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Research Article

Configuring the Expression of Wilms Tumor 1 in Oral Squamous Cell Carcinoma and Its Relationship with Clinicopathologic Features

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Abstract

Introduction: The most common oral cancer is Squamous Cell Carcinoma (SCC) that a wide variety of environmental factors has caused complex etiology in terms of prevalence presentation also a significant correlation between the levels of WT1 expression and prognosis of the disease has been reported.

Materials and Methods: For This descriptive - analytical study samples were collected from the archives of Pathology Department of Imam Reza Hospital Tabriz and Department of Pathology of Dentistry School. In this study, data from patients' records and results of Immunohistochemical staining (IHC) were collected in the laboratory. After a review of the histopathologic grade we compared them with the data records to ensure the accuracy grade and then were compared with the results of immunohistochemistry. Chi-square test and comparison of independent groups was analyzed using statistical software spss16.

Results: From 45 cases, expression of WT1 has been reported in 3 cases (6.2%) and all of them were well-differentiated. The mean age of the participants was 66.42 (±16.06) years, which 16 cases were female (35.6%) and 29 cases were male (64.6%). Histopathologic grade was well-differentiated in 36 cases (80%), 2 cases had poorly-differentiated grade (4/4%), and moderately-differentiation was reported in 7 cases (6.15%). There was not significant relation between histopathologic grade and WT1 expression.

Conclusion: Further studies about investigating of the possibility of treatment with immunotherapy based WT1 peptide as an effective treatment in cases with increased expression of WT1, are suggested.

Keywords: Oral Cancer; Wilms' Tumor; Staining; Expression; Gene

Introduction

Squamous cell carcinoma (SCC) is the most common type of oral cancer, and a wide variety of environmental factors contribute to its complex etiology [1]. The Wilms' tumor (WT1) gene is a tumor suppressor gene found on chromosome 13p11. Mutations in WT1 play a role in the pathogenesis of Wilms' tumor [2,3], a pediatric renal neoplasm. WT1 plays an important role in the development of the kidney. Sites of variant tissues during nephrogenesis and germline mutations in WT1 are associated with abnormal development of the urogenital tract and are frequently associated in Denys-Drash syndrome [1-5].

In healthy individuals, WT1 is expressed in the kidneys, gonads, urinary tract, decidua, skeletal muscle, mesothelium, muscles, and bladder. High levels of WT1 have been reported in breast and lung cancers [6], and high levels of WT1 in leukemia are significantly correlated with poor prognosis [7-9].

Expression of WT1 varies across solid tumors such as colon, lung, breast, testicular, ovarian, urinary tract, liver, and thyroid. WT1 is an active factor in oncogenic leukogenesis and can also act as a tumorigenic factor in solid tumors [10-11].

WT1 overexpression has also been reported in many cancers such as pancreatic cancer, urinary tract and male genital organ cancers, soft tissue cancer, and malignant melanoma [12]. Mounting evidence suggests a role for WT1 in tumorigenesis. As such, WT1 peptide-based immunotherapy has been proposed as a useful method with a high potential for cancer therapy.

Very few studies have reported on the association of WT1 with SCC. Because of the mechanism of action of WT1, the association with oral SCC (OSCC) remains unclear [13]. In 2013, Mikami et al. studied the relationship between OSCC and WT1 expression, and they determined that WT1 may play an important role in the pathogenesis of some subgroups of OSCC [14].

Due to the limited research about the role of WT1 in OSCC and because of the different causes of cancer in different societies, the aim of this study was to explore the relationship of WT1 expression with clinicopathologic variables in OSCC. A definitive role for WT1 in OSCC carcinogenesis would suggest the use of WT1 peptide-based immunotherapy as a novel approach to cancer treatment.

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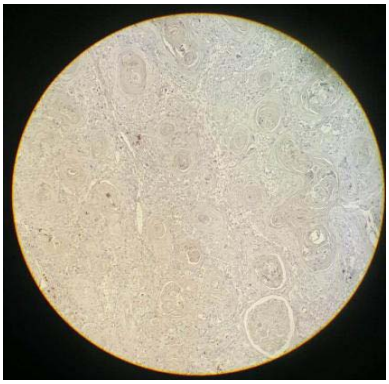

Figure 1: Immunohistochemical analysis of WT1 expression at 400×magnification.
This sample was negative for WT1 expression.

