RESEARCH ARTICLE

Prevalence of Human Papillomavirus subtypes 16 and 18 among Yemeni Patients with Cervical Cancer

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Abstract

Background: The Human Papillomavirus (HPV) is a DNA tumor virus that causes epithelial proliferation. There are more than 100 HPV subtypes, of which 13 subtypes are regarded as high risk subtypes that can cause cancers of epithelial mucosal surfaces. High risk human papilloma viruses (HR-HPV) subtypes 16 and 18 plays a major role in the etiology of cervical cancer worldwide. Therefore, the aim of this study was to screen for the existence of HPV16 and HPV18 among Yemeni women with cervical lesions. Methodology: Formalin fixed paraffin wax processed tissue blocks were retrieved for 200 patients (150 were previously diagnosed with cervical cancer and the remaining 50 were diagnosed with different benign conditions). Results: Of the 200 cervical cancer tissue specimens, HR-HPV 16 was identified in 74/200 (37%) samples and couldn’t be recognized in 126/200(63%) tissue samples. HR-HPV 18 was identified in 32/200 (16%) specimens and couldn’t be recognized in 168/200(84%) tissue specimens. Conclusion: HR-HPV subtypes were prevalent among Yemeni women with cervical cancer, with significant increase of HR-HPV subtype 16 over the HR-HPV subtype 18.

Keywords: HPV16- HPV18- Yemeni- cervical cancer- prevalence

Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted diseases worldwide, which is strongly involved in the pathogenesis of genital cancers such as cervical cancer (Zandberg et al., 2013). The majority of individuals who involve in multi-sexual activity becoming infected at some stages of their lifetime (Baseman and Koutsky, 2005). More than 130 HPV types have been well-known and categorized into low- or high-risk groups according to their potential for oncogenesis depending on persistent infection (Zur Hausen, 2009). According to the International Agency for Research on Cancer (IARC), the HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66, regarded as high risk Human Papillomaviruses (HR-HPV). The HPV types 6, 11, 40, 42, 43, 44, -53, 54, 61, 72, and 81, regarded as low-risk Human Papillomaviruses (LR-HPV) (Steben and Duarte-Franco, 2007). HPV genome encodes for early structural genes (E1 to E8) and late structural genes (L1 and L2). The late-coding regions yield structural proteins whereas the early-coding regions, mainly E6 and E7, are responsible for malignant transformation (Pyrri et al., 2011). E6 and E7 proteins of HR-HPVs have transforming activity that leads to autonomous or synergistic immortalization of the cell whereas; low-risk LR-HPVs have weak immortalization activity. This difference is thought to correlate with the ability of E7 proteins to make the degradation of the Rb gene rather than just their affinity for the Rb gene. HR-HPV E6 and E7 proteins can also induce mitotic abnormalities through mitotic spindle checkpoints, whereas, this is not realized in LR-HPV (Jemal et al., 2011).

A strong association has previously been built for the responsibility of HPV in oncogenesis of cervical cancer, and it is involved in other genital cancers (Parkin and Bray, 2006). Moreover, the influence of HPV is more far-reaching than initially supposed, with a strong link between infection and the development of other cancers such as, oral, pharynx and esophageal cancers (Zandberg et al., 2013).

Cervical cancer is the second greatest females’ cancer in developing countries with an estimated 445,000 new cases in 2012 (84% of the new cases world wide). Approximately 270,000 women died from cervical cancer in 2012; more than 85% of these deaths cases occurred...
in less developed countries. HPV types (16 and 18) are known to be responsible of 70% of cervical cancers and precancerous cervical lesions (WHO, 2015). HR-HPV types 16 and 18 are frequently associated with invasive cervical cancer than other types. This indicates that HR-HPV types 16 and 18 are more carcinogenic than other HPV types. Moreover, HR-HPV type 16-related cancers occur at a younger age than other cervical cancers (Pinkowish, 2009).

However, there is relatively complete paucity of data from Yemen regarding the association between HPV and cervical cancer. The few previous reports in this context have investigated HPV types other that HR-HPV types 16 and 18. Therefore, the aim of this study was to find out the prevalence of HR-HPV types 16 and 18, which is essential for any future strategies in term of control, prevention and vaccination.

