Identification and Genotyping of High-Risk HPV in Cervical Swaps with Real-Time PCR in Women Attending Balikesir University Hospital

Mine Islimye Taskin¹, Ertan Adali², Tevfik Yavuz³, Coskun Cuce³, Mehmet Unlu³
1Department of Obstetrics and Gynecology, ²Department of Microbiology, ³Department of Public Health, Balikesir University Faculty of Medicine, Balikesir, Turkey

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Abstract
As oncogenic types of human papilloma virus (HPV) are associated with a higher risk of cervical cancer, this study was undertaken to investigate high-risk human papilloma virus (HR-HPV) prevalence among Turkish women in Balikesir. Cervical samples were collected from 204 women for cytological screening and HR-HPV testing by Xpert HPV PCR test. HR-HPV prevalence and its relation with cytological results and epidemiologic data were analyzed by SPSS. The prevalence of HR-HPV was 5.4% (13 of the 204 women). Women with abnormal cytological screening results had a significantly higher risk of HR-HPV positivity compared with women with normal cytological results (3.2% vs. 46.7%) (p<0.01). Age at first sexual intercourse and number of abortions were correlated with HR-HPV positivity (p<0.05), but there was no association with age, number of sexual partners, or contraception methods. We found that other HPV types apart from HPV type 16 and 18 are the most common types in our population. HR-HPV was positive in 16.7%, 80%, and 50% of the ASCUS, LSIL, and HSIL, respectively. The prevalence of cervical HR-HPV infection is 5.4% in our population. Further studies with larger sample sizes are needed for the development of new screening strategies and second generation HPV vaccines.

Keywords
HPV; HR-HPV Types; Cervical Cancer; PCR

Özet
Onkojenik human papilloma virus tipleri (HPV) yüksek oranda servikal kanser gelişim riski ile beraberdir ve bu çalışma Balıkesir ilindeki kadınlarda yüksek riskli HPV tiplerinin (HR-HPV) prevalansının araştırılması için düzenlenmiştir. 204 kadın hastanın servikal örnekleri servikal sitolojik değerlendirme ve Xpert HPV PCR testi ile HR-HPV tiplerinin belirlenmesi için toplanmıştır. HR-HPV prevalansı ve sitolojik sonuçlar ile ilişkisi ve epidemiyolojik veriler SPSS programı ile değerlendirilmiştir. HR-HPV sıklığı %5.4 bulunmuştur (204 hastanın 13’si). Anormal servikal sitolojili kadınlarda HR-HPV pozitifiğinin normal sitolojili kadınlara göre daha yüksek olduğu saptanmıştır (%3.2 vs. %46.7) (p<0.01). İlk cinsel ilişki yaş ve abortusların sayısı ile HR-HPV pozitifiği korele iken (p<0.05); yaş, seksüel partner sayısı ya da kullanılan kontraseptif metot HR-HPV pozitifiği ile korele bulunmamıştır. Çalışmamızın sonuçları da bir diğer öncü bir çalışmamızın psikoloji ile birlikte değerlendirildiğinde ki bizi en fazla ilgilendiren bireylerin geleneksel HPV tiplerini içermesinin bir neden olduğunu düşünüyoruz. HR-HPV, ASCUS, LSIL, HSIL için sırasıyla %16.7, %80, %50 pozitif saplanmış. Bu sayede HR-HPV tiplerinin servikal kanser riskini belirlemek için bir yol açmış olabilir. Dolayısıyla bu çalışmamızda bulduğumuz verilerin daha büyük bir deneysel bir çalışma yaparak daha da güçlendirilmesi ve daha da genişletilmiş bir birikim oluşturmak için gereklidir.

Anahtar Kelimeler
HPV; HR-HPV Tipleri; Servikal Kanser; PCR

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Corresponding Author: Mine Islimye Taskin, Department of Obstetrics and Gynecology, Balikesir University School of Medicine, 10145, Çağış Kampüsü, Balikesir, Turkey. T.: +90 2666121010/4550 F.: +90 2666121294 E-Mail: minetaskin1302@yahoo.com.tr
Introduction
Cervical cancer is the third most common cancer among women worldwide. Approximately 75-80% of cervical cancer cases are seen in developing countries, where efficient cervical screening is insufficient [1]. In Turkey, cervical cancer is the 8th most common female cancer, with an age standardized incidence rate of 4.2 in 100,000 and age standardized mortality rate of 1.6 in 100,000 [2].