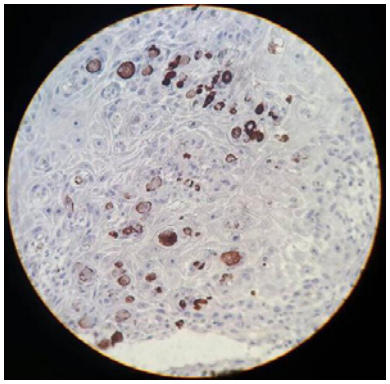

Figure 2: Immunohistochemical analysis of WT1 expression at 400×magnification.
This sample was positive for WT1 expression.

Materials and Methods

For this descriptive analytical study, samples were collected from the archives of the Pathology Department of Imam Reza Hospital Tabriz and the Department of Pathology of the Dentistry School of Tabriz medical sciences university. The study was approved by the Research Ethics Committee of Tabriz University of Medical Sciences. Data from patient records and results of immunohistochemical (IHC) staining were collected. After a review of the histopathologic grade, we compared the samples with the patient records to ensure the accuracy of grading. We then compared the grading with the results of the IHC.

Tissue samples

A total of 45 cases of OSCC diagnosed between 2006 and 2014 were selected from the paraffin-embedded tissue archives in the Pathology Department at the Dentistry University and Imam Reza Hospital of Tabriz. Formalin-fixed, paraffin-embedded tissue sections (4lm thick) were used for histopathological analyses. The sections were placed in a microwave for 10 min in 10mm citrate buffer (pH 6.0) for antigen retrieval. Peroxidase activity in the sections was blocked with 0.3% H_2O_2 and they were incubated with mouse anti-human WT1 monoclonal antibody (1:100, M3561; Dako) at room temperature for 1 hour. The samples were then processed with Dako Envision + Dual Link System-HRP (K4063; Dako) for 45min, and immunoreactive WT1 protein was visualized with 3,3′-diaminobenzidine using Dako Cytomation Liquid DAB+ Substrate Chromogen System (K3467; Dako). Statistical analysis was performed by Chi-square test, and for comparison of independent groups, we used SPSS software v.19 (SPSS, Chicago, IL, USA) [16].

Results

The samples used in this study were taken from 45 different patients. The mean age of the subjects was 66.42 (± 16.06) years. Sixteen were female (35.6%) and 29 (64.6%) were male.

Three samples (6.7%) out of 45 were positive for WT1, and 42 samples were negative (Figure 1 and 2). The majority of the samples (36, 80%) were well-differentiated, 2 samples (4.4%) had a poorly-differentiated grade, and in 7 samples (15.6%), moderate differentiation was reported.

This study aimed to investigate the expression of WT1 in OSCC and its relationship with histopathologic grade. The three cases with WT1 expression were all well-differentiated. According to a regional-grade chart, eight cases were in the buccal area with a histopathologic grade of well-differentiated. Of 20 cases in the tongue, 15 were well-differentiated, and 5 had a moderate grade. Thirteen cases were from the gingival area, of which, 10 had a well-differentiated grade, 1 had a reported moderate grade, and 2 were poorly-differentiated. There was no statistically significant difference between the location and grade of the tumors (P = 0.26).

In evaluating the relationship between WT1 expression and involved area, the cases with WT1 expression were in the tongue, buccal area, and conflict zone of the oral floor (one each). No WT1 positive samples came from the gingiva.

We found no significant relationship between WT1 expression and conflict region (P = 0.927).

One WT1 positive sample was from a woman, and the remaining two came from men. There was no significant correlation between gender and WT1 expression (P = 0.715).

Of the 16 samples from women, 12 were well-differentiated and 4 had a moderate histopathology grade. Of the 29 samples from men, 24 were well-differentiated, 3 had a moderately-differentiated grade, and 2 had a histopathologic grade of poor. There was no significant relationship between gender and histopathologic grade (P = 0.958). There was also no significant relation between histopathologic grade and WT1 expression (P = 0.044).

