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Original Article

Immunophenotyping of HPV Types 16 and 18 among Sudanese Patients with Oral Lesions

Ibrahim A. M. Ginawi,[1] Ebtihag A. Mahgoub,[2] Hussain G. Ahmed[2]

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Abstract

Objective: The aim of this study was to screen patients with oral lesions for the presence of Human Papilloma Virus (HPV) types 16 and 18.

Methods: Sixty patients aged between 11-80 years with a mean age of 46 years were examined using immunohistological techniques. All samples were retrieved from RICK during the period from August 2009 to August 2010. Out of 60 patients, 50 had Oral Squamous Cell Carcinomas (OSCCs) and the remaining ten had benign oral lesions, included as internal control.

Results: Of the 50 patients with OSCCs, 10 (20%) showed positive immunohistochemical results for HPV types 16 and 18 of which 50% were detected among males and 50% were demonstrated among females. The ten positive findings were Immunophenotyped as follows: five were positive with HPV type 16, four with type 18 and one was positive for HPV types 16 and18. All patients with benign oral lesions were negative for HPV immunohistochemistry.

Conclusion: The study suggests the role of HPV 16 and 18 in the etiology of oral cancers in different parts of Sudan. However, the use of molecular techniques such as PCR are needed to confirm the results of immunohistochemistry in the role of the HPV in developing of OSCC in Sudan.

Keywords: Oral carcinoma; HPV; Immunohistochemistry.

Introduction

Oral cancer (OC) incidence and mortality rates vary widely across the world. The highest rates are registered in developing countries and more common among males than among females.[1] Incidence rates of oral cancer have been rising in most regions of the world.[2]

The association between Human Papilloma Virus (HPV) infection and cervical cancer is well established.[3,4] HPV infection has also been postulated as a potential risk factor for Oral Squamous Cell Carcinoma OSCC. Several studies have detected HPV DNA in a considerable proportion of oral cancers, with wide variations from 0% to 100% prevalence in oral tissues. This perhaps reflecting the inherent variations in the different populations,[5-7] as well as the detection methods used.[8,9]

Oral cancer surveys in Sudan found that squamous cell carcinoma was the commonest malignant lesion representing 66.5%. They also found that its prevalence was higher in men than in women.[10] In Sudan, OC is the fifth most common cancer type with about 920 cases per year, comprising 9% of the cases reported annually in Africa.[11,12] This is strongly attributed to the use of local type of snuff known as Toombak, a very popular material in the Sudanese community.[13,14] The association between Toombak dipping and OC has been investigated thoroughly during the last three decades.[15-18]

Many studies found a relatively high frequency of oral malignant neoplasms, particularly squamous carcinomas in men from Northern Sudan and of the Gaalein tribe, who lives in northern Sudan. Eastern side has shown relatively high occurrence of OSCCs with the absence of HPV infection, but no study from there has explored the possible causes.[19,20]

However, most of the previous studies from Sudan focused on the relationship between oral cancer and tobacco or alcohol abuse.[21-24] However, few studies from Sudan have investigated the association between HPV and oral cancer.[25] Therefore, the objective of this study is to determine the rate of HPV among OSCC patients.

Methods

In this retrospective descriptive study immunophenotyping of HPV was performed for 50 Sudanese patients with OSCC and 10 patients with oral benign lesions. The most common cancer site for the lesions was the tongue, which was implicated in 14 cases (28%), followed by the lower lip, palate, lower jaw and floor of the mouth constituting 11 (22%), 10 (20%), 8 (16%) and 7 (14%) respectively. The male: female ratio was 1.22: 1.00. The study also includes the subjects' history, age, occupation and socio-economic status. Sixty paraffin embedded tissue blocks and data from patients with oral benign and malignant lesions were retrieved for this study from two histopathology laboratories (total converge sample). Patients' files lacked demographic and behavioral information of the patients such as smoking, alcohol consumption and snuff dipping.

Ibrahim A.M. Ginawi
Department of Community Medicine, Hail University, Hail, Saudi Arabia.
E-mail: iginawi@gmail.com

Ebtihag A. Mahgoub, Hussain G. Ahmed
Department of Histopathology and Cytology, FMLs, University of Khartoum, Khartoum, Sudan

The study was approved by the Faculty Research Board of Sudan University for Science and Technology in collaboration with Radio Isotope Centre Khartoum (RICK).

