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Oral cancer in Sudan and the crucial role of the PTEN gene mutation

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ABSTRACT

Objective: This study aimed to screen for mutations in the PTEN gene among Sudanese patients with oral cancer using molecular and immunohistochemical techniques, as well as to identify the frequency of oral cancer patients with PTEN gene alterations. **Methods:** One hundred formalin-fixed, paraffin-processed tissue blocks from patients previously diagnosed with oral cancer, along with their associated data, were obtained from various histopathology laboratories in Khartoum City. **Results:** Among the 100 patients diagnosed with Oral Squamous Cell Carcinomas (OSCCs), 30 patients (30%) exhibited a loss of PTEN expression as determined by immunohistochemical analysis. Among the 30 negative patients, 23 were male and 7 were female. Recombination of exon 9 of the PTEN gene was achieved in all samples from 100 patients. **Conclusion:** The results of this study indicate the involvement of PTEN gene mutation in the etiology of oral cancers in Sudan. Additional studies involving a greater number of exons for the PTEN gene are necessary.

Keywords: Oral cancer, PTEN, Mutation, Sudan

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents the predominant form of oral cancer, associated with significantly elevated morbidity rates [1]. OSCC is part of a diverse category of head and neck cancers, specifically originating from the mucosal epithelium of the lips, buccal mucosa, hard palate, anterior two-thirds of the oral tongue, floor of mouth, gingiva, and retromolar trigone. The primary oral sites impacted by OSCC include the lateral border of the oral tongue and the buccal mucosa, influenced by various risk factors [2]. As much as 46% of oral cancers can be prevented through the avoidance of risk factors and early detection of precancerous lesions [3]. Reducing or eliminating alcohol consumption, avoiding direct or indirect exposure to tobacco products, and decreasing betel quid chewing may lower the risk of oral cancer. Reducing processed meat consumption, moderating coffee intake, consuming appropriately prepared green tea, increasing fish and citrus fruit intake, maintaining oral hygiene, preventing periodontal diseases and HPV infections, and minimizing mechanical stimulation of the oral mucosa can provide protective effects against oral cancer [4]. The incidence of oral cancer in Sudan is notably high, primarily linked to the consumption of N-nitrosamine-rich oral snuff [5]. The delayed presentation and diagnosis of OSCC contribute to the high incidence of patients with advanced disease stages. In Sudan, cases of delayed presentation, especially among individuals with risk factors like Toombak dipping and alcohol consumption, often exhibit extensive lesions and a broad area of field cancerization. This condition is marked by genetic and epigenetic alterations in histologically normal-appearing tissues, which are associated with an elevated risk of recurrence and the development of second primary tumours. This requires more intensive treatment and is typically linked to worse outcomes [6]. Multiple genetic mutations have been linked to the etiology of oral cancer. The most prevalent genes are TP53, NOTCH1, CDKN2A, SYNE1, PIK3CA, ROS1, and TAF1L [7]. Growing evidence indicates that phosphate and tension homology (PTEN) plays a critical role in the

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immunosuppression of the tumour microenvironment (TME) across various cancers [8]. Loss of PTEN has been observed across multiple tumour types and is associated with poor clinical outcomes. Alongside PTEN mutation, various mechanisms play a role in the loss of PTEN during tumorigenesis. The natural selection process of PTEN-deficient tumour cells is not yet fully understood [9]. The relationship between PTEN mutation and OSCC remains ambiguous. This study aimed to screen for PTEN mutations in OSCC tissues from Sudanese patients.

MATERIALS AND METHODS

This retrospective descriptive study involved the retrieval of 100 tissue samples of OSCC, processed with formalin fixation and paraffin wax embedding. Specimens and data were sourced from various histopathology laboratories in Khartoum City between 2021 and 2023.

Immunohistochemistry

Serial sections of 5 microns in thickness were cut from formalin-fixed paraffin-embedded tissue blocks. The sections were deparaffinized, rehydrated, and washed in phosphate buffered saline. An immunohistochemical assay for AR was done on consecutive paraffin sections using the streptavidin-biotin technique. PTEN's primary antibodies were monoclonal mouse anti-human antibodies. After antigen retrieval, slides were treated with primary antibody, then secondary biotinylated antibody. The sections were rinsed in PBS and then incubated with streptavidin peroxidase. Finally, the chromogen Diaminobenzidine (DAB) was applied, and the sections were counterstained with hematoxylin.

