10.21608/adjc.2024.259861.1455 Advanced Dental Journal
Volume 6 (2024) | Issue 4| Pages 702-709

Original Article

Expression Of TGF-B In Oral Papillary Squamous Cell Carcinoma and Different Histological Grades of Oral Squamous Cell Carcinoma: An Immunohistochemical Comparative Study

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Submitted: 2-1-2024
Accepted: 28-2-2024

Abstract

Aim: The aim of the study is to investigate Transforming growth factor beta immune-expression in oral papillary squamous cell carcinoma and then compare its expression in oral squamous cell carcinoma with different histological grade to correlate its expression with the prognosis.

Subjects and methods: This will be accomplished by collecting archival blocks of oral papillary squamous cell carcinoma and oral squamous cell carcinoma with different histological grades, then staining the specimens with transforming growth factor beta, and then calculating the area percent for each group.

Results: Results showed that the epithelial expression of transforming growth factor beta was significantly high in well differentiated squamous cell carcinoma with statistically significant results. On the contrary, there was no difference in the epithelial expression of transforming growth factor beta between oral papillary squamous cell carcinoma, moderately differentiated and poorly differentiated cases with statistically insignificant results. Transforming growth factor beta showed the highest expression in the stroma of poorly differentiated squamous cell carcinoma with statistically insignificance results with the other three groups.

Conclusion: Oral papillary squamous cell carcinoma may has a favorable prognosis than well differentiated squamous cell carcinoma.

Keywords: Oral Papillary Squamous cell carcinoma, Well-differentiated Squamous cell carcinoma, Moderately differentiated Squamous cell carcinoma, Poorly differentiated squamous cell carcinoma, Transforming growth factor beta.

Introduction

Oral cancer is considered the sixth most common type of cancer in the world [(1)]. Over 90% of oral cancers is diagnosed as Oral squamous cell carcinoma (OSCC) [(2)]. OSCC has many risk factors, including extrinsic and intrinsic [(3)]. Tobacco usage, betel chewing, alcohol consumption, sun exposure, and HPV infection are considered extrinsic factors [(4)]. These risk factors lead to genetic or epigenetic alterations, that in turn cause the development of OSCC [(5)]. According to the degree of differentiation, they are classified into well, moderately and poorly differentiated OSCC [(5)].

Oral papillary squamous cell carcinoma (OPSCC) is considered an unusual recent variant of OSCC, which was supposed to have a good prognosis [(6)]. It appears as a solitary lesion with exophytic papillary growth [(7)]. Histologically, it exhibits malignant keratinizing or non-keratinizing epithelium with finger-like papillary projections that have

fibrovascular cores [8]. The epithelium shows frank cellular and nuclear signs of dysplasia such as enlarged nuclei, prominent nucleoli, and a marked increase in nuclear cytoplasmic ratio with evidence of invasion into the underlying connective tissue [9]. It differs from conventional OSCC in that OPSCC has limited invasion, a lower rate of metastasis, and a better survival rate [6].

Tumor microenvironment (TME) is the interaction of tumor cells with the surrounding tissues and immune cells creates the complex, integrated system known as TME [10]. The presence of a TME promotes tumor growth by increasing tumor cell motility, proliferation, and immune evasion [11]. TME is formed of cellular and acellular components including fibroblasts, myofibroblasts, macrophages, extracellular matrix, and blood vessels [12].

TGF-β plays a significant role in ECM degradation which is mediated by Matrix metalloproteinases (MMPs). These MMPs cause the degradation of basement membrane components, leading to the infiltration of tumor cells into blood and lymphatic vessels [13]. Moreover, the carcinogenic role of TGF-β in the activation of various cells is one of the most recently investigated role of TGF-β [14].

Therefore, the aim of the study was to compare the immuno-histochemical expression of TGF-β in OPSCC and compare its expression with different histological grades of OSCC inorder to detect if there is a difference prognosis between OPSCC and different histological grade of OSCC.

Subjects and Methods

Participants and staining

Archival blocks of OPSCC and different histological grades of OSCC were collected from Oral and Maxillofacial Pathology, faculty of Dentistry, Cairo University according to the histopathological criteria for OPSCC and OSCC as stated in the WHO classification 2022 of head and neck tumors. Cases with underlying systemic condition, cases who received treatment, other variants of OSCC were excluded. A total of 40 blocks of OSCC were recruited, where 10 were OPSCC, 10 were well-differentiated OSCC, 10 were moderately-differentiated OSCC, and 10 were poorly-differentiated OSCC. The specimens were stained histologically by Hematoxylin and Eosin (H&E); according to [15], and immunohistochemically by TGF-β, according to [16] at National cancer institute, Cairo university. It was accepted by the ethical committee of Faculty of Dentistry, Cairo University. The manuscript id is ADJC-2401-1455.

