

## Human Papillomavirus Type 16 in Sudanese Patients with Upper Respiratory and Digestive Tract Squamous Cell Carcinoma

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**Abstract:** HPV was detected in cervical and oral cancers however to the best of the knowledge, there is no study covering its presence in Upper Respiratory and Digestive Tracts Squamous Cell Carcinomas (UR and DT SCCs) in Sudan. This study aimed at finding out the relation (if any) between HPV type 16 and UR and DT SCCs in Sudanese patients and to study the histopathology of these tumors. It is a descriptive, cross-sectional study in which ninety five formalin-fixed paraffin embedded tissues of UR and DT SCCs were used for DNA extraction (Real Genomics, Genomic DNA extraction kit [Mini] (Tissue), Real Biotech Corporation (RBC), Taiwan) and conventional polymerase chain reaction to detect the presence of HPV type 16 in those tissues. Statistics Package of Social Sciences was used for data analysis. Person Chi-square test for statistical significance with the 95% confidence level was used. Out of 95 patients with UR and DT SCCs there were 47 (49.5%) males. HPV DNA type 16 was detected in 10.53% of cases. The relation between the site of UR and DT SCCs and the gender as well as the site and the degree of differentiation was found to be statistically significant ( $p:0.035$  and  $0.009$ , respectively), however there was no significant relation between those sites and the age categories that were studied ( $p:0.097$ ). Researchers concluded that HPV type 16 is unlikely to be a causative agent in UR and DT SCCs, however further studies are needed to support that and look for other viral types as a possible cause for these tumors.

**Key words:** HPV type 16, PCR, digestive tracts, cancer, gender, Sudan

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### INTRODUCTION

Certain Human Papilloma Virus (HPV) types are recognized as important human carcinogens (WHO, 2007). They are recognized as important causes of cancer of the anogenital tract and may be involved also in the etiology of cancers of Upper Respiratory and Digestive Tracts (UR and DT) (Franceschi *et al.*, 1990). UR and DT cancers account for 15% of all cancer cases in men worldwide (Parkin *et al.*, 1993) and approximately 270 additional cases occur in women per year (Franceschi *et al.*, 1990; Munoz and Castellsague, 1994).

In developing countries, UR and DT cancers are the most frequent cancers in males and the third most frequent in females (Parkin *et al.*, 1993). Tobacco smoking, alcohol drinking and betel nut chewing are associated

with high risk of up to 80% of upper aerodigestive tract cancers. This risk is further aggravated by nutritional deficiencies (Franceschi *et al.*, 1990). UR and DT Squamous Cell Carcinoma (SCC) can arise from oral, oropharyngeal, nasopharyngeal or esophageal epithelium. Knowledge of human papilloma viruses' prevalence and incidence in UR and DT SCC is crucial for treatment and prevention of these tumors.

Many studies had connected the infection with HPV to the etiology of UR and DT SCC in different parts of the world (Franceschi *et al.*, 1990). In Sudan, HPV was detected in oral and cervical SCC (Jadelkareem and Mergeny, 2010; Salih *et al.*, 2010). However to the best of our knowledge, there is no study covering its presence in UR and DT SCC. Therefore, this study was an attempt to find out the relation (if any)

between HPV type 16 and UR and DT SCC in Sudanese patients using conventional Polymerase Chain Reaction (PCR). It also aimed to study the histopathology of these tumors and relate it to gender and age.

**MATERIALS AND METHODS**

This is a descriptive, cross-sectional study. All patients who were diagnosed histologically to have UR and DT SCC at Dr. Elmubarak Private Laboratory and the Department of Histopathology at The National Health Laboratory, Khartoum, Sudan in the period 2005-2009 were subjected to the study. Exclusion criteria included unavailability of the paraffin embedded block and <1 cm<sup>3</sup> biopsy size.

Microsoft Excel and Statistics Package of Social Sciences (SPSS) were used for data analysis. Person Chi-square test for statistical significance (p-value) with the 95% confidence level and confidence intervals were used. Ethical consent was obtained from ethical committee of the Faculty of Medical Laboratory Sciences Research Board, University of Medical Sciences and Technology, Khartoum, Sudan.

**DNA extraction:** A DNA extraction kit (Real Genomics, Genomic DNA extraction kit [Mini] (Tissue), Real Biotech Corporation (RBC), Taiwan) was used to extract the genomic DNA from the Formalin Fixed Paraffin Embedded Tissue (FFPET) specimens. The written manufacture instructions were strictly followed.

**Polymerase Chain Reaction (PCR):** The virus was detected using conventional Polymerase Chain Reaction (PCR) as it was recorded as one of the most reliable diagnostic tools (Kleter *et al.*, 1999). The methods adopted were that of Barrow and Feltham (1993). Forward and reverse primers were reconstituted according to the manufacture instructions. HPV 16 forward sequence used was 5' TCA AAA GCC ACT GTG TCC TGA 3' and HPV backward was 5' CGT GTT CTT GAT GAT CTG CAA 3'.

**RESULTS AND DISCUSSION**

About 95 cases were included in this study. Of them, 47 (49.5%) were males. HPV DNA was detected in 10 (10.53%) samples out of the 95 samples. Out of these,

5 were males' samples. The difference between males and females was found to be statistically insignificant (p = 0.972). The studied patients were categorized in eight groups according to the age range. The relation between the age categories and HPV positive UR and DTSCC was not statistically significant (p = 0.769).

