Genotyping of High-risk Human Papilloma virus (HPV) among Iraqi women in Baghdad by Multiplex PCR

Abstract

Human papilloma virus (HPV) infection has a causative factor for cervical cancer. Early detection of high risk HPV types might help to identify women at high risk of cervical cancer. The aim of the present study was to determine the occurrence of the high-risk HPV infection in population of Iraqi women in Baghdad using Multiplex PCR to determine the percentage and genotyping of Human Papilloma Virus and to put the best prevention and control program in Iraqi women.

Key words: Human Papilloma Virus (HPV), PCR, cervical neoplasia Pap smear

Introduction

Human papillomavirus infection has a central role in the etiology of cervical cancer [1]. More than 100 HPV types have been described and 40 can infect the anogenital tract [2]. Genital HPV types are categorized according to their association with cervical cancer [3]. About 20 are classified as high-risk (HR) types and are associated with cervical cancer and precancerous lesions, as well as low-grade cervical pathology. Worldwide, HPV types 16 and 18 cause approximately 70% of cervical cancers; HPV types 31, 33, 35, 45, 52 and 58 account for an additional approximately 20% of cases, although there is substantial geographical variation in the relative frequency of different HR types [4]. Low risk
HPV types, including HPV6 and 11, cause low-grade cervical lesions, genital warts and recurrent respiratory papillomatosis.

Cervical cancer is the second most common cancer among women globally with 400,000 cases of invasive cervical cancer diagnosed yearly [5]. Evidence from epidemiological studies conducted worldwide clearly indicates that HPV infection is the cause of cervical cancer [1,6]. Whereas cervical cancer prevention currently relies on screening and treatment of precancerous lesions, new technologies such as HPV testing [7] and the development of HPV prophylactic vaccines [8] will offer alternatives for prevention and control in the near future [9].

Women positive for HPV DNA in cervical cells have a higher risk of developing cervical cancer [10], which accounts for about 10% of newly diagnosed cancers in women worldwide [11]. In women with abnormal cervical cytology, HPV was reported to have a high prevalence [12]. The advent of polymerase chain reaction (PCR) procedures increased the detection level, and therefore, the estimated prevalence of HPVs in normal or abnormal cytological samples of women [13]. It has been suggested that the molecular identification of HPVs (HPV testing in cervical scrapes) may be very useful for primary screening or secondary triage of patients with certain lesions [14].

Women who are infected with high risk HPV type have an approximately 100-fold increased risk of developing cervical cancer than non-infected women. Therefore, it has been suggested that high risk HPV detection might be used as a tool to identify women at high risk of cervical cancer, in addition to Pap smears [15].

In Iraq the prevalence of HPVs in women has never been reported. The aim of this study, to determine the occurrence of HPV infection in population of Iraqi women in Baghdad by using Multiplex PCR to determine the percentage and genotyping of Human Papilloma Virus present in Iraqi women and to put the best prevention and control program in Iraqi women.

Materials and Methods

A total of 856 women between 16 to 70 years of age participated in this study from January 2009 to March 2010. The population was consecutively recruited from the Gynecology Departments of Women Health Center at Al-Elwiyia Teaching Obstetrics Hospital, Al-Samarrai Hospital of infertility and cytological department in central public health laboratory in Baghdad, study done in Central Public Health Laboratory / Molecular Biology Department. The groups were comprehensive assigned to receive screening by HPV-DNA testing 856 women of which 218 women testing cytologically. The study was approved by ethical committee in CPHL /MoH /Baghdad. All subjects underwent their scheduled examination, which included the placement of a speculum in the vagina, visualization of the cervix, and collection of cervical cells using a cotton swabs, sample material was rinsed into a liquid medium (supplied with DNA extraction sorb A kit, Sacace company), transported to molecular biology department in central public health laboratory for HPV molecular analysis. For 218 cases used a wooden spatula and endocervical brush for the preparation of a standard cervical smear. Pap smears were classified by local cytopathologists.

