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## Detection of Mucosal HPVs, HPV16 and HPV18 in Cervical Squamous Cell Carcinomas in Sudan

<sup>1,2,3</sup>Zahir A. Hilmi, <sup>2,4</sup>Nusiaba H. Abdo, <sup>3</sup>Bandar H. Aloufi, <sup>2</sup>Suheir R. Rehan, <sup>2</sup>Yousif, F. Hamedelnil and <sup>5</sup>Husam, M. Abdallatif

<sup>1</sup>University of Gezira, Center for Bioscience and Biotechnology, Faculty of Engineering and Technology, P. O. Box 20 Wad Medani, Gezira State, Sudan.

<sup>2</sup>Sudan University of Science and Technology, Department of Microbiology, College of Medical Laboratory Sciences, P. O. Box 407 Postal code 11111, Khartoum State, Sudan.

<sup>3</sup>University of Ha'il, Department of Biology, Faculty of Science, P. O. Box 2440, Ha'il, Kingdom Saudi Arabia.

<sup>4</sup>Elaj Al Riyadh Medical Center, Riyadh, Kingdom Saudi Arabia.

<sup>5</sup>International University of Africa, College of Medical Laboratory Sciences, Khartoum State, Sudan.

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### ABSTRACT

**Background:** Human papillomavirus (HPV) is the principal etiology for cervical cancer. Few contradictory researches were published in Sudan. **Objectives:** This research aimed to explore the roles of HPV16 and HPV18 in cervical cancer in Sudan. **Materials and Methods:** The DNA was extracted from sections of paraffin embedded tissues of Sudanese women (n, 63) with oral squamous cell carcinomas and controls (n, 17). The patients DNA samples (100ng/μl) were amplified by mucosal HPV GP5+/GP6+, HPV16 type specific and HPV18 type specific primers. **Results:** GP primers detected mucosal HPVs in 75% (n, 47) of the patients, and none of the controls. HPV16 and HPV18 types were identified from 70% (n, 33) and 9% (n, 4) of the GP positives. HPV16 was identified from all tumor differentiations degrees specially moderately differentiated tumors, while HPV18 was identified from moderate and well differentiated tumors. HPV16 was isolated from all ages but mostly isolated from older patients, while HPV18 was isolated from younger patients. Ten unknown mucosal HPV types were detected. HPV16 and HPV18 were mostly detected in house wives ethnically from Central Sudan. **Conclusion:** HPV 16 is the most important determinant for development of cervical cancer in Sudan, with less contribution of HPV18. HPV screening and vaccination program has to be established in young Sudanese women.

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## INTRODUCTION

Cervical cancer is the third most common cancer in women and the fifth most common overall cancer worldwide with an estimated incidence of 553,119 new cases and 288,109 deaths in 2010 (Ferlay *et al.*, 2012). HPV is the principal sexually transmitted oncogenic DNA tumor virus that causes cervical cancer (zur Hausen 1982; 1996; Gissmann *et al.*, 1983; Durest *et al.*, 1983; 1987; and Walboomers *et al.* 1999). About 75% of women in USA were reported to have HPV infection at least once lifespan (Bosch *et al.*, 2003). High-risk mucosal HPV 16 and HPV 18 were isolated and identified as the etiological agents of cervical cancer (Durest *et al.*, 1983; Boshart *et al.*, 1984 and Crum *et al.*, 1984; Muñoz *et al.*, 1992 and Bosch *et al.*, 1992). HPV16 and 18 were isolated from about 95% of cervical cancers (zur Hausen, 2009). Early oncoproteins E6 and E7 of HPV 16 and 18 were confirmed to transform cervical cells by degradation of tumor suppressor proteins P<sup>53</sup> and P<sup>Rb</sup>, respectively (Werness *et al.*, 1990; Dyson *et al.*, 1992; zur Hausen, 1996, 1999; 2000 and 2009). Based on epidemiology and carcinogenic potentials, HPVs were categorized into high and low risk viruses. Cervical cancer caused by persistent infection with high risk HPVs in patients who are unable to mount sufficient immune response (Vici *et al.*, 2014).