UR and DTSCC can occur in different anatomical sites and nasopharyngeal neoplasms accounted for 50.5% of this study population. The relation between these sites and HPV was studied and it was found to be of no statistical significance (p = 0.097). Histopathological examination of the studied UR and DTSCC revealed neoplastic lesions with different grades of differentiation. In females, 52.5% of the nasopharyngeal neoplasms were undifferentiated corresponding to only 20.7% in males. The poorly differentiated nasopharyngeal neoplasms were found to be higher in males (44.8%) than in females (21.1%). The same case applied to the well differentiated type (27.6 and 15.8%, respectively). The percentage of the moderately differentiated type was found to be 6.9% in males and 10.5% in females. The degree of differentiation was also studied in relation to HPV and there was no statistically significant relationship between them (p = 0.897).

The relation between the site of SCC in the UR and DT and the gender (Table 1) as well as the site and the degree of differentiation (Table 2) were found to be statistically significant (p = 0.035 and 0.009, respectively), however there was no statistically significant relation between those sites and the age categories that were studied (p = 0.097).

It was reported in some studies that HPV type 16 was the most common high risk strain detected in biopsies of oral SCC (Smith *et al.*, 2007) while it was found in only 1.54 (Jadelkareem and Mergeny, 2010) and 5% in others (Khovidhunkit *et al.*, 2008). In this study, the oral carcinoma was found to constitute only 1.1% of the UR and DT SCCs and it was negative for HPV type 16. Tobaccos smoking, alcohol drinking and snuff dipping were reported as risk factors for upper aerodigestive tract cancer. This risk is further shown to be aggravated by nutritional deficiencies (Elbeshir *et al.*, 1989; Franceschi *et al.*, 1990; Munoz and Castellsague, 1994; Ahmed and Mahgoob, 2007). Bearing in mind that all of these factors are prevailing in Sudan, the elevated level of oral cancer (68.5% out of 1,916 studied cases) reported in a descriptive epidemiologic study of oral

Table 1: The relationship between sites of upper respiratory and digestive tracts squamous carcinomas and the gender

Site/sex	Nasal	Nasopharangeal	Pharyngeal	Oropharyngeal	Oral	Tonsillar	Supraglottic	Hypopharyngeal	Pyiriform fossa	Post-cricoid
Male	43.8	60.4	0	50	0	33.3	33.3	0	100	0
Female	56.3	39.6	100	50	100	66.7	66.7	100	0	100

p = 0.035

Table 2: The relationship between sites of upper respiratory and digestive tracts carcinomas and degree of differentiation

Site/degree of differentiation	Nasal	Nasopharyngeal	Pharyngeal	Oropharyngeal	Oral	Tonsillar	Supraglottic	Hypopharyngeal	Pyriform Post-	
									fossa	cricoid
------(%)-----										
Well	25.0	22.9	100	50	0	16.7	44.4	50	40	25
Moderate	6.3	8.3	0	0	0	16.7	22.2	0	60	0
Poor	31.3	35.4	0	0	0	66.7	11.1	0	0	0
No	37.5	33.3	0	50	100	0.0	22.2	50	0	50
Not detected	0.0	0.0	0	0	0	0.0	0.0	0	0	25

p = 0.009

neoplasm in Sudan (Idris *et al.*, 1970) may be due to one of those factors instead of HPV. In one study, sixteen cases of nasopharyngeal carcinoma were examined for the presence of HPV types 16 and 18 genomes using PCR on paraffin wax embedded biopsy specimens. No DNA of either HPV subtypes was detected in those cases (Dickens *et al.*, 1992). Nasopharyngeal carcinoma constituted about 50.5% of UR and DT SCCs in this study; 33.3% of it was found to be undifferentiated neoplasms. The incidences of UR and DT SCCs were approximately similar in males and females but differ in site. This difference did not affect the incidence of HPV in both sexes.

In the current study, the poorly differentiated nasopharyngeal neoplasms were found to be higher in males (44.8%). The same applied to the well differentiated type (27.6 and 15.8%, respectively). The site of the tumor affects greatly the degree of differentiation. The researchers have no explanation but there may be a role for the differences in structure or function of the cells of that site that cause them to react differently in the presence of the etiological factor.

HPV was not detected in 103 cases of esophageal carcinomas studied in Japan (Saegusa *et al.*, 1997). Nevertheless, another study found type 11 to be more frequent in esophageal cancer (Matsha *et al.*, 2002). In China, HPV DNA was detected in 8% of Esophageal Squamous Cell Carcinomas (ESCC) (Chen *et al.*, 1994). In their trial to examine the potential roles of HPV in ESCC development, Shuyama *et al.* (2007) examined the presence of HPV DNA in paraffin-embedded tissue samples collected from two different areas with different ESCC incidence rates in China that is Gansu (n = 26) and Shandong (n = 33). In Gansu where ESCC incidence is much higher than in Shandong, HPV DNA was detected in 17 cases (65%) compared to only two (6%) in Shandong. HPV genotype 16 was detected in 79% of HPV-positive samples.

In this study, 10.53% of the investigated patients were found to harbor HPV type 16. This percentage was higher than that of others (Dickens *et al.*, 1992; Chen *et al.*, 1994; Saegusa *et al.*, 1997). On the other hand, this percentage was much lower than that of

Shuyama *et al.* (2007). The limitation of this study is that only type 16 was investigated. The same samples might harbor other types of the virus.

### CONCLUSION

There is a great variation of HPV DNA detection rates among UR and DT SCCs different anatomical sites and in different parts of the world. The role of HPV in the pathogenesis of UR and DT SCCs though has been extensively studied but it is still mysterious and further studies are needed to verify the role of HPV type 16 and other types as causative agents of these cancers.

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