Materials and Methods

In the present study 200 formalin fixed paraffin embedded tissue (PET) blocks were obtained from 150 patients previously diagnosed with cervical cancer and the remaining 50 with benign cervical lesions, their ages ranging from 21 to 75 years with a mean age of 47 years. The sample size represents a full coverage of patients whom their samples were referred to histopathology laboratories which provide diagnostic services for 6 provinces. All samples were confirmed by histopathologic diagnosis regardless to age and histological type. Samples were sequentially recruited from 6 Yemeni provinces (Aden, Azaal, Al-Janal, Tohama, Hadhramout and Saba’a) between March 2013 and July 2014. Specimens of patients with a history of neo-adjuvant treatments or another cancer or cancer that metastasized from other organs were excluded. Personal and demographic data as well as, clinico-pathological characteristics were obtained from each patient’s file.

DNA Extraction

Three cuts of 3-μm-thickness sections of PET were cut and put into 1.5 ml micro-tubes. Positive controls (HPV 16 infected SiHa cells and HPV 18 infected HeLa cells embedded in paraffin wax) and negative controls (blank paraffin wax block) were also used for quality control. Total DNA from PET was extracted from each specimen using DNA extraction kit purchased from Sacace biotechnologies-Casera –Italy. The 268 bp fragment of the β-globin gene amplification was used to assess the quality of DNA in PETs. The primers were GH20 (5’ GAA GAG CCA AGG ACA GGT AC3’) and PC04 (5’-CAA CTT CAT CCA CGT TCA CC-3’). Positive specimens for β-globin gene were used and indicated that the samples were accessible for the subsequent analysis.

Polymerase chain reaction (PCR) for detection of HPV

PCR was applied to amplify the HPV E6/E7 gene of HPV 16 and 18 using type-specific primers (type-specific PCR, TS PCR). Below were primer sequences (Wang, et al., 2008) : HPV16 E6: forward 5’-CTG CAA GCA ACA GTT ACT GCG ACG-3’, reverse 5’-CAT ACA TCG ACC GGT CCA CC-3’, product of 315 bp; HPV 18 E7: forward 5’-GAG CCG AAC CAC AAC GTC AC-3’, reverse 5’-GGA TGC ACA CCA CGG ACA AC-3’, product of 152 bp.

Gel-electrophoresis

The PCR products were visualized in 2% Agarose gel with 0.5 μg/ml Ethidium bromide. Ten micro liters of 100 bp DNA ladder and PCR product was loaded on the gel. Gel-electrophoresis was made at 120 V and 36 mA for 60 minutes. Images were reserved by Gel documentation system (Gel mega, digital camera and software in a computer).

Statistical Analyses

Data management was done by using the Statistical Package for Social Sciences (SPSS version 16; SPSS Inc, Chicago, IL). SPSS was applied for analysis and to perform Fisher exact test for statistical significance (P value < 0.05 was considered significant). The 95% confidence level and confidence intervals were used.

Results

Of the 200 cervical cancer tissue specimens, HR-HPV 16 was identified in 74/200 (37%) samples and couldn’t be recognized in 126/200(63%) tissue samples. Out of the 74 infected specimens, 72/74 (97.3%) were found among cervical cancer’s patients and 2/74 (2.7%) were found among those with benign cervical lesions. The prevalence of HR-HPV type 16 was 72/150 (48%). With 95% confidence level, the risk associated with relationship between HR-HPV subtypes 16 and 18 and malignant cancers occurs at a younger age than other cervical cancers (Pinkowish, 2009).