The Family Papillomaviridae, contains 29 genera formed by 189 papillomavirus (PV) types isolated from; humans (120 types), nonhuman mammals (64 types), birds (3 types) and reptiles (2 types) (Bernard *et al.*, 2010). Human PVs were members of five genera (Alpha-, Beta-, Gamma-, Mu- and Nu-PVs), with some biological properties shared within the genera. For instance, in the Alpha-papillomavirus genus, 15

**Corresponding Author:** Zahir A. Hilmi, University of Gezira, Center for Bioscience and Biotechnology, Faculty of Engineering and Technology, P. O. Box 20 Wad Medani, Gezira State, Sudan  
E-mail:zahir.hilmi@gmail.com

different species were found (de Villiers *et al.*, 2004 and Bernard *et al.*, 2010). *Papillomaviridae* are small DNA viruses with icosahedral non-enveloped 55nm capsid. All PVs contain a circular dsDNA genomes of  $\approx$  8 kb in size and typically contain eight genes (E1, E2, E4, E5, E6, E7, L1 and L2) with ten open reading frames -ORF (Bernard *et al.*, 2010; de Villiers *et al.*, 2004; Cann, 2005; zur Hausen, 1996 and Munger & Howley, 2002).

HPVs are large common heterogeneous group of up to 124 viruses and each type is known by a number (Bernard *et al.*, 2014 and de Villiers *et al.*, 2004). HPV absolutely species-specific and extremely tissue specific with tropism for either cutaneous or mucosal surfaces at specific sites. HPVs cause a variety of benign cutaneous or mucosal warts and papillomas (*condyloma acuminatum*) and cancer. Cutaneous HPVs contribute indirectly to skin carcinogenesis with sun UV (Jackson & Storey, 2000). Forty mucosal HPV types were isolated from ano-genital area: uterine cervix, vulva, vaginal wall, penis and anus. The International Agency for Cancer Research (IARC) showed that HPV 16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51, 56, to be the most common high risk HPV types associated with invasive cervical cancer (Munoz *et al.*, 2003, 2006; Smith *et al.*, 2007; Li *et al.*, 2010 and Bernard *et al.* 2013). HPV 16 accounting for over 50% and HPV 16 and 18 for >70% worldwide (Li *et al.*, 2010).

This study aimed to explore the role of HPV in cervical cancer in Sudan and to know the high risk mucosal HPVs responsible for cervical squamous cell carcinomas. The present study detected human papilloma viruses from Sudanese cervical squamous cell carcinomas. Also identified HPV16 and HPV18 from cervical cancer and linked them with the degree of tumor differentiation, age, sites and jobs. This study detected the presence of mucosal HPVs other than 16 and 18 in cervical cancer in Sudan.

## MATERIALS AND METHODS

### *Patients and controls samples:*

In this retrospective study, 63 samples of paraffin embedded sections of cervical cancer and 17 non cancerous controls were obtained from Sudan. All patients samples were confirmed histologically as cervical squamous cell carcinomas at the Department of Histopathology of the Sudanese National Health Laboratory. Their ages ranged from 20 to 85 years and their mean age was  $55.5 \pm 14.8$  years. Their tumor differentiation degree were; 30 moderately differentiated (47.6%; 30/63), 18 poorly differentiated (28.6% ; 18/63) and 15 well differentiated (23.8%; 15/63). Most of the patients and controls were house wives from Central Sudan.

### *DNA extraction:*

DNA was extracted using Viral-spin R Viral DNA extraction Kit (Intron Biotechnology, South Korea). This kit utilizes advanced silica-gel membrane technology for DNA isolation. The concentration of extracted DNA was measured and stored at  $-20^{\circ}$  C.

### *PCR analysis:*

#### *GP PCR for mucosal HPVs:*

The DNA quality and human genome integrity of some samples were tested by PCR for  $\beta$  Globin gene. Total cellular DNA (100ng/sample) of cervical cancer (n = 63) and controls (n = 17) were amplified by general primers GP5+/GP6+ (Oligo Microgen- Korea) that amplifying highly conserved regions within the L1 of mucosal HPVs associated with cervical cancer. The sequences of GP5+ forward was: 5'-TTTGTTACTGTGGTAGATACTAC-3' and for the GP6+ backward 5'-AAAATAAACTGTAAATCATATTC-3'.

The GP sequence was amplified using 40 PCR cycles with annealing temperature of  $55^{\circ}$  C for 60 sec were performed. Ten  $\mu$ l of PCR products were loaded into 2% agarose gel containing 2 $\mu$ l Ethidium Bromide. Electrophoresis was carried at 100 volt for one hour. The gel documentation system was used for photography. The GP5+/GP6+ positive diagnostic band for mucosal HPVs was 140 to 150bp (see plate 1).

