

Prevalence of Human Papilloma Virus Subtypes 52,56,58,59 and 66 Among Yemeni Patients With Cervical Cancer

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ABSTRACT

Background: Knowledge of high risk Human Papilloma Virus (HPV) subtypes might be helpful for development of strategies for decreasing the burden of risk of cervical cancer. Therefore, the aim of this study was to screen for some HR-HPV subtypes that are less common in many regions.

Methodology: A total of 150 tissue samples obtained from patients with cervical cancer in addition to 50 tissue samples obtained from patients with benign cervical lesions, were investigated for the presence of HPV subtypes 52,56,58,59 and 66 by Polymerase Chain Reaction (PCR).

Results: The prevalence of HPV subtypes 52,56,58,59, and 66, among cases was 0.6%, 0%, 4%, 3.3% and 0% respectively.

Conclusion: HPV subtypes 58 and 59 have a considerable contribution to etiology of cervical cancer in Yemen that requires further consideration.

INTRODUCTION

Cervical cancer is the third most common cancer in women worldwide, leading to about 300,000 deaths each year. Most cervical cancers are caused by human papillomavirus (HPV) infection [1]. HPV are double-stranded DNA viruses that infect the epithelium tissues and cause common skin warts. More than 100 HPV types have been recognized; they are distinguished by the genetic sequence of the outer capsid protein L1 [2]. About 40 types infect the mucosal epithelium; these are classified according to their epidemiologic relationship with cervical cancer. Infection with low-risk, or nononcogenic types, such as types 6 and 11, can cause benign or low-grade cervical cell abnormalities, genital warts and laryngeal papillomas. High-risk, or oncogenic, HPV subtypes act as prime causes of cervical cancer [3]. High-risk subtypes (currently including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 73, 82) can cause cervical precancerous and cancerous lesions. High-risk HPV types are detected in 99% of cervical cancers. Type 16 is the cause of approximately 50% of cervical cancers worldwide, and types 16 and 18 together account for about 70% of cervical cancers. Two oncogenes encoded in the HPV genome, E6 and E7 play critical roles in cervical cancer development [4].

The most common clinically important manifestation of persistent genital HPV infection is cervical intraepithelial neoplasia, or CIN. Within a few years of infection, low-grade CIN "called CIN1" may develop in to CIN2, CIN3 and further to cervical carcinoma [5].

Estimates of the population prevalence of HPV infection among women around the world range from 2% to 44% [6]. The wide variation in estimates is largely explained by differences in the age range of the populations studied and the sensitivity of the DNA assay used for detection of HPV infection. Overall, these DNA-based studies, combined with measurements of type-specific antibodies against HPV capsid antigens, have shown that most (>50%) sexually active women have been infected by one or more genital HPV types at some point in time [7]. (Therefore the aim of the current study was to screen for some HR- HPV subtypes that are less common in many regions.

MATERIALS AND METHODS

In this study 200 women with cervical lesions were retrospectively investigated for the presence of HPV subtypes 52,56,58,59 and 66 by Polymerase Chain Reaction (PCR). Of the 200, Formalin fixed paraffin embedded tissue samples, 150 tissue samples were obtained from patients with cervical cancer and the remaining 50 tissue samples were from patients with benign cervical lesions.

DNA Extraction

DNA was extracted according to the steps described in DNA extraction kit purchased from Sacace biotechnologies- Casera-Italy. The pellet obtained from previous steps was treated with 300 µl of Reagent 2 (lysis buffer) in addition 100 µl of sample, vortexed, incubated at 65°C for 5

min and centrifuged at (12000-16000 g) for 10 min and transfer the supernatant into new tube (sterile 1.5 ml Eppendorf tube) for DNA extraction. Vortexed vigorously sorbent and added 20 µl to each tube, Vortexed for 5-7 sec and incubated all tubes for 3 min at room temperature, then this step was repeated. Then all tubes were centrifugated for 30 sec at 5000 g and used a micropipette with a plugged aerosol barrier tip, carefully removed and discarded supernatant from each tube without disturbing the pellet. Tips was Changed between the tubes. 500 µl of Washing Solution was added to each tube. Vortexed vigorously and centrifuged for 30 sec at 10000 g. Supernatant was removed and discarded from each tube. This step was repeated and incubated all tubes with open cap for 5–10 min at 65°C. The pellet was resuspended in 100 µl of DNA-eluent. Incubate for 5 min at 65°C and vortex periodically. The tubes were centrifuged for 1 min at 12000x g. The supernatant was containing DNA ready for amplification stored at - 20°C until used.