DNA Extraction

Formalin-fixed paraffin-embedded archival tissues were sectioned 20 mm thick and placed in a separate clean sterile Eppendorf tube with a tight cover (to minimize contamination, each specimen was cut with a new clean microtome knife). Then it was dewaxed with xylene and rehydrated in graded ethanol using centrifugation. DNA was recovered using phenol-chloroform extraction and ethanol precipitation. The tissues were lysed with sodium dodecyl sulfate and proteinase K overnight at 37 ^{0}C. The proteins were then precipitated with phenol solution, and the DNA was recovered using ethanol precipitation,

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before being resuspended in Tris-EDTA (pH 7.2) solution.

DNA quantification

To test DNA quantity after extraction, we used a Nano-Drop spectrophotometer.

PCR

All samples were evaluated for PTEN mutations using PCR using genomic primers for PTEN exons 9 and primers for the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene from chromosome 12p as a reference. AAG GCC TCT TAA AAG ATC ATG was the forward primer, while TTT TCA TGG TGT TTT ATC CCT C was the reverse primer for PTEN exon 9. The amplification product measured 700 pb. The GAPDH primer sequences were: AGT ACG CTG CAG GGC CTC ACT CCT T (sense chain) and AAGAGC CAG TCT CTG GCC CCA GCC A (antisense chain). PCR was done with 20 ng genomic DNA as a template in this mastermix, which contained 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. The reaction volume was 50 ml. Initial denaturation takes four minutes, followed by 35 cycles of 1 minute at 94˚C, 1 minute at 52˚C, and 10 minutes of extension at 72˚C. The PCR products were separated on 2% agarose gels using electrophoresis with 0.5 mg/ml ethidium bromide and read using a UV reader.

Data Analysis

Data management was done using Statistical Package for Social Sciences (SPSS version 24). 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

A study was conducted on 100 patients diagnosed with oral squamous cell carcinoma, utilizing molecular and immunohistochemical methods to detect the PTEN gene. The ages of the participants ranged from 33 to 89 years, with a mean age of 55.8 years. Among the 100 study subjects, 76 were male and 24 were female, resulting in a male-to-female ratio of 2.84:1. Many study subjects were aged over 45 years, with the following distribution: 46-55 years (29%), 56-65 years (25%), 66-77 years (22%), and 76+ years (15%). Most males were observed in the age groups of less than 45 years and 46-55 years,

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comprising 20 patients. This was followed by the age groups of 56-65 years, 66-75 years, and 76 years and older, which included 16, 12, and 6 patients, respectively. In relation to females, the majority of males were observed in the age range of <45 years, followed by 56-65, 46-55, 66-75, and 76+, representing 9, 6, 5, 3, and 3, respectively, as indicated in Table 1.

Table 1. Demographic distribution of the study population categorized 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 of patients (38%) were diagnosed with well differentiated OSCC, followed by moderately differentiated OSCC and poorly differentiated OSCC (31 each). For men, the majority were diagnosed with well differentiated OSCC (30 cases), followed by moderately differentiated and poorly differentiated (24 and 20, respectively). For females, most patients were diagnosed with poorly differentiated OSCC, followed by well differentiated OSCC and moderately differentiated OSCC, representing 8 and 7, respectively, as shown in Table 2.

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

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

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The majority of patients presented with lower lip lesions, followed by cheek mucosa, upper lip, buccal mucosa, and tongue, accounting for 77.2%, 41.7%, 37.2%, 23.9%, and 20.1%, respectively. For males, the most prevalent lesion site was the lower lip, followed by the upper lip, with 27.2% and 25.6%, respectively. Females' most prevalent site was the lower lip, followed by the cheek mucosa, which accounted for 50% and 26.9%, respectively, as illustrated in Figure 1.