#### *HPV16 and HPV18 type specific PCR:*

All samples positive for GP mucosal HPVs (n, 47) were rescreened using HPV16 and HPV18 type specific primers to identify HPV16 and HPV18. HPV16 was detected using HPV 16 forward 5'-TACCTACGACATGGGGAGGA -3' and that of HPV16 reverse primer 5'-GCAATTGCCTGGGATGTTAC -3'. Forty PCR cycles were carried for HPV16 type specific primers, with annealing temperature of  $56^{\circ}$  C/60 sec. Ten  $\mu$ l of PCR products were loaded into 2% agarose gel with Ethidium bromide for electrophoresis. The HPV16 diagnostic band was 194bp (see plate 2).

For HPV18 type specific PCR, the sequence for forward primer was; 5'- TGGTGTGTTGCTGGCATAATC -3' and for HPV18 reverse primer was; 5'- GCAGCATCCTTTTGACAGGT -3'. Forty PCR cycles were carried for HPV18 type specific primers, with annealing temperature of  $56^{\circ}$  C for 60 sec. Ten  $\mu$ l of PCR

products were loaded into 1.5% agarose gel with Ethidium bromide for electrophoresis. The HPV18 diagnostic band for was 339bp.

SPSS 12 and Excel 2007 were used for statistical analysis and to link the virological data with cervical cancer patients and controls.

## RESULTS AND DISCUSSION

Many decades ago high risk mucosal HPVs (16 and 18) were confirmed to be the etiology of cervical cancer (Durest *et al.*, 1983; Boshart *et al.*, 1984; zur Hausen, 1996; 1999; 2000 and 2009). Oncoproteins E6 and E7 of HPV 16 and 18 degraded the tumor suppressor proteins P<sup>53</sup> and P<sup>Rb</sup> (Werness *et al.*, 1990 and Dayson *et al.*, 1992). Few researches in Sudan detected HPV in 2%; 40%; and 94% of cervical cancers (Salih *et al.*, 2010; Elsbali *et al.*, 2012 and Eltahir *et al.*, 2012). This research aimed to explore the role of high risk HPV16 and HPV18 in cervical squamous cell carcinomas in Sudan. Also to study the link between these HPVs with tumor differentiation, age and sites.

In this research the GP5+/GP6+ PCR detected mucosal HPVs in 75% of cervical cancers, whereas all controls were negative (Table-1). The results of this study (75%) as less than those of previously published; Saudia (82%), Egypt (86%), Sir Lanka (90%), Sudan (94%) South Africa (97%) and 100% in 22 countries by IARC - Walboomers *et al.*, 1999 (Alsbaih *et al.*, 2013; Abd El-Azim and Lotfy, 2011; Karunaratne *et al.* 2014; Eltahir *et al.*, 2012 and Richter *et al.*, 2008). Three meta analytic studies estimated HPV positivity in the world to be from 83% to 90% (Cliffland *et al.*., 2003; Smith *et al.*, 2007 and Ciapponi *et al.*, 2011). In contrast to Eltahir *et al.*, 2012 who detected HPV in 8% of Sudanese controls, this study did not detected HPV in controls.

Less HPVs positivities were reported from Sudan (2.2%, 50% ), Iran (26%) and Egypt (50%) (Salih *et al.*, 2010; Elsbali *et al.*, 2012; Farjadian *et al.*, 2003, and Abd El-Azim and Lotfy, 2011). Low HPV positivities might be due to poor infrastructure of research in HPV molecular biology, and using HPV DNA detection strategies of suboptimal sensitivity (Clifford *et al.*, 2003).

In the present study, 70% (33/47) of the HPV types identified were HPV16 (70% = 33/47) and HPV18 (9% = 4/47) (Table 1). This study showed that HPV16 and 18 were responsible for about 80% of HPV positive cervical cancers in Sudan, however other authors in the world estimated that HPV16 and 18 to be responsible for 70% of cervical cancers (Clifford *et al.*, 2006 and de Sanjosé *et al.*, 2010 and Bernard *et al.*, 2013). Our findings on the HPV16/18 prevalence (80%) are more than that reported before about Africa (70%) ( Smith *et al.* 2007).