Discussion

Squamous cell carcinoma is the most common tumor in head and neck malignancies. The latest advances in the treatment of oral cancer have not significantly enhanced survival, and only about half of all individuals diagnosed with head and neck cancer will survive five years [15].

Recently, WT1 peptide-based cancer immunotherapy has emerged as a possible new cancer treatment, and over the last eight years, clinical trials of WT1 peptide-based cancer immunotherapies have shown effective clinical responses for specific types of cancers. Oka et al. performed a phase I clinical trial for a WT1-based vaccine

in patients with different types of cancer and confirmed that, except for a local inflammatory response with erythema at the injection site, there were no observable damages to healthy tissues. Morita et al. evaluated the toxicity of a weekly treatment of WT1 injection in patients with solid malignancies and concluded that WT1 peptide-based immunotherapy is acceptable for patients. Further understanding of the role of WT1 in OSCC, however, will facilitate the development of new treatment strategies [13,15,16].

In another study, Oji et al. examined the expression levels of WT1 mRNA in tissue samples obtained from patients diagnosed with head and neck SCCs using real-time PCR and found that all 4 cases in the mouth floor, 5 of 9 cases in the gingiva, and 17 of 25 cases in the tongue displayed overexpression of WT1 mRNA (68.4%). The rates of WT1 mRNA expression were higher than what we observed. In their study, endothelial cells from blood capillaries, muscle cells, and myoepithelial cells in the sample tissues also expressed WT1 protein, and blood capillaries proliferated more in the tumor stroma than in intact mucosa [10]. According to the results of Mikami et al., the frequency of WT1 protein overexpression in OSCC tissue samples was 6.9%, and all of the cases with WT1 expression were well-differentiated. Similarly, in our study, the frequency of WT1 overexpression was 6.2%, and all of the cases with WT1 expression were well-differentiated.

Our results disagree with those of Oji et al., who reported that high WT1 expression levels were significantly correlated with poor histologic tumor differentiation and advanced tumor stage in head and neck SCC. However, the samples in our study had similar demographics as the study by Eshghyar et al., who found similar results [18].

Normal oral epithelium maintains its structure by a continuous renewal of cells, in which, cells produced by mitotic

division in the lower cell layers of the basal epithelium migrate to the surface to replace cells that have been shed. This phenomenon is also frequently seen in the cancer nests of well-differentiated OSCC samples, where the basal layer proliferates in this fashion. In this study, although WT1 was not expressed in the basal layer of normal epithelium, it was expressed in the basal layer of infiltrating and proliferating cancer nests of the same tissue specimen. This suggests that WT1 is important to the neoplastic proliferation of cancer cells. Amassing more data from OSCC cases displaying overexpression of WT1 is necessary to determine the epidemiologic correlations that exist between WT1 and OSCC. Based on observations of the morphological features of tissue specimens and the expression patterns of WT1, WT1 appears to play an important role in the pathogenesis of some types of OSCC, particularly in the proliferation of cancer cells [19].

Two separate phase I clinical studies were performed by Tsuboi et al. and Oka et al. using WT1 peptide-based cancer immunotherapies on patients with OSCC with high WT1. Both studies demonstrated a decrease in WT1 expression and were considered to be effective. Thus, our findings and this phase I clinical studies suggest that although the pathogenesis of OSCC is diverse, WT1 peptide-based immunotherapy could have a role as a new treatment option for OSCCs that have WT1 overexpression [20].

Conclusion

WT1 expression was reported in 6.2% of all studied cases in this survey. Additionally, the WT1-positive samples were well-differentiated. However, the association of WT1 expression with the proliferation of malignant cells remains inconclusive. Collecting more data to examine the relationship between WT1 expression and any relevant clinicopathologic variables in oral SCC is essential.

Acknowledgment

This study has been approved as a student project at the Gingiva Research Center of Tabriz University of Medical Sciences and has been accepted by the Ethics Committee of the Tabriz University of Medical Sciences.

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