Polymerase Chain Reaction (PCR) Amplification of HPV

Type specific primers for HPV subtypes 52,56,58,59, and 66 were used. DNA in cervical malignant lesions. Amplification was performed according to HPV subtypes 52,56,58,59, and 66kit from Sacace-Biotechnologies S.r.l. Caserta –Italy. The final reaction volume of 40 µl containing 20 µl mix-1(contained in PCR tubes), 10 µl of mix-2 and 10 µl of extracted DNA (sample). Negative control, positive HPV16 DNA and positive control 18 DNA tubes contained 10 µl of DNA buffer, 10 µl of HPV 16 DNA and 10µl of HPV18 DNA respectively. Samples and controls were amplified using Gene Amp PCR system 9700.

RESULTS

This study investigated 200 patients with cervical lesions, their ages ranging from 21 to 75 years with a mean age of 46 years. The presence of HPV subtypes 52,56,58,59 and 66 was investigated among 150 cases (patients with cervical cancer) and 50 controls (patients with benign cervical lesions). Positive detections were established among cases in 1/150 (0.6%), 0/150(0%), 6/150 (4%), 5/150 (3.3%) and 0/150(0%) of HPV subtypes 52,56,58,59, and 66, respectively. Amongst controls, only HPV subtype 58 was identified in 1/50 (2%). HPV infections were found in one case of Cervical Intraepithelial Neoplasia grade 1 (CIN1), two cases of CIN2, three cases of CIN3, five cases of squamous cell carcinoma of the cervix (SCC) and one case of Adenocarcinoma (CA), as indicated in Table 1, Figure 1. HPV subtype 58 is the most frequent representing 6/12 (50%) and detected in SCC (4/6 (66.7%) and remaining 2/6 (33.3%) CIN2. HPV subtype 59 was also identified in 5/12 (41.7%), of which 3/5 (60%) were identified in CIN3, as indicated in Table 1, Figure 2.

Table 1: Distribution of Cases by HPV subtypes 52, 56, 58, 59, 66 and pathology.

HPV subtype	CIN1	CIN2	CIN3	SCC	CA	Total
HPV52	0	0	0	0	1	1
HPV56	0	0	0	0	0	0
HPV58	0	2	0	4	0	6
HPV59	1	0	3	1	0	5
HPV66	0	0	0	0	0	0
Total	1	2	3	5	1	12

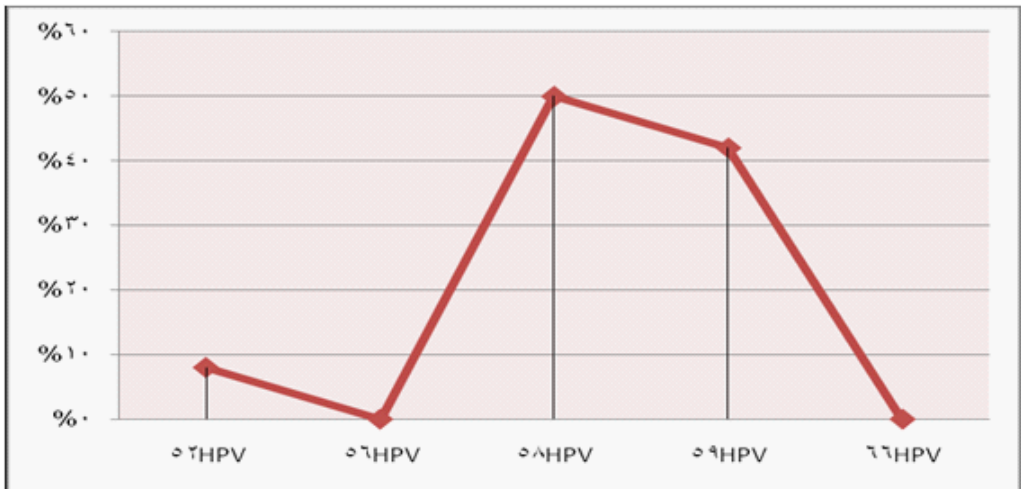


Figure 1: Description of cases by positive HPV subtypes 52, 56, 58, 59 and 66.

