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Expression Analysis of Transforming Growth Factor-Beta (TGF-β) in Oral Squamous Cell Carcinoma

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Research Article

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) is the most common cancer in the head and neck region. More than 45% of the cancer cases in India are diagnosed as squamous cell carcinoma. Although much progress has been made to control OSCC in recent years, the management or treatment option for OSCC remains challenging. Interestingly, Transforming Growth Factor (TGF-β) has a significant role in the extracellular microenvironment and variety of cellular processes such as cell proliferation, differentiation, apoptosis and migration. Thus, the aim of this study is to evaluate the levels of TGF-β in OSCC patients and their adjacent normal tissue.

Materials and Method: A total of 20 OSCC and adjacent normal tissues were collected from the department of oral and maxillofacial surgery, Saveetha dental college and hospitals. The tissues were processed for hematoxylin and eosin staining and expression studies. The data were shown as mean ± standard deviation and $p<0.05^*$ was statistically significant.

Results and Conclusion: Our histological observation findings showed that there was epithelial cell injury in the OSCC samples, which contributed to the creation of more keratin pearls. Additionally, our findings showed that as compared to normal tissue, OSCC samples had significantly increased TGF-β expression levels. As a result, we conclude that TGF-β was found to be an important oncogene in the tumour growth and metastasis of various cancer, including OSCC. Thus, inhibition of TGF-β can be postulated to be a potential treatment against OSCC.

Keywords: Oral squamous cell carcinoma; TGF-β; Hematoxylin and eosin staining; Gene expression; Treatment

INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is a prominent oral cancer that accounts for almost 90% of oral malignancies and has the ability to influence the immune system *via* direct or indirect suppression. Tobacco, alcohol consumption, Human Papilloma Virus (HPV) demographic/clinical and histopathological variables such as stage, grade may all play role in cancer prognosis. High recurrence rates despite several treatments suggest that existing therapies and prognostic indicators are ineffective. These demand for the discovery of novel diagnostic, prognostic, and perhaps therapeutic markers for SCC.

Interestingly, Transforming Growth Factor (TGF-β) has a significant role in the extracellular microenvironment and variety of cellular processes such as cell proliferation, differentiation, apoptosis and migration. Recent reports have suggested that TGF-β acts as a tumour suppressor in normal epithelial cells and in the early stages of cancer development. However, as a tumour grows, TGF-β becomes a potent tumour promoter in the epithelium and even boosts TGF-β synthesis, promoting tumour development and metastasis. The interaction of tumour cells and the immune system is critical in carcinogenesis, and better understanding of dysregulated pathways may lead to the identification of new targets. As a result, the diagnostic and prognostic roles of biomarkers must be evaluated. Thus, the aim of the present study is to evaluated the expression levels of TGF-β in OSCC of south Indian population [1].

In this study, the expression analysis of TGF-β were determined in the participants with OSCC tissues and their adjacent normal tissues. It was observed that TGF-β levels were significantly higher in the OSCC tissues when compared to the normal tissues. Thus, studying the expression levels of TGF-β helped in understanding the onset and progression of OSCC [2].

MATERIALS AND METHODS

Sample collection

20 malignant tissues and their adjacent healthy tissues from patient with OSCC were collected from the department of oral and maxillofacial surgery of Saveetha Dental College and Hospitals (SDCH), Chennai, India. The institutional ethical committee of SDCH approved the study execution. Before the sample was processed, each subject gave their signed informed consent. The samples were kept at -80°C for further analysis [3].

Inclusion and exclusion criteria

Participants with squamous cell carcinoma and tissue sub site (buccal mucosa) were chosen from those who met the following criteria: >18 years of age; ability to give informed permission. Participants having any of the following medical disorders, such as renal failure, uncontrolled hypertension, tongue carcinoma on the border, or any other active cancers, were disqualified from the study [4].

Histopathological analysis

Before being embedded in paraffin, samples of malignant tissues were put in 10% formaldehyde. Hematoxylin and eosin (H and E) was used to stain the paraffin embedded tissues, which were divided into sections with a thickness of 5 µm. Following that, a light microscope was used to analyse the tissue samples [5].

RNA isolation and quantitative real time PCR

Total RNA was extracted from cancerous and normal tissues using TRIzol reagent (Invitrogen, Carlsbad, USA), as directed by the manufacturer. The nano drop 2000 lite spectrophotometer (Thermo Fisher Scientific, Waltham, US) was used to determine the concentration and purity of the isolated RNA. Using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, total RNA was reverse transcribed into complementary DNA (cDNA) in a total volume of 10 µl. The reaction mixture is incubated in PCR (MiniAmp plus thermal cycler, Thermo Fisher) with 30°C for 10 mins, 42°C for 30 mins, 95°C for 5 mins, and final incubation at 4°C. The cDNA derived is quantified in nano drop lite and stored at -20°C until further analysis. The cDNA obtained is subjected to expression studies using SYBR Green (Takara, Japan) for the gene TGF-β and GAPDH was used as a housekeeping control gene. The primer sequence for TGF-β (Fwd- 5'-TACCTGAACCCGTGTTGCTCTC-3'; Rev-5'-GTTGCTGAGGTATCGCCAGGAA-3') and GAPDH (Fwd- 5'-GTCTCCTCTGACTTCAACAGCG-3'; Rev- 5' ACCACCCTGTTGCTGTAGCCAA-3'). The following thermo cycling settings were used to amplify all the samples in duplicate: Denaturation for 30 seconds at 95°C, followed by 40 cycles at 95°C for 5 sec and 60°C for 30 sec. Finally, the expression levels of TGF-β were calculated by using the 2-^^Cq method [6].