Histopathology was performed using conventional hematoxylin and Eosin Mayer's method. The diagnosis was based on clinical examination and histological features of the biopsy. OSCCs were graded as well, moderate and poorly differentiated using Smith and Pindborg criteria. [24]

Serial sections on poly-L-lysine–coated slides for immunohistochemistry (IHC) and one section on a regular slide for hematoxylin and eosin stain were prepared from each case. The immunohistochemistry staining was performed as described previously.[27,28] Slides were heated overnight at 56°C, followed by deparaffinization through graded ethyl alcohols and rehydration. Before immunostaining with antibodies, the tissues were treated with 10 mM sodium citrate buffer at 100°C for 15 minutes for antigenic retrieval. The samples were incubated with 0.3% hydrogen peroxide (Merck, Germany) in methanol for 30 minutes to inhibit endogenous Peroxidase activity, washed 3 times with phosphate buffered-saline (PBS). After blocking nonspecific sites with horse normal serum (DAKO, Denmark) diluted in phosphate buffer (PBS), the slides were rinsed with distill water 2 × 5 minutes in PBS. Primary antibodies were incubated for 8 hours in a humidity chamber (Dako, Denmark), the procedure was performed by applying the avidin-biotin-Peroxidase complex method. After 2 × 5 minutes in rinse PBS, secondary antibody (LSAB2, DAKO) was incubated for 30 minutes in the same chamber. Detection of the primary antibody was obtained using the Strepto ABC, LSAB2 system (DAKO) according to the manufacture instructions. The slides were counter stained with hematoxylin, mounted and analyzed with light microscope. All slides were performed at the same time and submitted to standard methods. Known positive and negative cases were used as external controls. All slides (conventional histopathology and immunohistochemistry) were reviewed by two independent pathologists unaware to the original diagnosis.

Data were analyzed using SPSS program to obtain frequencies and percentages.

Results

In this study, a total of 50 patients with OSCCs and 10 patients with benign oral lesions were examined for HPV, their ages ranging from 11 to 80 years with a mean age of 46 years. Out of the 50 patients with OSCCs, 10 were found positive for HPV, while none of the benign lesions was found positive. Of the 10 positive cases, 5 were revealed as positive for HPV type 16, 4 for HPV type 18 and the remaining one was found positive for both HPV types.

The relationship between HPV and age was indicated in Fig. 1. Most positive cases were detected among age ranges 31-40 and 41-50 years. With regard to the tumor grade, the majority of patients were detected at the well differentiated grade representing 33 (66%) followed by moderate and poorly differentiated carcinomas, constituting 13 (26%) and 4 (8%), respectively. It appeared that among OSCC patients, the older patients tended to have a well differentiated grade of OSCC. In addition, older patients are more likely to have cancer, to have higher/worsening grades of cancer, and more likely to have HPV infection in general, as indicated in Table 1.

Regarding the gender and grade of oral squamous cell carcinoma, 26 (52%) were males, of whom 17 (34%) were diagnosed as well differentiated and 7 (14%) were moderately differentiated; while 2 (4%) were poorly differentiated squamous cell carcinoma. While 24 (48%) were females, of whom, 16 (32%) were diagnosed as well differentiated, 6 (12%) as moderately differentiated and 2 (4%) as poorly differentiated squamous cell carcinoma. (Table 1)

Of the 5 positive cases for HPV subtype 16; 4 (80%) were detected among females and one (20%) was among males. Of the 4 positive cases for HPV subtype 18; 3 (75%) were males and one (25%) was a female. The case that was found positive for both HPV subtypes was male.

The distribution of HPV infection according to the patient's residence was: 1 (2%) patient from Khartoum, 1 (2%) patient from the south and 8 (16%) were from northern Sudan. (Fig. 2)

Table 1 shows the relationship between the patient's residence and the grade of OSCCs. The majority of patients were coming from Northern Sudan 22 (44%) followed by Khartoum 11 (22%), south 7 (14%), east 6 (12%), west 3 (6%) and central Sudan 1 (2%). Among the OSCC patients from Northern Sudan, 15 (30%) were with well differentiated, 6 (12%) were with moderate, and 1 (2%) was with poorly differentiated OSCC.

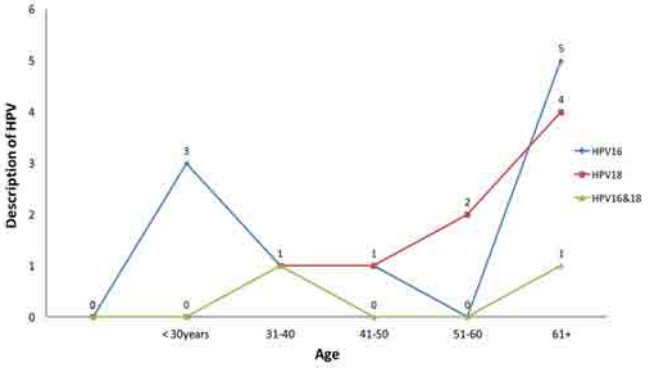

Figure 1: Description of HPV infection by age.

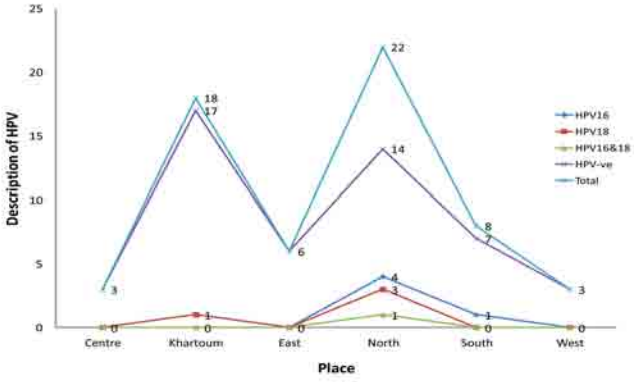

Figure 2: Description of HPV infection by Residence.