The remaining unknown other HPV types (n = 10; 20%)(see Table- 1) may be for (HPV; 31, 33 and 35) as in de Oliveira *et al.*, 2013 or may be HPV 31, 58, 33, 45, and 52 as reported before in Latin America by Ciapponi *et al.*, 2011 or may be for HPV (45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 70) as in Clifford *et al.*, 2003). In Colombia, Muñoz and Bravo (2012) reported that (HPV 16, 18, 45, 33, 31, 52, 58 and 35) account for about 90% of cervical cancer. Smith *et al.*, 2007, estimated that HPV31, 33, 35, 45, 52 and 58 to be responsible for 18% of cervical cancers.

### GP HPV negatives:

In this study HPV was negative in 25% (16/63) of the cervical cancer patients (Table 1). This may be due to absence of HPV DNA in the carcinoma cells or a false-negative PCR result due to integration of HPV DNA in the cervical carcinoma which may have disrupted PCR primer target sequences or resulted in loss of the L1 ORF. HPVs may have hit and escape phenomenon. The present study did not type for a broad range of HPV types. Presence of yet unknown HPV types that not amplified by the existing PCR primers, could not be ruled out (Clifford *et al.*, 2003). In this study paraffin embedded sections were used and fixation process may degraded integrated DNA that lower the sensitivity of PCR. PCR may detected only DNA of viruses with capsid which known to be resistant to organic solvents (de Villiers *et al.*, 2004). However, HPVs were detected in 99% of cervical fresh tissues (de Oliveira *et al.*, 2013). Usually very low viral load in these cases may give false negatives as invisible DNA signals or bands. Nevertheless, substantial methodological variations that may have influenced on the overall HPV positivity.

In contrast to Oliveira *et al.*, 2013, who detected more HPVs in older patients (> 50 years), this research detected HPVs in all ages. This may suggest more virulent oncogenic HPV variants in Sudan that may induces cervical cancer in younger women or may have inherited mutations in their tumor suppressor genes. However, comparison of their tumor suppressor genes and HPV oncogenes sequences with others from the world may give more acceptable explanations. In Sudan, Altahir *et al.*, 2010, studied P<sup>53</sup> and P<sup>Rb</sup> mutations in cervical cancer patients did not revealed such associations.

**HPV 16:**

This study identified HPV16 in 70% of HPV positive samples (Table 1) which is less than Abatia *et al.*, 2013 in Sudan (83%) and Ethiopia (91%). The HPV16 positivity in this study was more than those reported before in three meta analytic studies; 55% (Smith *et al.*, 2007), 53.2% (Ciapponi *et al.*, 2011) and 57.9% (Bernard *et al.*, 2013). Another meta analytic study estimated different HPV16 positivities in Asia (46%) and in North America and Australia (63%) (Clifford *et al.*, 2003). This might indicate different prevalence for HPV16 and HPV18 from continent to continent. Many authors indicated the presence of HPV16 variants, that may have various clinical outcomes and age linkages (Wentzensen *et al.*, 2013, and Zuna *et al.*, 2011).

Our finding that HPV16 was the most frequent HPV type in cervical cancer (70%), agrees with other authors from; Sudan (83%), Saudia (71%), Sirlanka (67.3%), and the World (Abatia *et al.*, 2013; Alsbeih *et al.*, 2013 and Karunaratne *et al.*, 2014; zur Hausen, 1987; Das *et al.*, 1999 and Bosch *et al.*, 2002). However, Ciapponi *et al.*, 2011 reported less HPV16 prevalence from Latin America (53.2%); Argentina (59.5%), Brazil (53.2%) and Mexico (54.9%). zur Hausen, 1987 showed that HPV16 DNA in cervical cancer present as episomal and not integrated and present in 50 to 80% of cervical cancers. Accordingly, HPV 16 is a stronger carcinogen and important determinant for the development of cervical cancer.

**HPV18:**

The present study identified four HPV18 isolates (9%)(Table 1), which is similar to reports from; Egypt (10%) and Sir Lanka (9.2%) (Abd El-Azim and Lotfy, 2011 and Karunaratne *et al.*, 2014). Our results were less than those of Bernard *et al.*, 2013 and Ciapponi *et al.*, 2011 who reported HPV 18 positivity of 12.8% and 12.6%, respectively. More HPV18 isolates were reported from; Argentina (17.6%), Brazil (15.8%) and Mexico (12.8%) (Ciapponi *et al.*, 2011). Less HPV18 positivities were isolated before from Sudan (Abate *et al.*, 2013 and Elsbali *et al.*, 2012) and Saudia (4%) (Alsbeih *et al.*, 2013). In agreement to other authors, this research confirmed that HPV18 was less frequent and it was not the major oncogenic HPV in cervical cancer (zur Hausen, 1987 and 2000). HPV18 was reported to be more prevalent (37.0% to 41%) in cervical adenocarcinoma and to be 12.8% in squamous cell carcinomas (CSCCs) which is in agreement with our results in CSCCs (IARC, 1995; Smith *et al.*, 2007 and Clifford *et al.*, 2003).