Image analysis

Capturing microscopic images were done in Analytica research center, Elharam, Cairo, Egypt, using SOPTOP EX20 biological microscope (China), HD camera (model No. XCAM1080PHB) and Imageview software at X40, X100 and X200 magnification powers. Immunostained sections were examined using high power fields (X400) and the most homogenous areas of the positive reaction were chosen for evaluation.

Area percent measurement

Immunostained sections were examined using high power fields (X400) by light microscope; SOPTOP EX20 biological microscope (China) and HD camera (model No. XCAM1080PHB), and the most homogenous areas of the positive reaction were chosen for evaluation. The image analyzer computer system applying Imagej 1.53e software (USA) was used for automated measurement of area percent of TGF-β positivity in the dysplastic epithelium Figure (1) and in the stroma Figure (2). It was performed in a standard frame area of 5.04X106 µm2, five fields were measured per case.

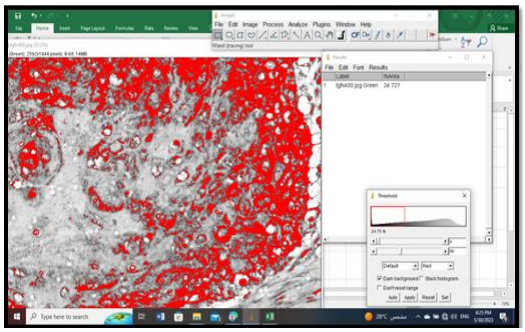

Figure 1: Area percent of TGF-B in the epithelium.

Copy display photo of Imagej image analyzer showing red binary colour marking the area percent of TGF-β positivity in the dysplastic epithelium of OPSCC (X400, frame area= $5.04X10^6$ μm^2).

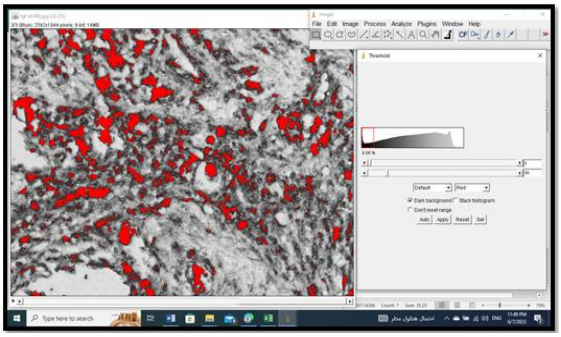

Figure 2 : Area percent of TGF-B in the stroma.

Copy display photo of Imagej image analyzer showing red binary colour marking the area percent of TGF-β positivity in the stroma of OPSCC (X400, frame area= $5.04X10^6$ μm^2)

Statistical methods

Statistical analysis of the results was performed using SPSS software. Shapiro-Wilk test of normality was used to test normality hypothesis of all continuous variables. Analysis of variance (ANOVA) test was used for evaluation of statistical significance of the difference the continuous data of each parameter among the studied groups, followed by Tukey Kramer Post hoc test for the statistically significant results. Upon comparison between two groups, unpaired T-test was used for evaluation of statistical significance of each parameter. For ordinal independent variables Kruskal Wallis test was used for evaluation of statistical significance of each parameter among the studied groups, followed by Tukey Kramer Post hoc for the statistically significant results. P-values ≤ 0.05 were considered statistically significant.

Results

Histopathological and Immunohistochemical findings

Microscopic examination of OPSCC sections stained with H&E revealed a verrucous lesion with irregular projections. The proliferating epithelium showed frank signs of dysplasia with obvious invasion into the underlying connective tissue. TGF-β in OPSCC showed strong cytoplasmic expression in the basal cell layer, as shown in Figure (3). In WDSCC, the dysplastic epithelial cells could be seen in large nests with numerous keratin pearls. TGF-β expression was strong as shown in Figure (4). In MDSCC, the lesions showed absence of cellular adhesion, keratin, and smaller sized nests. TGF-β expression was focal and weak as shown in Figure (5). The dysplastic individual cells in PDSCC invaded the underlying connective tissue. Dysplastic signs were clearly seen in all cases of PDSCC with pleomorphism, hyperchromatism, numerous abnormal mitotic figures and increased mitosis. PDSCC showing focal cytoplasmic expression of TGF-β in epithelium with some nuclear expression as shown in Figure (6).