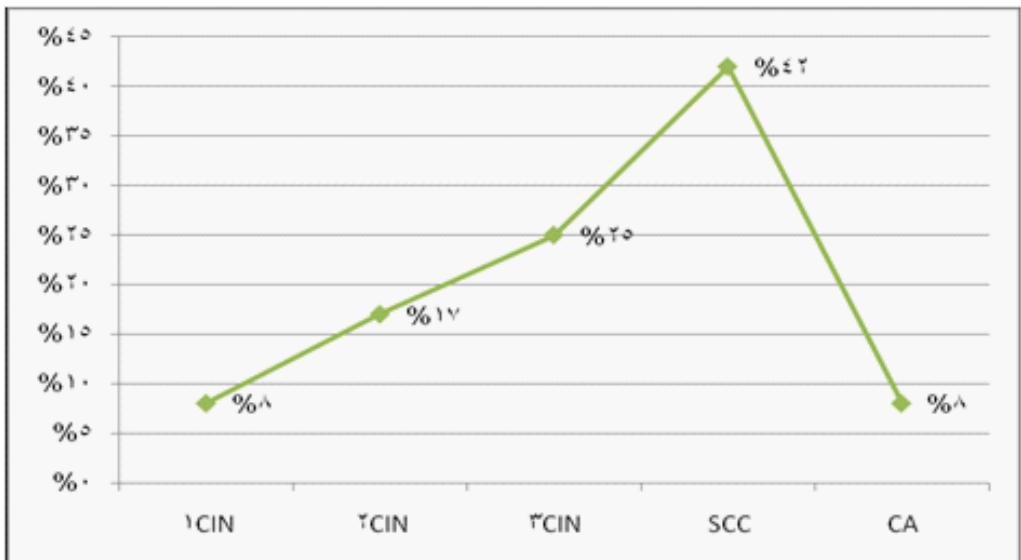


Figure 2: Description of cases by proportions of infections with HPV in different diagnostic findings.

DISCUSSION

About 20% of human cancers are related to viral infections [8] Of the estimated 12.7 million cancers cases in 2008 worldwide, 4.8% were assumed to be caused by HPV infection [9] HPV broad range of distribution around the world. Among women it ranges from 2% to 44%. (6) The most frequently focused types in relation to cervical cancer are HPV type 16 and 18 [10] Although, the HPV subtypes 58 and 59 are amongst the high risk HPV subtype, but they are less investigated in many HPV related cancer management settings. Therefore, in this study we tried to assess the burden of these subtypes. The role of these two subtypes as risk factors for occurrence of cervical cancer was previously reported [11].

However, HPV subtype 58, which is linked to a high risk of developing cervical precancerous and cancerous lesions, is rare worldwide but is frequently found in East Asia [12]. Overall HPV type 58 is the third most common oncogenic type in Asia, but causes only 3.3% of all global cervical cancer cases [13,14]. In Korea, HPV-58 is the second most common type diagnosed in women with abnormal cytological specimens (10.8% of all abnormal cytological specimens) [15]. Though the higher prevalence of HPV subtype 58 in East Asia is not completely understood, genetic characteristics and HPV subtype 58 variants with diverse oncogenicity are supposed to play a part [12,16]. Moreover, a considerable role of HPV type 58 toward the progress of squamous cell carcinoma in East Asia has been reported [17]. Additionally, HPV subtype 58 has been found in a relatively high percentage of patients with high-grade cervical dysplasia in China [12].

In this study most infections with HPV subtype 58 were identified in SCC. High prevalence of HPV58 among squamous cell carcinoma has been reported from China (28% in Shanghai, 10% in Hong Kong and 10% in Taiwan) and other countries in East Asia including Korea (16%) and Japan (8%) (Chan 2012, HPV 59 is a high-risk genital type that is frequently detected in genital specimens [18] and is associated with an increased risk of genital tract malignancy [19]. A comparative analysis of the HPV 59 genome with other HPVs showed close homology with HPV types 18 (71%), 45 (70%), and 39 (69%), types associated with a high risk of dysplastic cervical lesions [20]. The most conserved open reading frame, encoding the L1 major capsid protein, shares a 73–75% homology with HPV types 18, 39, and 45 [21].

In conclusion, the present results suggest that the assessment of prevalent of less prevalent HPV subtypes is important. In this study, we emphasize the high oncogenicity of HPV subtypes 58 and 59 for developing cervical cancerous lesions among Yemeni women. These findings are important when planning a HPV vaccination for the prevention of cervical cancer. Our findings also suggest that HPV subtypes 58 and 59 should be included in HPV vaccination regimens, if such initiative is created in Yemen.

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