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Table 1 Distribution of the Study Population by Age and HR-HPV Subtypes 16 and 18 Infection

|                | <35years | 36-45 | 46-55 | 56+ | Total |
|----------------|----------|-------|-------|-----|-------|
| HR-HPV 16      |          |       |       |     |       |
| Positive       | 18       | 27    | 24    | 5   | 74    |
| Negative       | 13       | 33    | 48    | 32  | 126   |
| Total          | 31       | 60    | 72    | 37  | 200   |
| HR-HPV 18      |          |       |       |     |       |
| Positive       | 6        | 9     | 13    | 4   | 32    |
| Negative       | 25       | 51    | 59    | 33  | 168   |
| Total          | 31       | 60    | 72    | 37  | 200   |
frequency of infection rates were identified among age range 46–55 years representing 13/32 (40.6%) followed by age range 36–45, <36 and 56+ years, constituting 9/32 (28%), 6/32 (18.8%) and 4/32 (12.6%), in this order.

On the other hand, when computing the percentage in each group, for HR-HPV subtype 16, the extreme percentage of infection was found in age group <35 years constituting 58% followed by 36–45, 46–55, and 56+ years, demonstrating 45%, 33%, and 14%, respectively. For HR-HPV subtype 18, the great percentage of infection was identified in age group <35 years constituting 19% followed by 46–55, 36–45, and 56+ years, establishing 18%, 15%, and 11%, as indicated in Figure 2.

In respect to the residence, the great majority of HR-HPV subtype 16 positive cases were coming from Aden representing 22/74 (29.7%) followed by Al-Janad, Azaal, and Tohama, constituting 18/74(24.3%),14/74 (19%) and 10/74 (13.5%), respectively. For HR-HPV subtype 18, most positive cases were form Al-Janad representing 10/32 (31.3%) followed by Azaal representing 9/32 (28%), as shown in Figure 3.

Nevertheless, when calculating the percentage within each entire residence, for HR-HPV subtype 16, the highest percentage of infection was found in the Aden representing 49% followed by Tohama, both (Azaal and Al-Janal) and Saba’a constituting 42%, 35%, and 33%, in this order. For HR-HPV subtype 18, the great proportion was identified in Azaal representing 22.5% followed by Al-Janal, Tohama and both (Hadhramout and Aden) constituting 19%, 12.5% and 9%, as indicated in Figure 4.

Regarding the relationship between infection with HR-HPV16 and 18 and diagnosis, most positive cases of HR-HPV16 were associated with Squamous Cell transformation was statistically significant, P < 0.001, as indicated in Figure 1.

HR-HPV 18 was identified in 32/200 (16%) specimens and couldn’t be recognized in 168/200(84%) tissue specimens. Out of the 32 infected specimens, 31/32 (96.9%) were found among cervical cancer’s patients and 1/32 (3.1%) was identified among those with benign cervical lesions. Moreover, co-infections of both types were identified among 10 patients, representing 10/74(13.5%) of HR-HPV 16 and 10/32 (31.3%) of HR-HPV 18. The prevalence of HR-HPV type 18 was 31/150 (20.7%).

As shown in Table 1, for HR-HPV subtype 16, the highest frequency of infection rates were identified among age range 36–45 years representing 27/74 (36.5%) followed by age range 46–55, <36 and 56+ years, constituting 24/74 (32.4%), 18/74 (24.3%) and 5/74 (6.8%), respectively. For HR-HPV subtype 18, the highest

### Table 2. Distribution of the Study Population by HR-HPV Subtypes 16 and 18 by Diagnosis

| HR-HPV | Diagnosis      | Benign | CIN1 | CIN2 | CIN3 | SCC | ADC | Others |
|--------|----------------|--------|------|------|------|-----|-----|--------|
| Positive | HR-HPV16     | 2      | 2    | 5    | 11   | 46  | 5   | 1      |
| Negative | HR-HPV16     | 48     | 13   | 8    | 5    | 34  | 14  | 3      |
| Total    | HR-HPV16     | 50     | 15   | 13   | 16   | 80  | 19  | 5      |
| Positive | HR-HPV18     | 1      | 1    | 3    | 4    | 9   | 12  | 2      |
| Negative | HR-HPV18     | 49     | 14   | 10   | 12   | 71  | 7   | 3      |
| Total    | HR-HPV18     | 50     | 15   | 13   | 16   | 80  | 19  | 5      |
Carcinoma (SCC) constituting 46/74 (62.2%) followed by Cervical Intraepithelial Neoplasia grade 3 (CIN3), CIN2 and Adenocarcinoma (ADC), representing 11/74 (14.9%), 5/74(6.8%), and 5/74(6.8%), respectively. For HR-HPV18, positive cases were linked to ADC, representing 12/32 (37.5%) followed by SCC, CIN3 and CIN2, constituting 9/32 (28%), 4/32 (12.5%) and 3/32(9.4%), in this order, as shown in Table 2.