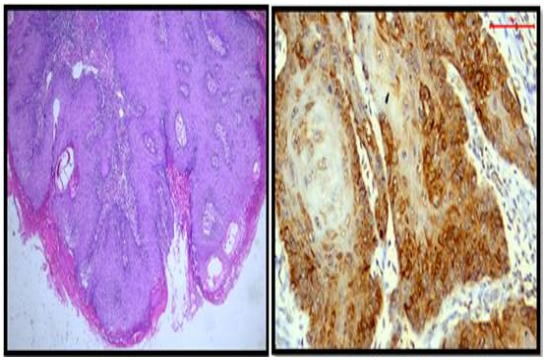

Figure 3: Oral papillary squamous cell carcinoma

A) Microscopic image of OPSCC showing a papillary lesion with interpapillary clefts filled with keratin. (H&E stain, Magnification x40). B) Microscopic picture of OPSCC showing strong cytoplasmic expression of TGF-β marker in the basal cells (Immunohistochemistry, Magnification x200).

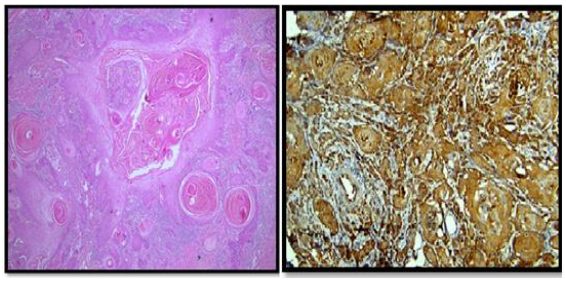

Figure 4: Well differentiated squamous cell carcinoma

A) Microscopic image of WDSCC showing numerous keratin pearls haphazardly arranged and minimal dysplastic changes (H&E stain, Magnification x40). B) Microscopic picture of WDSCC showing strong diffuse cytoplasmic expression of TGF-β, notice the perivascular invasion of dysplastic epithelium (Immunohistochemistry, Magnification x200).

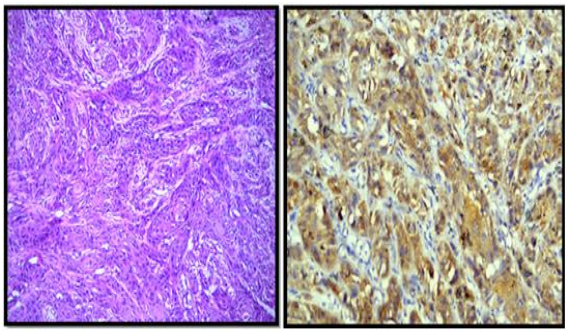

Figure 5: Moderately differentiated squamous cell carcinoma

A) Microscopic picture of MDSCC showing numerous small malignant epithelial cell nests and strands (tumor budding), with minimal keratinization invading the connective tissue (H&E stain, Magnification x100). B) Microscopic picture of MDSCC showing focal TGF-β expression, (Immunohistochemistry, Magnification x200).

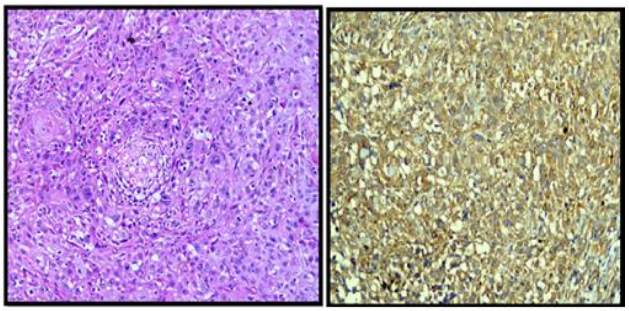

Figure 6: Poorly differentiated squamous cell carcinoma

A) Microscopic picture of PDSCC showing individual malignant cells infiltrating the connective tissue with bizarre looking nuclei and abnormal mitosis (H&E stain, Magnification x200). B) Microscopic picture of PDSCC showing focal weak cytoplasmic expression of TGF-β in epithelium with few nuclear expression (Immunohistochemistry, Magnification x200).