The DNA from cervical samples was extracted and purified, using DNA-Sorb-A DNA extraction kit (REF K-1-1/A VER 29.03.06 Sacace company), according to the manufacturer's instructions. Briefly, added to each tube 300 μl of Lysis Solution. and 100 μl of Samples, incubated for 5 min at 65°C. Then Centrifuged (12000-16000 g.). Transferred the supernatant into a new tube for DNA extraction, 20 μl of Sorbent were added, and incubated for 3 min at room temperature, centrifuged for 30 sec at 5000g, and then discard supernatant. 500 μl of Washing Solution were added to the pellet, vortex and centrifuged for 30 sec at 10000g and then repeated this step. Incubate with open cap for 5-10 min at 65°C. Resuspend the pellet in 100 μl of DNA-eluent. The entire extracted DNA was stored at -20°C. HPV detection and typing was performed using HPV High Risk Typing PCR Kit (Sacace Company, Italy, Catalog No. V- 25-50R). HPV High Risk Typing was an in vitro nucleic acid amplification test for qualitative detection and genotyping of HPV types 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 66 in the cervical swabs. Each PCR-mix-1 tube contained primers directed against regions of four HPV types and globine gene used as Internal Control. The reaction done by Multiplex -PCR reaction of 25 μl and contained: 5 μl of PCR-mix-1, 10 μl of 2.5X buffer-red, 0.5 μl of Hot Start Polymerase, 15 μl of Reaction Mix, 1 drop (25 μl) of Mineral Oil and 10 μl of DNA sample. Reaction was run for 42 cycles, under the following condition: 15min at 95°C, 30 sec at 95°C, 30 sec at 63°C and 40 sec at 72°C. The final elongation step of 1 min at 72°C was performed to complete the elongation. Amplification products were visualized under UV light after electrophoresis for 45 min through a 3% agarose gel containing ethidium bromide. ( Sacace Company) The statistical analysis system [16] was used to data analysis, the least significant difference –LSD test was used to compare between means, and the chi-square test was used to compare between percentages.
Results
The study encompasses 856 women with a range 16-70 years age groups. HPV-DNA was detected in 106 (12.38%).

Figure (1, 2) shows the age distribution of population under investigation, discriminating HPV positive and negative women; two peaks in age groups 21 to 30 and in 31 to 40 years old are clearly in evidence. Women younger than 21 years and older than 40 years had a lower HPV prevalence. Twelve high risk HPV types were detected. Single infection were found in 87 women 82.07% and multiple infections were detected in 17 (16.04%) cases, 15 women have 2 genotype infection and 2 women have 3 genotypes infection Figure (3).
HPV was detected in 106 (12.38%) of the study population. Our results show that the overall HPV prevalence twelve genotypes were identified, including HPV-33 (18.60%), HPV-35 (18.60%), HPV-56 (18.60%), HPV-39 (10.85%), HPV-52 (10.08%), HPV-18 (7.75%), HPV-16 (4.65%), HPV-59 (4.65%), HPV-58 (2.32%), HPV-31 (1.55%), HPV-45 (1.55%) and HPV-66 (0.77%) Figure(4).

Groups testing cytologically with HPV DNA testing was 218 (27.36%) out of 856 women. Women with non malignant cytology was 198 (90.83%), 24(12.12%) of them was HPV positive and 174(87.88%) of them was HPV negative, and those with neoplastic cytology was 20(9.17%) women , 5(25%) of them was HPV positive and 15(75%) of them was HPV negative Table (1). Women with neoplastic cytology 16 (80%) was Cervix Intraepithelial Neoplasia grade 1 (CIN 1), 3 of them was HPV positive , 3(15%) of neoplastic cytology was (CIN 2), 2 of them was HPV negative and 1 woman was HPV positive , and 1(5%) woman with cervical carcinoma was HPV positive Table(2) .