Table 1: Relationship between grade of cancer and demographical factors.

Age group	Grade of OSCC			Total
	Well differentiated	Moderately differentiated	Poorly differentiated	
< 30 years	1	1	0	2
31-40	4	1	0	5
41-50	9	7	2	18
51-60	14	3	2	19
61+	5	1	0	6
Total	**33**	**13**	**4**	**50**
Gender				
Males	17	7	2	26
Females	16	6	2	24
Total	**33**	**13**	**4**	**50**
Centre	0	0	1	1
Khartoum	8	2	1	11
East	5	1	0	6
North	15	6	1	22
South	5	3	0	7
West	1	1	1	3
Total	**33**	**13**	**4**	**50**

Discussion

The epidemiological association of HPV with OSCC is well established.[29,30] The prevalence of oral carcinomas reported to be associated with HPV has varied widely. This is due to differences both in the population studied and in the sensitivity of the assay used for HPV detection.[31] The prevalence of potentially malignant oral mucosal lesions and conditions shows wide variations between developed and developing countries. The primary risk factors for development of both OSCCs and potentially malignant lesions are considered to be similar. However, social habits of tobacco use and alcohol consumption have been strongly attributed to development of these lesions.[32] Squamous cell carcinoma was the most common malignant lesion (66.5%) found in the cases of oral cancer in Sudan.[33] It was also found that its prevalence was higher in men than women.[33] The use of Toombak (snuff dipping) has been stated to play a major role in the etiology of oral cancer in Sudan.[34] Since toombak use is a confounder factor in this setting, the absence of data regarding toombak use is a limitation in this study. The proprotion of positive cases for HPV in this study was found to be 20% (10/50), which is in agreement with reports from different countries.[35-37] Notably, spread of HPV infection is greatly varied in different countries around the globe; though some studies have reported lower frequencies, while others have reported very high proportions.[38,35] Nevertheless, HPV subtype 18 was relatively higher than the subtype 16, albeit, several studies have reported the predominance of subtype 16.[38,39]

In Sudan, most studies of oral cancer have attributed its etiology to the use of Toombak (tobacco rich Nitrosamine).[10-14] To the best of the authors' knowledge; no study has investigated the presence of HPV by immunohistochemical methods from Sudan, except only one study in this context, which examined the presence of HPV in tissues from Sudanese Patients with oral cancer.[40] They found similar findings as reported in the present study; although, somewhat relatively higher proportions of HPV subtype 16 was associated with OSCCs. However, several studies have reported that HPV subtype 16 is more frequently associated with oral cancer than HPV subtype 18.[38,39] However, one of the limitations of this study is that, the immunohistochemical results were not confirmed by molecular techniques such as, Polymerase Chain Reaction (PCR).

The mean age for all oral lesions was 46 years in the present study. Skinner and colleagues reported their patients to be in their fifth and sixth decades of life.[35] However, Bayat reported a mean age of 56.3 years, which is very close to our study findings.[41] With regard to the gender ratio, a similar finding was previously reported from Sudan that oral cancer is more common in males.[34] Moreover, a considerable number of patients present with advance stages of the disease (34%), among whom the male and female distribution was relatively similar.

The great majority of patients in this series were coming from Northern Sudan (44%) followed by Khartoum (22%). However, many studies have reported that the highest incidences of many cancers are from Northern Sudan.[38,40,41] Though Northern Sudan is inhabited by the lowest population estimate (approximately 64,000), compared with Khartoum (approximately 7000.000). The possible reasons behind this high incidence of OC in Northern Sudan are yet to be discovered.

One of the major limitations of this study is the method used for the detection of HPV in terms of sensitivity measures. Several methods have been used to diagnose infection with HPVs including cytological screening by Pap smear, electron microscopy, immunohistochemistry; but these methods have some disadvantages such as non-standardization and subjectivity, insufficient sensitivity and low predictable value.[44] The most perspective ways of HPV diagnosis is a direct detection of DNA of the human papilloma virus of high carcinogenic risk by the polymerase chain reaction and In-situ hybridization (ISH). Therefore, the number of patients infected might be more than what is reported in the current study. If HPV ends up being a significant causal factor in OSCC in a country that has high rates of OSCC, then perhaps more HPV screening should occur prior to the onset of cancer. Education at an early age could help prevent HPV infections which might impact cancer risk.

Another weakness in this study was the lack of demographic and behavioral information of the patients such as smoking, alcohol consumption and snuff dipping. This data is difficult to obtain in this setting as files were lacking.

Conclusion

Overall, the study found that there is association between HPV16 and 18 infections and OSCC in different regions of Sudan. The role of HPV 16 and 18 in association with demographical factors need to be investigated. Further studies using PCR are urgently recommended with large sample size and involving all parts of the Sudan to get conclusive results on the actual link of HPV infection and the development of OSCC in the Sudan.

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