

Frequency of PTEN gene Mutation in Oral Squamous cell Carcinoma

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Abstract:

Objective: *The aim of this study was to screen for the mutation in PTEN gene among Sudanese patients with oral cancer by Molecular and Immunohistochemical techniques and to identify the frequency of oral cancer patients with PTEN gene* **Methods:** *One hundred Formalin fixed paraffin processed tissue blocks from patients previously diagnosed as having oral cancer with their related data retrieved from different histopathology laboratories in Khartoum City.* **Results:** *Of the 100 patients with Oral Squamous Cell Carcinomas (OSCCs), 30 (30%) were found with Loss of PTEN expression by Immunohistochemical methods. Out of 30 negative patients, 23 were males and the 7 were females. Exon 9 of PTEN gene was successfully recombined in all 100 patients samples.* **Conclusion:** *The findings of the present study suggest the role of PTEN gene mutation in the etiology of oral cancers in Sudan. Further studies with involving more exons for PTEN gene are required.*

Key words: PTEN gene mutation, Oral Squamous cell Carcinoma

INTRODUCTION

Oral cancer is the 8th most frequent cancer in the world among males and the 14th among females and accounting for nearly 3% of all cancer cases (de Camargo, et al. 2010). In the United States (US), cancers of the oral cavity account for 2.3% of all cancers with 30,000 new cases diagnosed each year. In Nigeria the (OC) rate 1.18% of all cancers where there are 1,205 new cases diagnosed annually. The incidence rates of oral cancer are 3.7% for men and 2.6% for women in the Sudan. (Jemal, et al. 2011). Prevalence of oral cancer is 3.2% in Sudan (GLOBOCAN, 2008).

Several lifestyle risk factors for the development of oral cancer are familiar, including tobacco products, alcohol, infections, dietary factors, chemical irritants and frank carcinogens. Prevalence of oral cancer is 3.2% in Sudan and the disease is mainly attributed to N-nitrosamine rich oral snuff consumption

Tumor suppressor PTEN (Phosphatase and tensin homolog on chromosome 10) is a dual-specific phosphatase. PTEN consists of 9 exons and encodes a 403-amino acid protein. PTEN acts as a negative regulator of the phosphatidylinositol-3-kinase (PI3K) pathway, thus controlling a variety of processes related to cell survival, proliferation, and growth (Salmena, et al. 2008; Wang and Jiang, 2008). Somatic PTEN mutations and deletions or epigenetic silencing are common in multiple tumor types, including breast, endometrium, and thyroid, prostate, lung, melanoma, leukemia and lymphoma (Pe´rez, et al. 2007; Jotta, et al. 2007). In much neoplasm PTEN deletion cooperate with other genetics alteration to enhance tumerogenesis and may determine clinical behavior of the tumor (Hollander, et al. 2011).

MATERIALS AND METHODS:

In this retrospective descriptive study, 100 formalin fixed paraffin wax processed tissue samples of oral SSC were retrieved. All specimens and data were obtained from the archive of different histopathology laboratories in Khartoum City during the period from 2014 to 2015.

Immunohistochemistry

Formalin fixed paraffin-embedded tissue blocks were cut in 5 microns thick serial sections. The sections were de-paraffinized, rehydrated and rinsed in phosphate buffer saline (PBS). An Immunohistochemical assay for AR was performed on consecutive paraffin sections using streptavidin–biotin method. Monoclonal mouse anti-human antibodies were used as primary antibodies for PTEN. After antigen retrieval, slides were incubated with primary antibody, followed by secondary biotinylated antibody. Sections were washed in PBS and then incubated with streptavidin peroxidase. Finally chromogen Diaminobenzidine (DAB) was used and section were counterstain with haematoxylin.

DNA Extraction

Formalin-fixed paraffin-embedded archival tissues were sectioned in 20 mm thickness and put in a separate clean sterile eppendorff tube with tight cover (each specimen was cut using new clean microtome knife to avoid contamination). Then it dewaxed by xylene and rehydrated in graded ethanol by mean of centrifugation. DNA obtained by phenol-chloroform extraction and ethanol precipitation. First the tissues lysed with sodium dodecyl sulfate and proteinase K overnight at 37 °C. Then, the proteins precipitated by phenol solution and, DNA recovered by ethanol precipitation, and resuspended in Tris-EDTA (pH 7.2) solution.