All cervical cancers are considered related to oncogenic human papillomavirus (HPV) infection. HPV infection is the most common sexually transmitted infection worldwide. HPV types are divided into two groups according to their neoplastic potential. Types that cause low grade cervical lesions and genital warts, mainly HPV 6 and 11, are called low risk HPV types, while those that cause cervical cancer are called high risk HPV (HR-HPV) types [3]. Among the HR-HPV types, HPV genotypes 16 and 18 are associated with approximately 71% of all cases of cervical cancer, and HPV genotype 45 is associated with approximately 6% of additional cases of cervical cancer [4]. HPV 16 is solely responsible for one half of cervical cancers. Multiple HPV-type infections constitute more than a quarter of infections [5,6].

There are more than 100 types of HPV, with more than 30 anogenital types and approximately 15 of these are oncogenic [6]. E6/E7 oncogenes of HR-HPV DNA integrate in cervical cells, lead to cell cycle modifications that support conditions favorable to HPV viral replication. Thus, HPV viral genome integration into the host DNA and E6/E7 oncoprotein expression correlate with the development of cervical lesions. So to detect the E6/E7 oncoprotein expressed early in cervical cells have an important role [7]. The ideal HPV test can be flexibly integrated easily into most environments and enable physicians to effectively risk stratify patients based on cytology and HR-HPV. Further, rapid comprehensive HPV results that include integrated high risk HPV 16 and HPV 18 testing strategies support quality decision making for colposcopy referral [8].

The prevalence of HPV infection differs from region to region. Different HPV prevalences have been reported among Turkish women. Because of the close relationship between HR-HPV and cervical cancer, the aim of the present study was to investigate the prevalence of HR-HPV with genotyping using the Xpert HPV test (Cepheid, USA) in women in Balikesir who presented to the gynecology outpatient clinic of a university hospital, and to correlate this to Pap smear results and risk factors.

Material and Method
This retrospective study was approved by the local Ethical Committee of Balikesir University. With a 95% confidence interval for detecting average 10% HPV positivity, 204 women were included in the study population. The study group included 204 women who were subjected to cervical HPV-DNA testing with simultaneous cervical Pap smear at the outpatient clinic of Balikesir University Faculty of Medicine between September 2014 and April 2015. Data were collected from patients’ files and the computer-based data record system. Women with a diagnosis of invasive or preinvasive cervical cancer and women who had never had sexual intercourse were excluded. Women admitted for routine gynecologic examinations or gynecologic complaints were included. Samples were obtained during gynecologic examinations and transferred using a liquid based medium to the microbiology laboratory daily. For the Pap test, the conventional cervical smear technique was used. Specimens were fixed in alcohol and the Bethesda system was used to classify the results [9].

HPV identification and typing were done by the Xpert HPV genotyping test (Cepheid, USA). Xpert HPV includes reagents for the simultaneous detection of thirteen high risk HPV (HR-HPV) types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and one possible HR-HPV type (HPV66), a human reference gene (HMBS = Hydroxymethylbilane synthase), and an internal Probe Check Control (PCC). The 14 targeted HPV types are determined in five fluorescent channels: 1. HPV 16, 2. HPV18 and HPV 45 ("HPV18/45"), 3. HPV 31, 33, 35, 52, and 58, 4. HPV 51 and HPV 59, and 5. HPV 39, 56, 66, and 68. The human reference gene (fluorescent channel 6) verifies specimen adequacy. The PCC verifies reagent rehydration, PCR tube-filling in the cartridge, probe integrity, and dye stability. In total, the assay utilizes six fluorescent channels for the detection of individual types of HPV, groups of HPV, and the human reference gene. Each fluorescent channel has its own cutoff parameters for target detection/validity. If a sufficient signal is detected by the human reference gene (i.e. the sample has sufficient cellularity), the assay results are reported as an overall “positive” if any type of targeted HPV is detected, but additionally, HPV 16, and HPV 18/45, and collectively the other high-risk HPV types detected by the assay are reported specifically as “positive” or “negative”. Test results for Xpert were categorized according to a priori cancer risk: HPV16 positive, else HPV16 negative and HPV 18/45 positive, else HPV16 and HPV18/45 negative and positive for other HR-HPV types, else HR-HPV negative.

Clinical data (age, age of menarche, number of deliveries, contraceptive method, smoking status, age at first sexual intercourse, number of sexual partners, and history of genital warts and pelvic inflammatory disease) were recorded. SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) was used to manage the data and to perform the statistical analyses. The chi-square test was used to compare the rate of HR-HPV infection and abnormal cytology in different groups of patients. The results were considered statistically significant if the p value was < 0.05.

