical College Hospital used BIOPSY to confirm that the oral cancer tissues were OSCC, while adjacent normal tissues and benign oral mucosa lesions were classified as either inflammatory lesions or normal oral mucosa;

(II) All patient medical records' data remained complete.

### Criteria for exclusion

(I) It was unknown what the patient's pathological and histological diagnosis results were;

(II) Preoperative history of radiation, chemical, or other related treatment was present in any of the cancer patients excluded in the study;

(III) Patient excluded in the study had a history of other malignancies.

### 3.1. Readyng the Sample

16 samples of oral squamous cell carcinoma, 16 normal tissues 0.5 centimeters away from the tumor's margin, and 4 control tissues from the extraction of an impacted third molar were taken from patients who had oral and maxillofacial surgery performed under the guidance and consent of an expert at the oral and maxillofacial surgery department of Rajshahi Medical Collage Hospital's Dental Unit.

According to the consent form of the oral and maxillofacial surgery department in the Dental Unit of Rajshahi Medical Collage Hospital, written consent was obtained from all patients whose tissue samples were used in this investigation. The IBSc, University of Rajshahi ethics committee has given its approval for the current study.

### 3.2. Extraction of RNA and Synthesis of cDNA

Trizol (Sigma Aldrich, USA) was used to extract RNA. Following RNA extraction, nanodrop (Thermo Scientific, USA) and electrophoresis (Biorad, USA) were used to assess the amount and quality of RNA, respectively. Next, the manufacturer's instructions were followed to perform cDNA synthesis using a Takara, Japan, cDNA synthesis kit.

## 4. Results

To the best of our knowledge, the present study is the first study to examine the expression of FGFR1 in OSCC cancer, and the results emphasized its oncogenic role in OSCC. In the present study, high expression of this gene was observed in OSCC tumor cells compared to normal cells. FGFR1 gene expression was normalized to the housekeeping  $\beta$ -actin gene.

### 4.1. Clinicopathological Features

Total patient taken for BIOPSY is about 20 patients but only 16 patients are positive for OSCC in BIOPSY. So, our total sample size is 16 in number, most of them are over age 60 years (62.5%), most of them are male 9 (56.25%) and most of them are Muslim 12 (75%). Among the male patient most of them are smokers 8 (50%) and among the female patient most of them are paan chewer 11 (68.75%).

Most common site is retromolar trigon 8 (50%) and alveolar ridge 4 (25%) and less common site is lip, floor of the mouth and hard palate. Most of them are poorly differentiated 8 (50%) and in stage III 6 (37.5%) and IV 9 (56.25%). (Table 1).

### 3.3. Primer Design

Using GenRunner and Primer 3 software, unique primer was created for FGFR1 gene in order to examine the expression of the gene in OSCC and normal tissues. oligo Analyzer and BLAST, respectively, carried out the primer's analysis in terms of optimal features and specificity.

### 3.4. PCR in real time

The real-time PCR method with SYBER Green (Takara, Japan) and the real-time PCR instrument (Applied Biosystem, ThermoFisher, USA) were used to examine the changes in gene expression at the molecular level. 5  $\mu$ L SYBER Green, 3  $\mu$ L nuclease-free water, 1  $\mu$ L cDNA, and 1  $\mu$ L primer mix (R + F) were added to the reaction mixture. In the end, the expression of the genes was normalized to that of  $\beta$ -actin.

### 3.5. Analysis of Statistics

The potential of the investigated genes as biomarkers was ascertained through the analysis of gene expression data. ANOVA was used by Prism 8 software to evaluate the data and their relationship at a significant level  $< 0.05$ . The data's normality was assessed using the Shapiro-Wilk normality test. The value of the count data was recorded in N%.