DNA quantification

To evaluate the DNA quantification after DNA extraction, we had analyzed DNA measurement using a Nano-Drop spectrophotometer.

PCR.

All samples screened for mutation of PTEN by PCR with genomic primers for PTEN exons 9, together with primers for the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene from chromosome 12p as reference. AAG GCC TCT TAA AAG ATC ATG was forward primer while TTT TCA TGG TGT TTT ATC CCT C was reverse primer for PTEN exon 9. The amplification product measured 700pb (Wang, et al.1997; Liaw,et al.1997).

The primer sequences for GAPDH were AGT ACG CTG CAG GGC CTC ACT CCT T (sense chain) and AAGAGC CAG TCT CTG GCC CCA GCC A (antisense chain) (Zhang, et al. 1994). PCR performed with 20 ng genomic DNA as a template in this mastermix, in a total reaction volume of 50 ml, containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 200 mM each dNTP, 1 unit of Taq polymerase, 0.5 mM each of PTEN primers, and 1 mM each of GAPDH primers. Four minutes of initial denaturation followed by 35 cycles of 1 min at 94°C, 1 min at 52° C and extension at 72° C for 10 min. The PCR products separated on 2% agarose gels by electrophoresis, containing 0.5 mg/ml ethidium bromide and read with UV reader.

Ethical consent

The study was approved by Faculty Research Board, Faculty of Medical Laboratory Science, Sudan University for Science and Technology and Technology & Radiation and Isotope Hospital. This in addition to the fact that, the authors followed the tenants of the Declaration of Helsinki.

DATA ANALYSIS

Data management was done using Statistical Package for Social Sciences (SPSS version 16). SPSS was used for analysis and to perform Pearson Chi-square test for statistical significance (P value). The 95% confidence level and confidence intervals were used.

RESULTS

100 patients with oral squamous cell carcinoma were investigated using molecular and Immunohistochemical detection for PTEN gene, their ages ranging from 33 to 89 years old with a mean age of 55.8 years. Of the 100 study subjects, 76 were males and the remaining 24 were females, giving males' females' ratio of 2.84: 1.00. The great majority of the study subjects were at age range > 45 years followed by age groups 46-55, 56-65, 66-77, and 76+ years, representing 29, 25, 22, 15, and 9 respectively. With regard to the males and age, the majority of males were found at the age ranges < 45 years & 46-55 years constituting 20 patients followed by 56-65, 66-77, and 76+ years, representing 16,12, and 6 in this order. With regard to the females the majority of males were found at the age range <45 years followed by 56-65, 46-55, 66-75 and 76+, representing 9,6,5,3 and 3, correspondingly as, indicated in Table 1.

Table 1. Distribution of the study population by age and gender

Age group	Males	Females	Total
<45 years	20	9	29
46-55	20	5	25
56-65	16	6	22
66-75	12	3	15
76+	6	3	9
Total	74	26	100

The majority the patients were diagnosed with well differentiated OSCC representing 38 patients followed by the moderately differentiated OSCC and poorly differentiated OSCC constituting, 31 for each. For men, most of them were diagnosed with well differentiated OSCC representing 30 patients followed by moderately differentiated, and poorly differentiated, constituting 24 and 20, in this order. For females, most of patients were diagnosed with poorly differentiated OSCC followed by well differentiated OSCC, and moderately differentiated OSCC representing, 8 and 7 correspondingly, as indicated in Table 2.