Nevertheless, in calculating the percentage of the positive cases within each diagnostic group, we found that for HR-HPV16, the highest proportion of infection was identified among CIN3 patients constituted 68.8%, followed by SCC, CIN2 and ADC, representing, 57.5%, 38.5% and 26.3%, respectively. For HR-HPV16, the highest percentage of infection was found in ADC constituted 63.2%, followed by others, CIN3, and CIN2, representing, 40%, 25% and 23%, respectively, as indicated in Figure 5.

**Discussion**

Cervical cancer is the fourth most common cancer that affects women worldwide (Ferlay et al., 2013). It is believed that more than 95% of cases of cervical cancers are associated with a history of persistent infection by HR-HPV (Walboomers et al., 1999; Elasbali et al., 2012). Striking variations in the incidence of cervical cancer exist between developed and developing countries. Even within developed or developing world, HPV infection rates differ significantly between geographic regions and population clusters.

In the present study we investigated HR-HPV types 16 and 18, since they were the most common cervical cancer associated types, and are believed to be responsible for over 70% of cases of cervical cancer (Koutsy, 1997). This is one of few studies of its kind, which highlights the HR-HPV types 16 and 18 prevalence in cervical cancers in Yemen. There are some studies investigated HPV from Yemen, but devoted to subtypes other than HR-HPV types 16 and 18. In a recent study from Yemen that investigated 84 patients with cervical cancer for the presence of HPV subtypes 16 and 18 applying both immunohistochemistry and molecular techniques; they found that 73.8% were infected with HPV subtypes 16 and 27.4% were infected with HPV subtypes 18 (Muñoz et al., 2004). This study shows relatively inconsistent higher prevalence rates from our findings. Another study from Yemen, found prevalence rates of HPV subtypes 52, 56, 58, 59, and 66, among cases were 0.6%, 0%, 4%, 3.3% and 0% respectively (Bensumaidea et al., 2014a). Another study investigated the presence of HPV subtypes 31, 33, 35, 39 and 45 among Yemeni patients with cervical cancer; the study identified HPV 31, HPV 33, HPV 35, HPV 39 and HPV 45 in 10/150 (6.7%), 6/150 (4%), 6/150 (4%), 5/150 (3.3%) and 10/150 (6.7%), respectively. The prevalence of these HPV subtypes among Yemeni cervical cancer patients was 24% (Bensumaidea et al., 2014b).

The findings of this study showed prevalence rates of 48% for HR-HPV type 16 and 20.7% for HR-HPV type 18. These findings show little variation from that reported by the International Association for Research in Cancer (IARC), in which they report prevalence rates of 53% for HPV subtype 16 and 15% for HPV subtype 18 (Ahmed et al., 2015). Studies on the prevalence of HPV genotypes in cervical cancer consistently indicate that infection with HR-HPV type 16 is the most common in a variety of studies from diverse regions (Muñoz, 2000; Zhi et al., 2015; Wang et al., 2015). Then followed by infection with other HR-HPV types including HR-HPV subtype 18 (Siddiqa et al., 2014). In general HPV infection rates differ significantly between geographic regions and population clusters, (Clifford et al., 2005; Koutsy, 1997). Particularly, in the Middle East countries (Elasbali et al., 2012). However, assessment of HR-HPVs in the pathogenesis of cervical cancer and other cancers in Yemen is essential in order to evaluate the status for future plan of prevention strategies including vaccination against HPVs. In this study, we present an additional evidence of the existing epidemiological evidences regarding the presence of HPV in cervical cancers in Yemen and the potential need for vaccination against HR-HPV infections, particularly HR-HPV 16 and 18 and its effect on human health in this conflicting country. Co-infections of both HR-HPV 16 & 18 types were identified in 13.5% of the patients. This percentage is much lower than reports from highly HPV endemic areas which ranging from 30% to 65% (Al Moustafa et al., 2014; Torres-Ibarra et al., 2014).