Table 1. Sociodemographic and clinic pathological Features of Oral Squamous Cell Carcinoma Patient

Patient's details	Total no. of patient (n)		16	Percentage, 100 %	
	Age	≥60	6	37.5	
		<60	10	62.5	
	Gender	Male	9	56.25	
		Female	7	43.75	
	Ethnicity or religion	Muslim	12	75	
		Hindu	4	25	
		Others	0	0	
	Risk factors or habits	Cigarette Smoking			
		Yes	8	50	
		No	8	50	
		Alcohol drinking			
		Yes	2	12.5	
No		14	87.5		
Paan chewing					
Yes		11	68.75		
No		5	31.25		
Tumour details	Primary		16	100	
	Recurrent at 2 years		0	0	
	Site of primary tumour	Tongue		2	12.5
		Floor of the mouth		0	0
		Lip		0	0
		Buccal mucosa		2	12.5
		Alveolar ridge		4	25
		Retromolar trigon		8	50
		Hard palate		0	0
	Degree of differentiation	Well differentiated		2	12.5
		Moderately differentiated		6	37.5
		Poorly differentiated		8	50
	TNM staging	I		0	0
II		1	6.25		
III		6	37.5		
IV		9	56.25		

Values are expressed as No. (%) unless otherwise indicated.

#### 4.2. Evaluation of Changes in Genes Expression and biomarker potential of FGFR1 gene

FGFR1 gene had a significantly different expression compared with normal. FGFR1 gene showed a significant increase in expression in tumor samples compared to tumor margin samples (normal) and control samples (Figure 1 A, B, C and D).

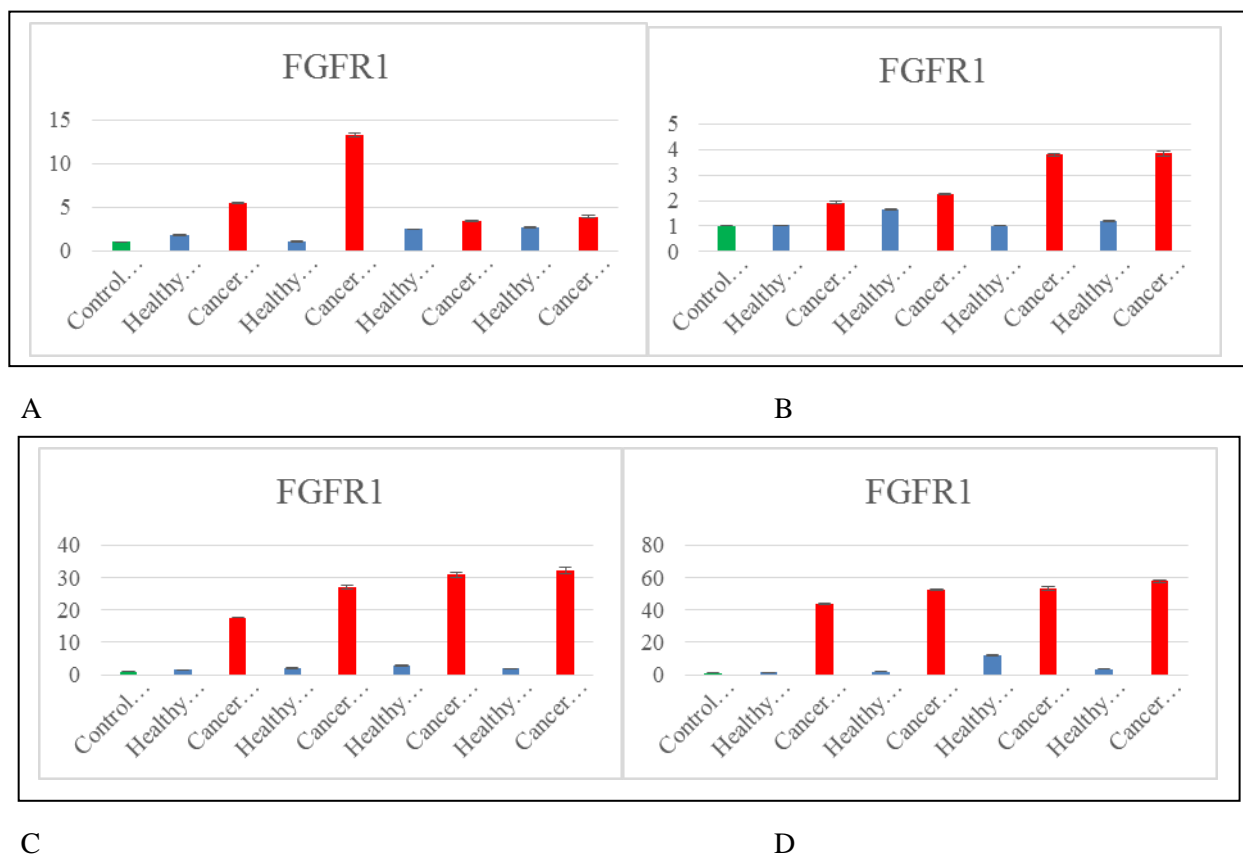


Figure 1. A, B, C and D showed a significant increase in expression of FGFR1 gene in tumor samples compared to tumor margin samples (normal) and control tissue.