Table 2. Distribution of the study subjects by Diagnosis and gender

Variable	Category	Males	Females	Total
Diagnosis	Well differentiated OSCC	30	8	38
	Moderately differentiated	24	7	31
	Poorly differentiated	20	11	31
	Total	74	26	100

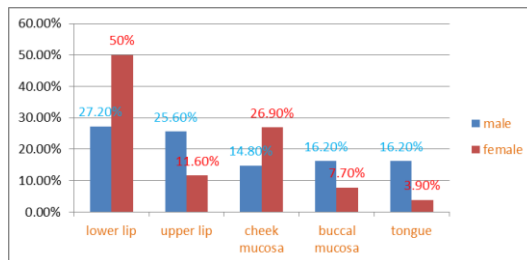


Figure 1. Description of study population by lesion site

Immunohistochemistry:

Loss of PTEN gene expression detect in 30 patients of whom 23 were males and 7 were females. Regarding the site of lesion negative PTEN gene expression was higher in lower lip scoring 14 patients followed by tongue and buccal mucosa accounting 5 for each and the upper lip were 4 cases and finally in cheek mucosa there were only 2 negative PTEN, as shown in Fig 1.

Table 3 indicates the distribution of immunohistochemical of PTEN gene in OSCC different grade Weak and moderate PTEN gene expression show on 21% of the study subjects and well staining account for 28 cases of study population.

Table 3. The Immunohistochemical of PTEN gene distribution in OSCC different grade.

	Results	Well differentiated OSCC	Moderately differentiated OSCC	Poorly differentiated OSCC	Total
PTEN expression	positive	29	19	22	70
	negative	9	12	9	30
PTEN Immunostainig	Low	8	8	13	29
	moderate	4	6	9	19
	Well	9	7	6	22

PCR:

Exon 9 of the PTEN gene was succefully recombine in the 100 samples of the study subjects.

DISCUSSION

Somatic PTEN mutations and deletions or epigenetic silencing are common in multiple tumor types, including breast, endometrium, and thyroid, prostate, lung, melanoma, leukemia and lymphoma (Pe´rez, et al. 2007; Salmena, et al. 2008; Jotta, et al.2010; Hollander.et al. 2011). A high frequency of mutations at the PTEN locus has been noticed in carcinoma of oral. However, the role of PTEN alternations and its association with outcome variables in the genesis of oral carcinoma is not understood fully.

In the present study absence of PTEN expression was establish in a reasonable number of the study population. The loss of PTEN expression may serve as a predictor of unfavorable prognosis for oral cancer patients (Zhao, et al. 2017). Previous studies have also showed relatively similar findings. The study by Snietura, et al. (2012) found that 42/73

(57.3%) patients with oral cancer has low PTEN expression. Xie, et al. (2011) investigated the expression of PTEN and dephosphorylated FAK. They investigated biopsy mucosal tissues from 34 cases of OSCC, 17 cases of OLP, 15 cases of OLK and 10 normal mucosa. PTEN and dephosphorylated FAK expression was significantly lower in the 34 cases of oral squamous cell carcinoma (73.5%) than in other non-malignant groups, and there were a positive correlation between the expression of PTEN and that of dephosphorylated FAK. The study indicate PTEN and dephosphorylated FAK potential involvement in the pathogenesis of the OSCC. In 2012, Alyasiri et al. investigated the expression of PTEN in 146 formalin-fixed paraffin embedded archival OSCC through immunohistochemical analysis. Sixty-one percent loss of PTEN expression were observed.

However, there is only one study from Sudan in this context. The study by Rahmani, et al. (2012) investigated a total numbers of 60 histopathologically confirmed cases of Squamous Cell Carcinoma and 15 cases of inflammatory lesion of oral specimens. PTEN expression was assessed by the use of anti-PTEN antibody through immunohistochemistry as directed by the manufacturer. There was progressive loss of PTEN expression from inflammatory lesion to OSCC ($p < 0.05$). Significant differences were found for PTEN expression between inflammatory lesion and OSCC. The difference in expression pattern of PTEN in gender did not reach statistical significance ($p > 0.05$). The loss of PTEN expression were correlated to poor differentiation, lymph node involvement and late stages. Thus, alteration of PTEN and bcl2 is likely an important molecular event in pathogenesis and carcinogenesis of oral carcinoma.

Furthermore, the difference in expression pattern of PTEN in gender, age and histological grade did not reach statistical significance

CONCLUSION:

The findings of the present study suggest the role of PTEN gene mutation in the etiology of oral cancers in Sudan. Further studies with involving more exons for PTEN gene are required.

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