Statistical analysis

The data were shown as mean±Standard Deviation (SD). The difference in the level of TGF-β in cancerous and adjacent normal tissues was compared using the student's t-test. $P<0.05$ was shown as statistically significant (*).

RESULTS

Clinical characterization of participants

According to our research data, only 16 participates were selected the study participants included 13 men and 3 women with mean ages ranging from 35 to 60. There were 11 individuals who chewed pan and gutka, 6 smokers, and 8 drinkers. According to tumour staging, 9 participants had T1, T2 stage tumours, while 7 people had T3 and T4 stage tumours. The histological grades of well and moderately differentiated squamous cell carcinoma were 8 each. Interestingly, study showed 4 non-habits cases who had OSCC. Table 1 represents the clinical information about the individuals [7].

Histopathological analysis of OSCC and normal tissue

Damaged epithelial cells were visible in the OSCC sections. The nucleus and cytoplasmic organelles disappeared during keratin synthesis, resulting in programmed cell death and fast tumour cell growth. The histopathological examination of well differentiated squamous cell carcinoma is shown in the Figure 1.

TGF-β expression in OSCC and normal tissue

The expression level of TGF-β is represented in the Figure 2. Our results have revealed that expression levels of TGF-β were found to be significantly ($p<0.05$*) up regulated in the OSCC samples when compared to their adjacent normal tissue [8].

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Table 1. Represents the clinical characteristics of the participants.

Clinical features		Total cases (n=16)
Age	<50	11
	>50	5
Gender	Male	13
	Female	3
Tobacco habits	Yes	12
	No	4
Non-Habits	Yes	-
	No	4
Histological grade	Well differentiated	8
	Moderate differentiated	8
Tumour staging	T1-T2	9
	T3-T4	7
Tissue sub site	Right buccal mucosa	8
	Left buccal mucosa	8

Figure 1 represents the histopathological examination of oral squamous cell carcinoma. Black arrow represents the keratin pearl formation, blue arrow represents the epithelial cells, yellow arrow represents the nucleus and red arrow represents the islands of malignant epithelial cells (Figure 2) [9].

Figure 1. Represents the histopathological examination of oral squamous cell carcinoma.