**Tumordifferentiation:**

As in this study a similar tumor differentiation of cervical cancer patients was reported from Sudan; moderate (47%), poorly (29%) and well (24%) (see Table 2) (Eltahir *et al.*, 2012). This study agree with other authors who detected mucosal HPVs and HPV16 in all stages of tumor differentiations (zur Hausen, 1987; Clifford *et al.*, 2003, and Smith *et al.*, 2007). HPV was detected mostly in 39.6% of total moderately differentiated samples and in 53.3% of HPV +ve moderate ( $P < 0.05$ ), then well differentiated and poorly differentiated (n, 11; 23.4%). This research isolated HPV16 from 70% of all HPV positive tumors, and identified HPV16 in HPV +ve tumors (n = 47), in moderately (36.2%) ( $P < 0.05$ ), well (19.1%) and poorly (14.9%)(Table 2).

In this study HPV18 was isolated from three moderately differentiated (6.4%), and from one well differentiated (2.1%) ( $P < 0.05$ ) (Table 2). This study detected ten mucosal HPVs by GP primers, their types were unknown as HPV16 and 18 type specific primers were used. These unidentified HPVs were detected in moderately (10.4%), poorly (8.5%), and well differentiated (2.1%). Our results showed that HPV mostly present in moderately differentiated tumors, and HPV16 present in all tumors. Also this study noticed that the unidentified HPV present also in poorly differentiated tumor, so these viruses may have different clinical outcome or virulent as HPV16. This study suggested that HPVs may required in early stages of tumor differentiation than late stages as poorly. This study confirmed the major role of HPV16 as strong carcinogenic virus causing 70% of cervical cancer in Sudan. Young Sudanese women has to be vaccinated against HPV16 and 18. The link between HPV and clinical outcome as tumor progression and differentiation, has to be clarified with an equal numbers of tumor differentiation stages.

**Age:**

The present study isolated more HPV16 from older ages  $\geq 56$  years (n, 18) ( $P < 0.05$ ), which is in contrast to Gargano, *et al.*, 2011 and Porras *et al.*, 2009, who linked HPV16 strongly to younger ages, (Table 3). This study identified three HPV18 isolates and six unidentified HPVs from younger ages. Ethnic variations and multiparty may explain our findings. Porras *et al.*, 2009 reported that 80% of cervical intraepithelial neoplasia - CIN3+ were associated with HPV 16 among young women ages 18-26 years compared with only 32% among women older than 55 years. This study and other authors identified HPV16 from all ages, and it is important alarm for cervical cancer regardless to age (Gargano, *et al.*, 2011 and Porras *et al.* 2009).

The present study agree with Munoz *et al.*, 2012 who thought that different HPV types may infect different age groups. HPV infection is most common in sexually active young women, 18 to 30 years of age and its prevalence sharply decreased after 30 years of age (Munoz *et al.*, 2012). However, cervical cancer is

more common in women older than 35 years, suggesting infection at a younger age and slow progression to cancer. Persistence of infection is more common with the high-risk oncogenic HPV types and is an important determinant in the development of cervical cancer (Munoz *et al.*, 2012).

Age is very important determinant for HPV infections and cancer. Age determines risk factor prevalence habits, smoking, marital status, exposure to high risk HPVs and the differential impact of specific HPV types, onset of sexual activities, lifetime number of sexual partners, parity, Multiparty, hormonal contraceptives using, menopausal status, virus prevention methods (condom), education and vaccination (Gargano *et al.*, 2011). Cancer is multistep disease that developed over a period of 10 to 20 years, and rely on accumulation of mutations in genome, so cancer developed in ages above 55 years (zur Hauzen, 2009). In Latin America and Caribbean the mean age of patients with cervical cancer was  $41.1 \pm 7.0$  years old (Ciapponi *et al.*, 2011). Oncogenic HPV viruses require another factors so virus/virus and virus/ cell interactions are very important. younger ages may have inherited cancer mutations in their oncogenes or tumor suppressor genes (P<sup>53</sup> and P<sup>Rb</sup>) (de Villiers *et al.*, 1997; Gargano *et al.*, 2011 and Porras *et al.* 2009). However, no mixed infections were detected in this study.