In regard to the relationship between age and HR-HPV type 16, the highest percentage was found in age range 36–45 years (36.5%), followed by age ranges 46–55 (32.4%) and <36 years (24.3%). Many studies showing that HPV16 positivity was significantly associated with younger age, particularly when early diagnosis is employed, such as in diagnosis of CIN. This increasing infection will decrease with increasing of age (Lebelo et al., 2015). In study investigated 317 women with CIN3, HPV subtype16 was recognized in 70% of those with age range of 16-25, 59% of 26-35, and 48% of ≥36-year-olds (P < 0.025). This link acquired the form of a tendency with decreasing HPV type 16 prevalence with increasing of age (P < 0.008). This means that HPV type 16 is commoner in younger women with high-grade cervical lesions. This report seems to show some sorts of discrepancies with our findings, which might be attributed to the large number of cervical cancers (69.3%) over the precancerous lesions (30.7%) in our series. A recent study has shown that HPV16 is responsible of absolute risk of ≥CIN3 equally in women aged 25-29 and ≥30 years (14.2% and 15.1%, respectively) followed by HPV31 (8.0% and 7.9%), HPV52 (6.7% and 4.4%) and HPV18 (2.7% and 9.0%). The positivity increased significantly with disease development for HR-HPV16 and HR-HPV18 which were accountable for 45.6% and 8.4% of ≥CIN3, respectively. Notable, HPV 18 was responsible for 50% of adenocarcinoma in situ and 50% of invasive cancer cases (Baandrup et al., 2012). This also explains our results, since they were dedicated to older population. About 84.5% of the studied patients in the present study were older than the age of 35 year-olds.

In regard to the relationship between age and HR-HPV type 18, the highest percentage was found in age range 46–55 years followed by age ranges 36-
45 and <36, representing, 40.6%, 28%, and 18.8%, respectively. Although, HR-HPV 18 shows relatively similar association in regard to the age, but some studies agree with our findings that it tends to occur at relatively higher age (Monsonego et al., 2015).

Furthermore, in the present study, HR-HPV16 was frequently identified in SCC and CIN. These findings were in consistency with several studies (Clifford et al., 2005; Newall et al., 2008; Clifford et al., 2003). On the other hand, most of cases of HR-HPV18 infection were found to be related to adenocarcinoma. Several studies have shown that HPV18 is more strongly linked with ADC than SCC (Ciapponi et al., 2011, Vandenbroucke et al., 2013; Chen et al., 2015).

In regard to the relationship between HR-HPV 16 &18 and residence, there is no specific justification for variations in the proportions of the infections, since all social practices and beliefs are relatively common all over the 6 provinces.

The limitations in this study include non-representativeness of the sample for all Yemeni provinces; exclusion of the other High risk and low risk HPV types, although these HPV types were reported elsewhere.

Further studies on the distribution of all HPV subtypes in Yemeni women of all Yemeni provinces are still required.

In conclusions, the rate of infection with HR-HPV 16 and 18 is relatively higher in Yemen with predominance of HR-HPV type 16. This study may provide valuable data for future overall management including; prevention, treatment of HPV infection, cervical cancer and endorse the urgent demand for HPV vaccines.

Ethical consent
This study was approved by concerned laboratories in Yemen and by the ethics board of the Faculty of Medical Laboratory Science, Sudan University for Science and Technology (where the study was carried out). All cervical tissues were obtained as a part of the specimens required for diagnosis.

Competing interests
The authors declare that they have no competing interests.

Authors’ contribution
- HGA and FDA: Contributed to the design of the study, data analyses, interpretation of results, and draft and approval of manuscript.
- SHB, FSA, BAM, MZA and IAA: Data Collection and Molecular identification, and draft and approval of manuscript.

“All authors read and approved the final manuscript.”

Acknowledgments
The authors would like to thank people at histopathology Laboratories at all 6 mentioned Yemeni provinces, for helping in the sample collection.

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