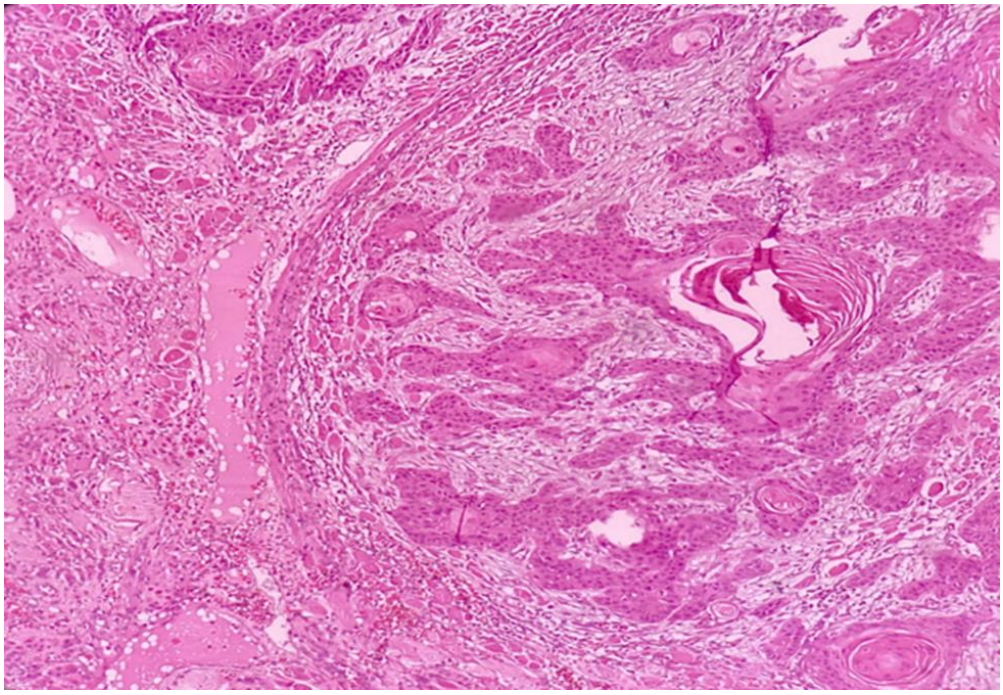
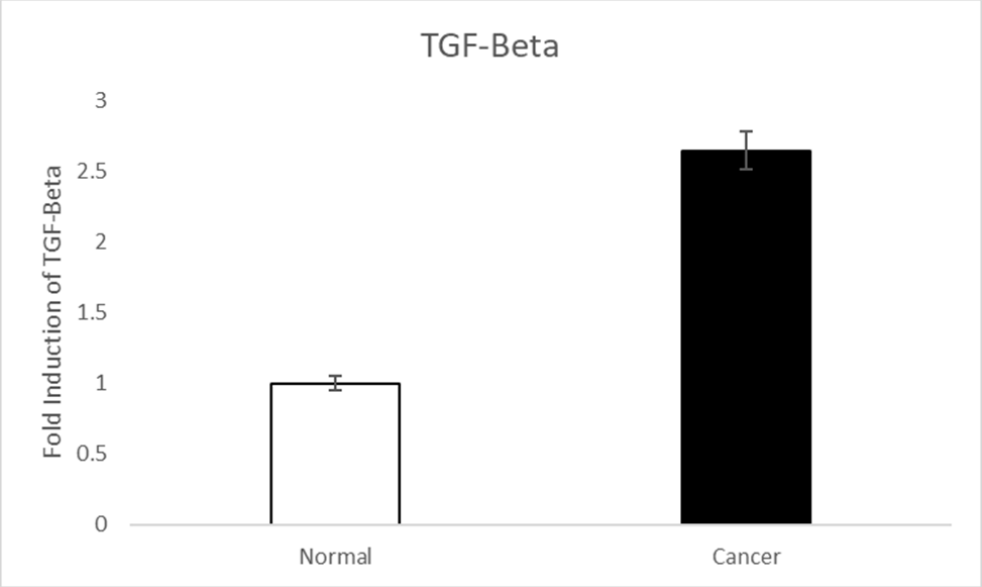


Figure 2. Represents the expression levels of TGF-β in the normal and OSCC tissue. Data are represented as mean ± standard deviation, $p<0.05^*$ is said to be statistically significant.



DISCUSSION

Head and neck cancer ranks sixth in terms of the frequency of systemic malignant tumours, with 90% of cases being OSCC with poor prognosis [10]. However, it is unclear how OSCC will evolve. On the one hand, chronic inflammation can draw immune and inflammatory cells to the tumour tissue, and as the tumour grew, the function of these cells shifted from immune monitoring and suppression to supporting tumour cell proliferation. TGF-βs are primarily produced by cancer cells and invading inflammatory cells in the tumor microenvironment [11,13]. There are three isoforms of TGF-β: TGF-β1, TGF-β2 and TGF-β3. They have distinct actions and are expressed differently in various cancer types. Previous research has shown that TGF-β within the tumour microenvironment can induce Epithelial to Mesenchymal Transition (EMT). In the present study, TGF-β expression levels were observed to be considerably ($p<0.05^*$) elevated in the OSCC samples when compared to the normal tissue. The histological analysis revealed significant keratin pearl formation, which caused nucleus disruption, programmed cell death, and tumour cell proliferation [14].

CONCLUSION

A study by Haga et al., suggested that TGF-β1 signalling suppression lowered sex-determining region Y- box9 (SOX9) expression and tumour invasion *in vitro* and *in vivo*, demonstrating that SOX9 is required for TGF-β1 mediated invasion. These findings have also demonstrated that Cancer Associated Fibroblasts (CAFs) promoted cancer migration and invasion *via* the TGF-β/SOX9 axis. Park, et al., reported that TGF-β was administered to TGFBR2 expressing and knockdown cells, and the culture supernatants were evaluated with a cytokine array kit. It was observed that TGF-β suppressed IGF-binding protein 3 (IGFBP3) levels and while increased MMP9 levels. Thus, these findings point to a new method for the regulation of osteoclastogenesis by oral cancer cells, which could be a potential therapeutic target for osteolysis caused by oral cancer invading the bone. Furthermore, more research with large sample size, using OSCC related cell lines and animal model studies are required to confirm the effectiveness of proinflammatory TGF-β cytokines as screening/diagnostic markers for

regular use in clinical practice.

AUTHOR CONTRIBUTIONS

All the authors read and approved the submitted version.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ETHICAL APPROVAL

This study was performed according with the Declaration of Helsinki and approved by institutional ethical committee of Saveetha dental college and hospital (IHEC/SDC/1995/22/OSURG/582).

CONSENT TO PARTICIPATE

Written informed consent to participate in the study was obtained from all the participants.

CONSENT TO PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIAL

The data are available on the special request of corresponding author.

FUNDING

Not applicable

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