#### **HPV and geographic sites:**

de Oliveira *et al.*, 2013 in Prasil attributed differences of HPV infections and types in two different geographic sites to different ethnic origins and characteristics and the as patterns of sexual behavior. This study agree with other authors from Sudan, that cancer patients were mostly from northern sites (central Sudan) and tribes (Idris *et al.*, 1994 and 1995). It is widely accepted that each continent have different HPV types and variants that have different biological and oncogenic pthogenicities (Bruni *et al.*, 2010 and Karunaratne *et al.*, 2014).

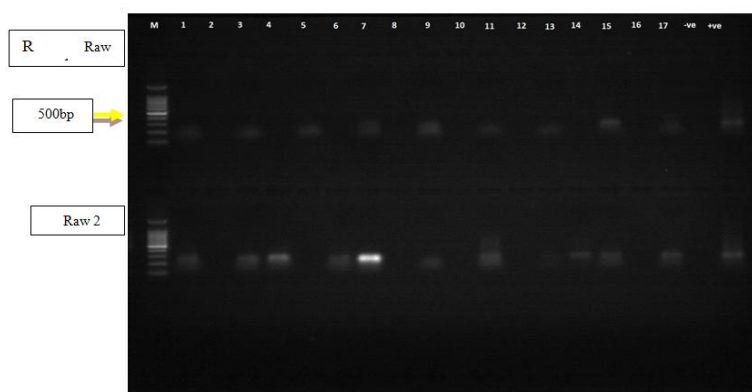
#### **General remarks:**

Reliable serological tools are not available and HPV culture is not possible, so molecular techniques are the only possible reliable methods for diagnosis and identification of HPVs (Dillner, 1999 and Koutsky *et al.*, 2005). Various molecular techniques such as PCR primers and probes have different DNA target sequence with variant annealing temperatures (specific PCR conditions), and have different sensitivities and specificities. Different results might be due to quality of histopathology, sample size and storage conditions (fresh, frozen tissues, paraffin embedded or exfoliated cells), viral load, researcher experience. New reagents for DNA extraction (DNA quality and quantity), staining and new PCR kits produce strongly visible bright bands. PCR regents has to be divided into aliquots and the reagents has to be placed in an ice pox during preparation.

L1 region is the most conserved part of HPV genome that targeted by many primers for detection of different HPV genotypes. Universal consensus or general PCR GP5+/GP6+ primers amplify a broad spectrum of HPV genotypes (de Roda Husman *et al.*, 1995). Type specific primers designed to amplify exclusively a single HPV genotype, multiple type-specific PCR reactions must be performed separately for the same sample. This method is laborious and expensive (Molijn *et al.*, 2005). However, multiplex PCR with type specific probes may be more economic. To obtain higher sensitivity, HPV general primers - GP has to be performed with low annealing temperature, and these GP PCR products (+ve or -ve) has to be subjected to a second round of HPV PCR with high risk HPV type specific primers. Using HPV type specific primers detected only targeted types and leave other HPV types that may produce false negatives. For more accurate sensitive results, the whole GP positive PCR product has to be precipitated and their bands cut and purified from the gel and cloned before using type specific primers. Commonly used HPV16 and 18 PCR type specific primers were designed from European variants and isolates not African ones and accordingly less sensitivities resulted (Wentzensen *et al.*, 2013 and Zuna *et al.*, 2011).

#### **Conclusion:**

In conclusion, mucosal HPVs were confirmed to be the causative agent for 75% of cervical squamous cell carcinomas in Sudan. HPV16 is the most prevalent tumor virus (70%) in Sudanese women with HPV positive cervical cancer. HPV18 has less contribution (10%) in causing cervical cancer, however mucosal HPVs other than 16 and 18 were isolated (10, 20%) and might contribute in cervical cancer in Sudan. Mucosal HPVs and HPV16 were isolated from all ages especially older Sudanese house wives from central Sudan. HPV16 was isolated from all tumor differentiation stages especially moderately differentiated tumors. HPV16/18 prophylactic vaccine in 9 to 10 years old girls will prevent 80% of HPV positive cervical squamous cell carcinomas in Sudan. There is a need to conduct larger group of women and in different part of Sudan to come with more concrete conclusion on best strains to be given as a vaccine.