Table (1): Cytologically with HPV-DNA testing (n-856)

<table>
<thead>
<tr>
<th>Cytology</th>
<th>No. of women cytology negative</th>
<th>% of women cytology negative</th>
<th>No. of women with HPV positive</th>
<th>% of women with HPV positive</th>
<th>No. of women with HPV negative</th>
<th>% of women with HPV negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non malignant cytology</td>
<td>198</td>
<td>90.83%</td>
<td>24</td>
<td>12.12%</td>
<td>174</td>
<td>87.88%</td>
</tr>
<tr>
<td>Neoplastic cytology</td>
<td>20</td>
<td>9.17%</td>
<td>5</td>
<td>25%</td>
<td>15</td>
<td>75%</td>
</tr>
<tr>
<td>Total</td>
<td>218</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (2): Neoplastic cytology in relation with HPV infection(n=20)

<table>
<thead>
<tr>
<th>Cytology (cells)</th>
<th>No. of women with normal cytology</th>
<th>% of women with normal cytology</th>
<th>No. of women with HPV positive</th>
<th>% of women with HPV positive</th>
<th>No. of women with HPV negative</th>
<th>% of women with HPV negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN I</td>
<td>16</td>
<td>80%</td>
<td>3</td>
<td>18.75%</td>
<td>13</td>
<td>81.25%</td>
</tr>
<tr>
<td>CIN II</td>
<td>3</td>
<td>15%</td>
<td>1</td>
<td>33.4</td>
<td>2</td>
<td>66.6</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>1</td>
<td>5%</td>
<td>1</td>
<td>100%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td></td>
<td></td>
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</tbody>
</table>

**Discussion**

Prevalence of HPV DNA in a sample of Baghdad females aged 16 to 70 years was 12.38%, with the highest prevalence of 47.44.34% among women aged 21 to 30 years.

Stoler [17] was reported that the incidence of cervical cancer in women under 25–30 years of age is extremely low, and the prevalence of HPV in the United States drops from approximately 40% at age 20 to 10–20% at age 30–40; this finding is concordant with this work were HPV prevalence was highest between 21 and 30 years old and followed by age groups (31 to 40 years old) Figure (1.2).

Regarding older women, there were no women on age groups ≥ 60 years old was HPV positive, and HPV infection decreased from ≥50 years old in our sample and disagreed with other researcher were Burchell [18] has showed that age-specific HPV prevalence among women was highest for younger women <20 and decreased in the middle age groups, to increase again at age 65 and older.

In the present study, it was intended to evaluate the prevalence of different HPV infection in a sample of women from the Baghdad, the prevalence of HPV infection in our study 12.38% was concordant with the range of 2 to 44% world-wide [18] and our results agreed with Kjear [19] has reported the overall HPV detection rate was 15.4%.

Of the total study population of HPV –DNA positive 82.07% was infected with a single type and 17.93% was infected with multiple HPV types: 16.04% were infected with two types and 1.89% were infected with three types Figure (3). The observed prevalence of multiple infections is agreed in comparison with the results from studies of different countries. Presence of multiple infections was 0% in Brazil [20], 5.3% in Morocco [21], 16.7% in Paraguay [22], and 28% in The Netherlands [23]. Stamataki [15] has reported that multiple infection in their study were 6.2% in total study population and reported that these differences in prevalence of multiple infections could be due to differences in the technique used or real differences in the prevalence of the HPV types in the populations studied.

In this study sample, the majority of HPV-positive women were infected with the high-risk HPV type 33, 35 and 56 with 55.8% Figure (4), which has a distribution of equal 18.60% respectively for each type, followed by HPV-39,52,18,16,59,58,45,66. Castellsague [24] reported that HPV-35 was as common in Africa as HPV-16 (in 8% of infections), followed by HPV-31, HPV-45, HPV-56, and HPV-58 (in 6% of infections). In Mozambique it was reported that HPV-35 was the most dominant type among women with no cervical disease and those with cervical dysplasia, another study by Kroupis [25] in a sample of Greek women with a history of cervical lesions reported a higher prevalence of HPV-53, and study by Dutra [26] reported were the majority of HPV-positive women were infected with the high-risk HPV 31 was 26.67%. Stamataki et al., 2010 [15] reported the prevalence of HPV-16 (5.3%) approximately was similar to that of our study was HPV-16 (4.65%).