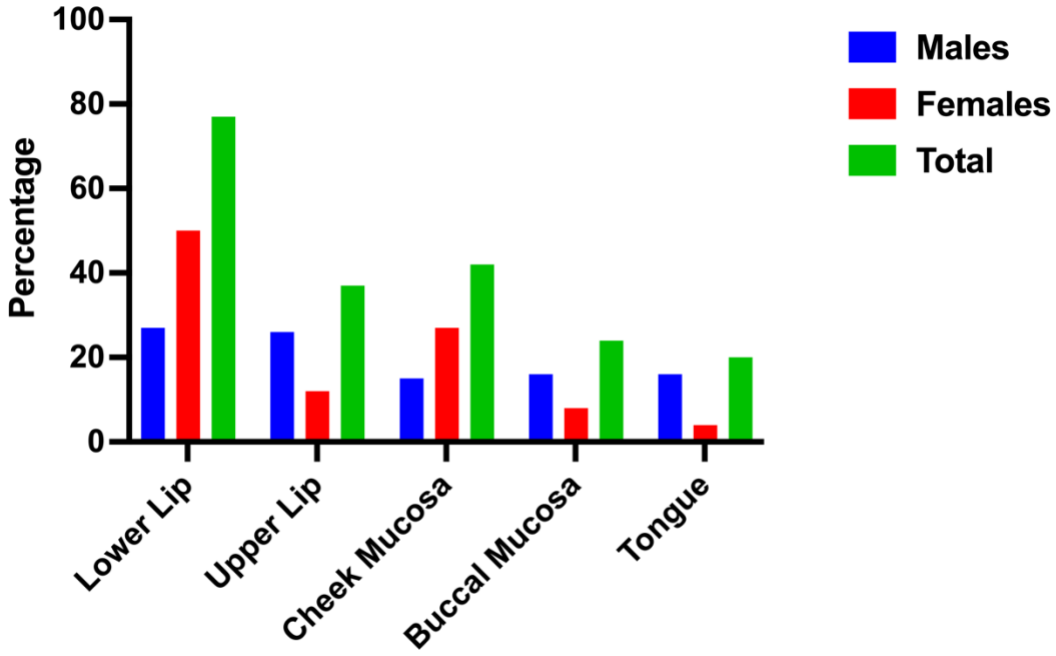

Figure 1. Description of lesion site by gender

Immunohistochemistry:

PTEN gene expression loss was detected in 30 patients, 23 of whom were men and 7 females. Regarding the site of lesion, negative PTEN gene expression was greatest in the lower lip with 14 patients, followed by the tongue and buccal mucosa with 5 each, the upper lip with 4 instances, and lastly the cheek mucosa with only 2 negative PTEN, as shown in Figure 1.

Table 3 shows the distribution of immunohistochemistry of PTEN gene in OSCC of different grades. Weak and moderate PTEN gene expression is seen in 21% of the research individuals, with 28 examples of well staining.

Table 3. Immunohistochemical analysis of PTEN gene distribution in OSCC of various grades.

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

PCR

Exon 9 of the PTEN gene was successfully recombined in 100 samples from the study subjects.

DISCUSSION

Oral cancer is one of the most common types of cancer in men in Sudan, mainly due to the use of toombak dipping, a type of smokeless tobacco that contains strong cancer-causing chemicals called tobacco-specific nitrosamines (TSNAs). TSNAs are a category of carcinogens derived from nicotine and associated tobacco alkaloids. NNK and NNN, two nicotine-derived nitrosamines, are potent carcinogens [10]. The results of the current investigation indicated that PTEN immunodepression was absent in 30% of the patients. Different studies have shown that the PTEN gene can be expressed negatively in a wide range of cases, from 29% to 96.3%, in OSCC[11-13]. Mutations in PTEN activate the phosphoinositide 3-kinase (PI3K) signalling pathway, resulting in the typical phenotypic alterations associated with cancer [14]. The PI3K/Akt pathway is crucial to numerous cellular activities and is inappropriately activated in malignancies, facilitating tumour development and progression [15]. The PI3K/AKT/mTOR pathway has been recognized as one of the most frequently altered signalling pathways in oral cancer, governing essential cellular and metabolic functions. Consequently, several proteins within the PI3K/AKT/mTOR pathway were employed as therapeutic targets for oral cancer, aiming to develop more selective pharmaceuticals with reduced off-target damage [16]. Exon 9 of the PTEN gene was successfully recombined in all instances in our investigation. In sporadic tumours, about 2% of documented sporadic PTEN mutations are located within exon 9, while 27% are found within exon 5. Specific PTEN mutations have been correlated with disease severity [17].

The current study's findings indicate that the vast majority of patients exhibit advanced stages of the disease, characterized by poorly to moderately

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differentiated OSCC. The delayed manifestation and diagnosis of OSCC contribute to the significant prevalence of patients with the advanced stage of the disease. In Sudan, cases with delayed presentation, especially those with risk factors like toombak dipping and alcohol consumption, often exhibit extensive lesions and a broad area of field cancerization, characterized by genetic and epigenetic alterations in histologically normal-appearing tissues, which are associated with an elevated risk of recurrence and second primary tumours. This requires more intensive treatment and is typically linked to worse outcomes [6].