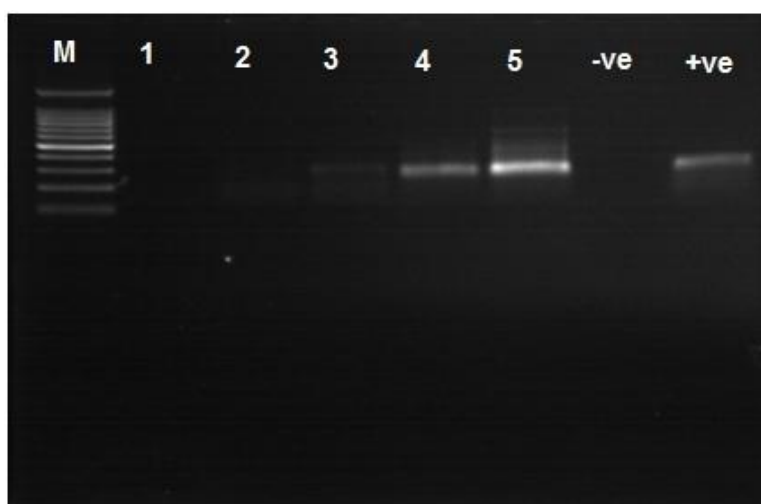
**Plate 1:** Mucosal HPV GP5+/GP6+ PCR in 1.5% agarose gel for Sudanese women with cervical cancer.

**Raw one:**

Lane M DNA ladder molecular marker. Lane 1 to 17 GP samples PCR product. Positive lanes [1, 3, 5, 7, 9, 11, 13, 15 and 17]. Negative lanes [2, 4, 6, 8, 10, 12, 14 and 16]. Controls; (-) negative and (+) positive.

**Raw two:**

Lane M DNA ladder molecular marker. Lane 1 to 17 GP samples PCR product. Positive lanes [1, 3, 4, 6, 7, 9, 11, 13, 14, 15 and 17]. Negative lanes [2, 5, 8, 10, 12, and 16]. Controls; (-) negative and (+) positive. (GP diagnostic band 140 to 150bp).



**Plate 2:** HPV 16 type specific PCR in 1.5% agarose gel for Sudanese women with cervical cancer.

Lane M DNA ladder molecular marker. HPV16 negatives lane [1, and 2] HPV16 positives lane [3, 4 & 5], Controls; (-ve) negative and (+ve) positive. (HPV16 diagnostic band 190 to 200bp).

**Table 1:** Detection of HPVs, HPV16 and HPV18 in Sudanese women with cervical cancer.

	GP5+/GP6+ PCR		HPV Type Specific PCR		
	HPV +ve	HPV -ve	HPV 16 +ve	HPV 18 +ve	Unknown HPV +ve
Number	47	16	33	4	10
Percentage	75%	25%	70.2%	8.51%	21.27%
Total	63 100%		47 100%		

**Table 2:** Isolation of HPV, HPV16 and HPV18 from Sudanese patients with cervical cancer with respect to degree of tumor differentiation.

	HPV +ve	HPV16 +ve	HPV18 +ve	Unknown HPV's +ve
<b>Tumor Differentiation</b>				
Moderate (n = 30) 47.6%	53.2% (25/47) P < 0.05	36.2% (17/47) P < 0.05	6.4% (3/47) P < 0.05	10.4% (5/47) P < 0.05
Well (n = 15) 23.8%	23.4% (11/47)	19.1% (9/47)	2.1% (1/47)	2.1% (1/47)
Poor (n = 18) 28.6%	23.4% (11/47)	14.9% (7/47)	Zero	8.5% (4/47)
	P < 0.05		P > 0.05	

**Table 3:** Isolation of HPV; HPV16 and HPV18 in Sudanese patients with cervical cancer according to age.

	HPV +ve	HPV16 +ve	HPV18 +ve	Unknown HPV's +ve
<b>Age</b>				
≤ 55 years	51.1% (24/47) P > 0.05	31.9% (15/47) P < 0.05	6.4% (3/47) P > 0.05	12.8% (6/47) P > 0.05
≥ 56 years	48.9% (23/47)	38.3% (18/47)	2.1% (1/47)	8.5% (4/47)

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