#### 4.3. The Relation of FGFR1 Expression with Sociodemographic and Clinicopathological Features

##### 4.3.1. Age, Gender and Religion

There were no significant differences in the expression of FGFR1 gene in different age, gender and religion in patients with OCSS. As a result, it seems FGFR1 cannot be valuable biomarkers for diagnosis based on age, gender and religion. The overexpression of the studied gene in patients with OCSS of different ages, gender and religion showed no significant relation with in the overexpression of the FGFR1 gene and sociodemographic variables (Figure 2).

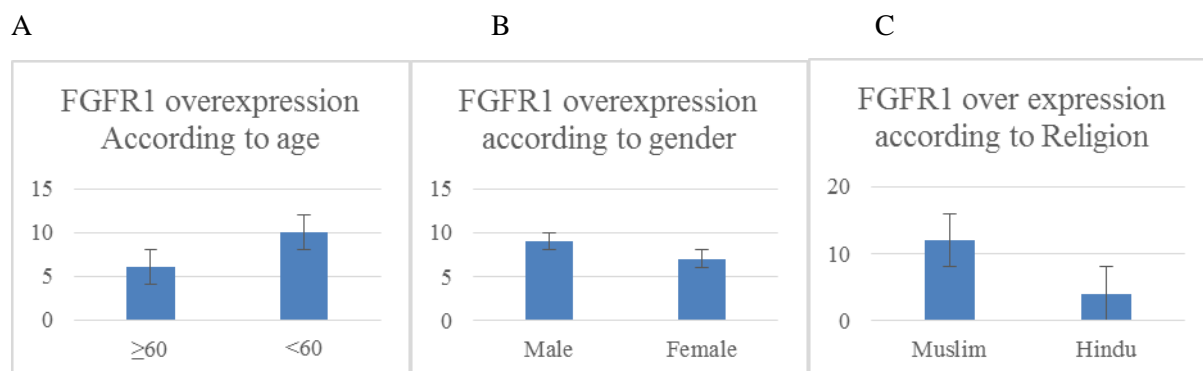


Figure 2. FGFR1 gene expression A, in terms of patients' age, B, in terms of patients' gender, and C, in terms of patients' religion.

#### 4.3.2. Site, Grade and Stage of the tumour

There were no significant differences in the expression of FGFR1 gene in different site, grade and stage of the tumour in patients with OCSS. As a result, it seems FGFR1 cannot be valuable biomarkers for diagnosis based on site, grade and stage of the tumour. The overexpression of the studied gene in patients with OCSS of different site, grade and stage of the tumour showed no significant relation with in the overexpression of the FGFR1 gene and clinicopathological variables (Figure 3).