The results of the present investigation indicated that the majority of patients exhibiting PTEN immune expression were linked to moderately differentiated cancer. This may indicate a reduction in the expression of the negative marker as cancer advances to later stages. PTEN is a tumour suppressor gene that encodes a dual phosphatase protein, which regulates membrane receptors and mediates cellular interactions with external stimuli. PTEN modulates cellular physiology, including division, differentiation/apoptosis, migration, and adhesion. PTEN expression was assessed using immunohistochemistry in OSCC and compared to a recognized histological malignancy grading system. Well-differentiated OSCC constituted 59.1%, while weakly differentiated OSCC comprised 40.9%. Based on PTEN expression, the cases were categorized as follows: 45.5% positive (the entire tumour exhibited staining), 22.7% mixed (presence of both negative and positive cells), and 31.8% negative (absence of staining in tumour cells). PTEN expression in OSCC correlated with the malignancy grade ($P < 0.0005$). Aggressive tumours exhibiting a high malignancy score did not express PTEN, although PTEN expression was evident in the epithelium next to the tumour. Negative cells were located at the tumour's invasive margin. This finding indicates that PTEN is associated with the histological pattern and biological behaviour of OSCC and may serve as a prognostic marker in the future. The function of PTEN in carcinogenesis and its potential as a biomarker warrant further investigation [18, 19]. The alarming increase of various cancers in Sudan [20,21], characterized by an unclear etiology, underscores the necessity for further research in this regard.

In conclusion, the findings of this study indicate that PTEN gene mutations contribute to the etiology of oral malignancies in Sudan. Additional research using a greater number of exons for the PTEN gene is necessary.

In conclusion, the findings of this study indicate that PTEN gene mutations contribute to the etiology of oral malignancies in Sudan. Additional research using a greater number of exons for the PTEN gene is necessary.

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Conflict of interest

The authors declare no conflict of interest.

Ethical considerations

Authorities at El-Obeid International Hospital granted permission to access the notified information.

Ethical approval

The Human Research Ethics Committee at MRCC has approved the study's proposal. Approval Number: HREC0017/PMRCC.3/25.

Data availability

Data regarding this study is available from the corresponding author.

REFERENCES

1- Liu J, Sun Z, Cao S, Dai H, Zhang Z, Luo J, Wang X. Desmoglein-2 was a novel cancer-associated fibroblasts-related biomarker for oral squamous cell carcinoma. BMC Oral Health. 2025 Jan 20;25(1):102. doi: 10.1186/s12903-024-05284-5.

2- Coletta RD, Yeudall WA, Salo T. Current trends on prevalence, risk factors and prevention of oral cancer. Front Oral Health. 2024 Nov 13; 5:1505833. doi: 10.3389/froh.2024.1505833.

3- Kijowska J, Grzegorczyk J, Gliwa K, Jędras A, Sitarz M. Epidemiology, Diagnostics, and Therapy of Oral Cancer-Update Review. Cancers (Basel). 2024 Sep 14;16(18):3156. doi: 10.3390/cancers16183156.

4- Xu H, Gao Z, Liu H, An L, Yang T, Zhang B, Liu G, Sun D. Associations of lifestyle factors with oral cancer risk: An umbrella review. J Stomatol Oral Maxillofac Surg. 2025 Jan 23:102234. doi: 10.1016/j.jormas.2025.102234.

5- Ahmed HG. Aetiology of oral cancer in the Sudan. J Oral Maxillofac Res. 2013 Jul 1;4(2): e3. doi: 10.5037/jomr.2013.4203.

6- Eltohami Y, Suleiman A. Survival analysis of Sudanese oral squamous cell carcinoma patients with field of cancerization. BMC Cancer. 2024 Apr 15;24(1):473. doi: 10.1186/s12885-024-12197-7.

7- Nakagaki T, Tamura M, Kobashi K, Koyama R, Fukushima H, Ohashi T, Idogawa M, Ogi K, Hiratsuka H, Tokino T, Sasaki Y. Profiling cancer-related gene mutations in oral squamous cell carcinoma from Japanese patients by targeted amplicon sequencing. Oncotarget. 2017 Jul 15;8(35):59113-59122. doi: 10.18632/oncotarget.19262.