It is clear that Pap smear screening has been very effective. It is equally clear that virtually all lesions encompassed by the term “cervical neoplasia” are HPV-associated. The epidemiological and molecular evidence supporting this is convincing. Epidemiological studies demonstrate that a positive HPV test is the most powerful independent risk factor for the development of both cervical dysplasia and invasive cancer. Once HPV status is accounted for, the relative risk associated with traditional factors such as sexual behavior becomes insignificant. In limited studies, HPV infection precedes and predicts for the development of cervical pre-cancer as well as invasive cancer. Furthermore, 93–100% of invasive carcinomas from around the world have been shown to be associated with a limited spectrum of HPV types [17].

In this study Groups testing cytologically with HPV -DNA testing was 218 of 856 women(27.36%). Women. With normal cytology was 198 (90.83%), 24(12.12%) of them was HPV positive, and those with abnormal cytology was 20(9.17%) women , 5(25%) of them was HPV positive. Table (1). These results agreed with other researchers Stamataki [15]. Women with normal cervical cytology who are infected with high risk HPV type increased risk of developing cervical cancer. Therefore, it has been suggested that high risk HPV detection might be used as a tool to identify women at high risk of cervical cancer, in addition to Pap smears. Other studies have yielded similar findings in cytologically
normal women as Clifford [4] were reported that HR-HPV-positive women with normal cytology, the relative frequencies for several of the common HPV types were similar to those in other European countries.

The proportion of women with abnormal cytology who are HR-HPV positive16 (80%) of these with abnormal cytology was cervix intraepithelial neoplasia grade I (CIN I) 3 of them was HPV positive, 3(15%) of abnormal cytology was cervix intraepithelial neoplasia grade II (CIN II). 1 woman was HPV positive, and 1(5%) woman with cervical carcinoma was HPV positive. Table (2). These results confirmed among prior published reviews of the prevalence of HPV types in invasive and preinvasive cervical disease, the worldwide meta-analysis, Olsen [10] was reported Women positive for HPV DNA in cervical cells have a higher risk of developing cervical cancer, Vizcaino [11] and Einstein and Goldberg [12] were reported In women with abnormal cervical cytology, HPV was reported to have a high prevalence. Prevention of HPV infection through vaccination is expected to dramatically reduce the morbidity and mortality associated with HPV infection. These vaccines are based on the major capsid protein of the virus, L1 proteins, which are capable of self-assembling into virus like particles (VLPs) when expressed in cells. These VLPs share great similarity to native HPV virions, are non-infectious and non-oncogenic and can induce high levels of neutralizing antibodies [26,27]. Two vaccines are currently available and they incorporate HPV types more frequently associated with cervical cancer. A bivalent VLP vaccine (Cervarix™ GlaxoSmithKline) is composed of the assembled VLPs of HPV16 and HPV18 L1, and a quadrivalent VLP based vaccine (Gardasil® Merck & Co) that includes HPV16, HPV18, HPV6 and HPV11; these last two types cause the majority of genital warts (approximately 90%) in both men and women [26,28]. The proportions of high-risk HPV infections preventable by a vaccine for HPV16 and HPV18 vary by region, being highest in Europe and lowest in Sub-Saharan Africa [29] However, the available vaccines do not contain HPV33, 35, 56 which is prevalent in our study. Determining whether any significant protection is provided between genotypes will be a prerequisite to increase the breadth of genotype coverage for prophylactic vaccines. If VLP vaccines are found to confer a high rate of type-specific protection but no significant cross-protection, alternative second-generation vaccines may be needed to protect against other high risk HPV types, such as HPV35 and HPV33 and HPV 56, 53, 31 to perform a major reduction in cervical cancer [30]. In conclusion: The prevalence of HPV in women attending an outpatient clinic was high showing the importance of the early screening as well as the necessity of preventive measures. Since, the vaccine against the most prevalent and high risk HPV subtypes is in use in some policies regarding prevention of HPV infection might help to reduce the risk of infection and cervical cancer. However, larger epidemiological studies in different regions of our country are needed in order to report the accurate prevalence of HPV infection. This finding has important implications for primary estimation and prevention of HPV infection and cervical cancer precursors.

References