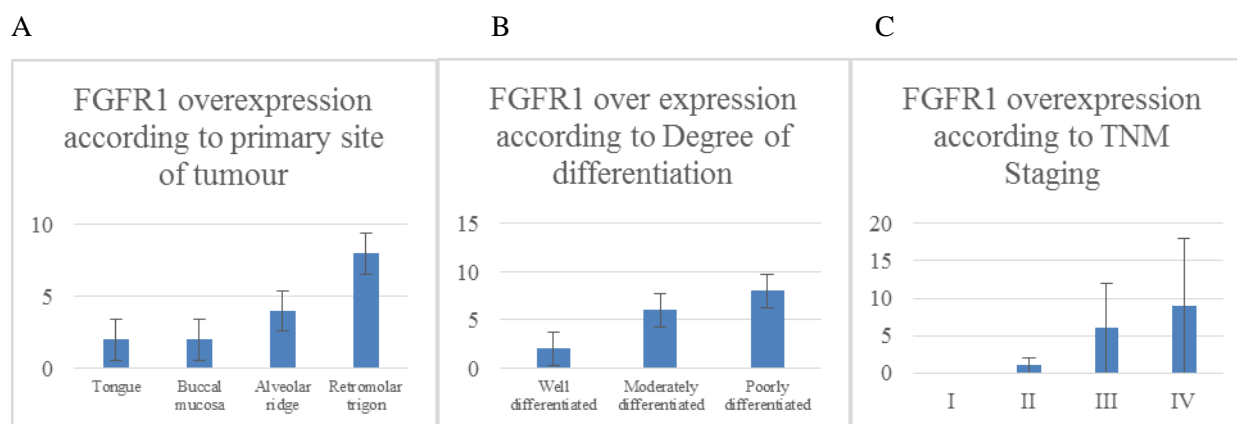


Figure 3. A, FGFR1 gene expression in terms of tumors' primary site, B, FGFR1 gene expression in terms of tumors' degree of differentiation and C, FGFR1 gene expression in terms of tumors' TNM stage

In general, OSCC tissues had a significant difference in the expression of the studied gene compared to normal tissue. Meanwhile, no association was found between the expression of this gene with sociodemographic and clinicopathological variables. The results showed that the FGFR1 gene has the potential to be used in the diagnosis of OSCC as biomarkers.

## 5. Discussion

The precise mechanism of OSCC is still unknown, despite the rapid advancements in tumor molecular biology techniques and OSCC metastasis. This is true of other neoplastic diseases as well.

Consequently, the pathological factors associated with oral cancer and its molecular mechanism serve as a foundation for subsequent immunotargeted therapies.

Over the past ten years, FGFR1 research has expanded, and different clinical characteristics

have been actively sought by examining the expression of FGFR1 using qPCR in a variety of malignant tumors. Oral SCC,<sup>11</sup> esophageal SCC,<sup>12,13</sup> lung cancer,<sup>14-16</sup> breast cancer,<sup>17,18</sup> and pancreatic cancer<sup>19</sup> have all been reported to have abnormal alteration or overexpression of FGFR1 proteins. A strong correlation has been noted between high expression status and a poor prognosis of patients. Initial results have been obtained with the use of FGFR inhibitors in the treatment of urothelial malignancies and cholangiocarcinoma,<sup>20</sup> while FGFR1 overexpression has been detected in head and neck cancer (HNSCC),<sup>21</sup> and it has been linked to a poor prognosis. Furthermore, FGFR1 was linked to a poor prognosis in HNSCC patients who did not have human papillomavirus (HPV), but not in those who did.<sup>22</sup> Furthermore, there was no or little expression in highly differentiated squamous carcinoma and high expression in low differentiation carcinoma, indicating a relationship between the overall degree of expression and tumor differentiation.<sup>23</sup> According to a recent study by Starska et al., FGFR1 activated tumor cell regeneration and vascular appreciation by activating the downstream PI3K/AKT kinase pathway. They also found that high expression levels were linked to increased tumor invasion rate, tumor lymph node metastasis, tumor recurrence, and poor patient prognosis in 137 surgically removed laryngeal SCC patients.<sup>24</sup>

The prognosis of oral cancer is determined by a number of independent factors, including the clinical stage, surgical margin, and systemic condition. These findings highlight the significance of early diagnosis, prompt treatment, a negative surgical margin, and a patient's good systemic condition.<sup>25</sup> FGFR1 may be a prognostic biomarker in HNSCC patients, particularly in HPV-negative patients, according to Dubot et al.'s analysis of deleterious genes recovered from 122 patients who had undergone primary surgery.<sup>26</sup> More research is required because earlier investigations have connected FGFR to a number of clinical characteristics, including the tumor's TNM stage, lymph node metastasis, and recurrence rate.<sup>23,24,27</sup>

## Conclusion

To the best of our knowledge, this study is the first to look at FGFR1 expression in OSCC, and the findings highlighted the protein's carcinogenic role in OSCC. FGFR1's function in carcinogenesis appears to vary depending on the kind of tumor. According to the study's findings, the FGFR1 gene is overexpressed in tumor samples, suggesting that it may be a useful biomarker for OSCC diagnosis. These results are consistent with the ongoing investigation. When compared to normal tissue, OSCC tissues generally showed a significant difference in the expression of the investigated gene.

To confirm whether increased FGFR1 is strongly associated with the incidence of oral cancer and can be further examined as an oncogenic factor, more research is necessary. A larger sample size is needed to fully understand the genetics and functional aspects of oral cancer in order to establish a foundation for early detection and screening. This experiment only included a small number of specimens.

**Conflict of interest:** None declared

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