Statistical analysis

Area percent of TGF-β in epithelium

The greatest mean of TGF-β area percent in epithelium was recorded in WDSCC, whereas the lowest mean was recorded in MDSCC. One-way analysis of variance (ANOVA) test revealed that the difference between all groups was statistically significant ($P<0.05$). Tukey's post hoc revealed that WDSCC group was significantly different from the other three groups. While, there was no significant difference among OPSCC, MDSCC and PDSCC groups **table (1).**

Area percent of TGF-β in stroma

The greatest mean of TGF-β area percent in stroma was recorded in PDSCC, whereas the lowest mean was recorded in MDSCC. However, one-way analysis of variance (ANOVA) test revealed that the difference between all groups was statistically insignificant ($P>0.05$) **table (2)**.

Table (1) : Mean of TGF-β area percent in epithelium all groups and significance of the difference using (ANOVA) test

POC	OPSCC	WDSCC	MDSCC	PDSCC
Mean	35.6 b,c,d	55.55 a	27.2 b,c,d	31.46 b,c,d
SD	7.915	6.8218	10.32	8.2359
Minimum	24.73	45.7	15.79	14.63
imum	47.67	62.55	44.7	39.24
F ratio	15.6			
P value	< 0.00001			

*significant at p<0.05

Tukey's post hoc test: means sharing the same superscript letter are not significantly different.

Table (2): Mean of TGF-β area percent in stroma in all groups and significance of the difference using (ANOVA) test

	OPSCC	WDSCC	MDSCC	PDSCC
Mean	12.4957	12.9414	9.3086	15.1357
SD	3.4485	9.2903	7.9474	8.6919
Minimum	8.15	2.35	3.3	2.15
Maximum	15.83	22.44	23.85	29
F value	0.68247			
P value	0.571428 (Not significant)*			

*significant at p<0.05

Discussion

The prognosis of OSCC differs according to histological grade and subtype [17]. OPSCC is an uncommon variant of OSCC which was supposed to have a good prognosis in comparison to different histological grades of OSCC [6]. So, the aim of the study is immunohistochemical staining of OPSCC and different histological grades of OSCC with TGF-β then to compare its expression in OPSCC with that in conventional OSCC. The correlation between the expression of TGF-β and prognosis is recommended.

In the current study, the immunohistochemical staining of TGF-β in OPSCC showed strong cytoplasmic expression in the basal and parabasal cell layers only of the malignant epithelium. These results were in agreement with [18] who found that oral potentially malignant lesions as erythematous lichen planus showed strong basal and parabasal TGF-β expression. This may explain that the pathological behavior and prognosis of OPSCC matches that of the oral potentially malignant lesions. They also found that the expression of TGF-β was also similar to microinvasive carcinoma which indicates favorable prognosis.

On the contrary, the immunohistochemical staining of TGF-β in epithelial cells of WDSCC showed significantly the highest expression in the present study. While MDSCC and PDSCC showed focal expression of TGF-β in the epithelium. These findings were in contrast to [19], who found that high-grade tumors were

associated with increased TGF-β. As well as, [20] contradicted our study as they reported that the expression of TGF-β; as the main inducer of EMT, increases with the advanced stage of cancer, so the percentage of TGF-β expression should be higher in PDSCC. The low percentage of TGF-β expression in MDSCC and PDSCC in the presented study could be attributed to the discohesiveness of the malignant epithelial cells in these groups, leading to lower area percent of expression than WDSCC in the fixed frame area. The expression of TGF-β in OPSCC does not significantly differ from moderately or poorly differentiated squamous cell carcinoma due to the presence of discohesive malignant cells.

However, the percentage of TGF-β expression in the stroma was the highest in PDSCC followed by the other three groups with statistically insignificant results. These results were in agreement with [21] who found that the percentage of TGF-β expression in the stroma increased with the histological grade and this was due to secretion of TGF-β by the TME with the tumor progression.

Conclusion

OPSCC may have a better prognosis than WDSCC

Recommendations

1) Further investigation of OPSCC using other prognostic markers.

2) Long period follow-up of OPSCC cases should be done to ensure its favorable prognosis.

3) TGF-β investigation in other variants of OSCC is also recommended.

Conflict of Interest

No conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry- Cairo university on: 31/5/2022 approval number: 3522

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