8- Du L, Zhang Q, Li Y, Li T, Deng Q, Jia Y, Lei K, Kan D, Xie F, Huang S. Research progress on the role of PTEN deletion or mutation in the immune microenvironment of glioblastoma. Front Oncol. 2024 Aug 14; 14:1409519. doi: 10.3389/fonc.2024.1409519.

9- Wang M, Pan Z, Chu X, Yao X, Duan X, Wan J, Lou X, Li W, Yan Y, Chen L, An J, Qin Z. Chemotherapy-induced PTEN-L secretion promotes the selection of PTEN-deficient tumor cells. J Exp Clin Cancer Res. 2024 May 11;43(1):140. doi: 10.1186/s13046-024-03059-y.

10- Ahmed HG, Mahgoob RM. Impact of Toombak dipping in the etiology of oral cancer: gender-exclusive hazard in the Sudan. J Cancer Res Ther. 2007 Apr-Jun;3(2):127-30. doi: 10.4103/0973-1482.34696.

11- Lee JI, Soria JC, Hassan KA, El-Naggar AK, Tang X, Liu DD, Hong WK, Mao L. Loss of PTEN expression as a prognostic marker for tongue cancer. Arch Otolaryngol Head Neck Surg. 2001 Dec;127(12):1441-5. doi: 10.1001/archotol.127.12.1441.

12- Zhao J, Chi J, Gao M, Zhi J, Li Y, Zheng X. Loss of PTEN Expression Is Associated with High MicroRNA 24 Level and Poor Prognosis in Patients with Tongue Squamous Cell Carcinoma. J Oral Maxillofac Surg. 2017 Jul;75(7): 1449.e1-1449.e8. doi: 10.1016/j.joms.2017.03.025.

13- Starzyńska A, Sejda A, Adamska P, Marvaso G, Sakowicz-Burkiewicz M, Adamski Ł, Jereczek-Fossa BA. Prognostic value of the PIK3CA, AKT, and PTEN mutations in oral squamous cell carcinoma: literature review. Arch Med Sci. 2020 Nov 13;17(1):207-217. doi: 10.5114/aoms.2020.100780.

14- Vidotto T, Melo CM, Castelli E, Koti M, Dos Reis RB, Squire JA. Emerging role of PTEN loss in evasion of the immune response to tumours. Br J Cancer. 2020 Jun;122(12):1732-1743. doi: 10.1038/s41416-020-0834-6.

15- He Y, Sun MM, Zhang GG, Yang J, Chen KS, Xu WW, Li B. Targeting PI3K/Akt signal transduction for cancer therapy. Signal Transduct Target Ther. 2021 Dec 16;6(1):425. doi: 10.1038/s41392-021-00828-5.

16- Rajendran P, Sekar R, Dhayasankar PS, Ali EM, Abdelsalam SA, Balaraman S, Chellappan BV, Metwally AM, Abdallah BM. PI3K/AKT Signalling Pathway Mediated Autophagy in Oral Carcinoma - A Comprehensive Review. Int J Med Sci 2024; 21(6):1165-1175. doi:10.7150/ijms.94566.

17- Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. Nat Rev Cancer. 2011 Apr;11(4):289-301. doi:

10.1038/nrc3037. Erratum in: Nat Rev Cancer. 2011 Jun;11(6):458.

18- Squarize CH, Castilho RM, Santos Pinto D Jr. Immunohistochemical evidence of PTEN in oral squamous cell carcinoma and its correlation with the histological malignancy grading system. J Oral Pathol Med. 2002 Aug;31(7):379-84. doi: 10.1034/j.1600-0714.2002.00142. x.

19- Qiu L, Gao Q, Liao Y, Li X, Li C. Targeted inhibition of the PTEN/PI3K/AKT pathway by YSV induces cell cycle arrest and apoptosis in oral squamous cell carcinoma. J Transl Med. 2025 Feb 3;23(1):145. doi: 10.1186/s12967-025-06169-z.

20- Abuelfadol EA, Abdalla MMM, Elsayid M, Agabeldour AA. Are Chronic lymphocytic leukemia blood parameters differing from Other leukemias subtypes. Medical Research Updates 2024;2(1): 37- 45. DOI: https//doi.org/10.70084/pmrcc.mruj2.14.

21- Hassan SS, Agebeldour AA, Ahmed HG. Histopathological characteristics of cervical cancer in El-Obeid tertiary hospitals: A 5- year retrospective study. Medical Research Updates Journal 2024;2(2): 55- 63. DOI: https//doi.org/10.70084/pmrcc.mruj2.22