Results
The mean age of the patients was 40.1±10.15. Their reasons for presenting to the gynecology clinic are summarized in table 1. Out of the 204 participants, 13 (6.4%) were HR-HPV positive and 191 (93.6%) were negative. Among the 13 HR-HPV-positive women, HPV 16 was detected in 3 (1.5%); other HR-HPV types were detected in 10 women (4.9%) by the Xpert HPV genotyping test. We did not detect HPV 18 in our study group (Table 2). Cervical cytological screening results of the entire group showed 92.6% women with normal cytology, 2.9% women with atypical squamous cells of undetermined significance (ASCUS), 0.5% women with atypical squamous cell not excluding high grade lesion (ASC-H), 2.5% women with low-grade squamous intraepithelial lesions (LSIL), 1% women with high-grade squamous intraepithelial lesions (HSIL), and 0.5% women with squamous cell carcinoma (table 3). Furthermore, HR-HPV was positive in 46.7% of women with...
cytologic abnormalities and in 3.2% of women without any cytologic abnormality. Women with abnormal cytological screening results had a significantly higher risk of HR-HPV positivity compared with women with normal cytological results (3.2% vs. 46.7%) (p<0.01). The distributions in the cytologically normal women of most common HPV types were as follows: HPV 16 (0.55%), HPV 18 (0%), and other HR-HPV types (HPV 31, 33, 35, 52, 58, 51, 59, 39, 56, 66, 68) (2.65%). The most common HPV types in the cytologically abnormal women were HPV 16 (13.4%) and other HR-HPV types (33.3%) (Table 4). HR-HPV was positive in 16.7%, 80%, and 50% of the ASCUS, LSIL, and HSIL, respectively. One woman whose smear result showed ASC-H was negative for HR-HPV and one woman whose smear result showed squamous cell carcinoma was positive for HR-HPV (Table 5).

Age at first sexual intercourse and number of abortions were found to be correlated with HR-HPV positivity (p<0.05) among all HR-HPV positive women (n=189) (Table 6). The mean age at first sexual intercourse was 21.31±7.5 for HR-HPV positive women and 23.46±4.10 for HR-HPV negative women. Comparison of the contraceptive methods in HR-HPV positive and negative women revealed that the statistical analyses did not detect any interrelation between the contraceptive method and HR-HPV positivity in our study (Table 6). Abnormal cervical cytology was seen in 53.8% of HR-HPV positive cases and in 4.2% of HR-HPV negative cases.

Table 1. Reasons for application to hospital

<table>
<thead>
<tr>
<th>Variables</th>
<th>SAYI (n)</th>
<th>HR-HPV (+) %</th>
<th>HR-HPV (+) %</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>39.62±7.87</td>
<td>40.1±10.30</td>
<td>0.0629</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>5(41.7)</td>
<td>47(25.1)</td>
<td>0.306</td>
<td></td>
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<tr>
<td>Gravida</td>
<td>2.33±1.56</td>
<td>2.14±1.80</td>
<td>0.570</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>1.42±1.00</td>
<td>1.59±1.39</td>
<td>0.728</td>
<td></td>
</tr>
<tr>
<td>History of PID</td>
<td>0(0)</td>
<td>42(22)</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>History of condyloma</td>
<td>2(15.4)</td>
<td>9(64.7)</td>
<td>0.150</td>
<td></td>
</tr>
<tr>
<td>Contraceptives</td>
<td>1(16.7)</td>
<td>7(67.5)</td>
<td>0.405</td>
<td></td>
</tr>
<tr>
<td>Hormonal</td>
<td>1(16.7)</td>
<td>7(67.5)</td>
<td>0.405</td>
<td></td>
</tr>
<tr>
<td>Intrauterine device</td>
<td>3(37.5)</td>
<td>22(204)</td>
<td>0.367</td>
<td></td>
</tr>
<tr>
<td>Condom</td>
<td>2(28.6)</td>
<td>38(306)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Coitus interruptus</td>
<td>1(16.7)</td>
<td>27(239)</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The prevalence of HR-HPV in the general population varies depending on the study cohort and other risk factors. Several studies about genotype distribution in different regions of Turkey and HPV prevalence have been reported. However, according to our review of the literature, there are no data about our restricted region, Balikesir in Turkey. There are also limited data about HR-HPV prevalence in Turkey, which leads to a high risk of cervical neoplasia. According to our results, HR-HPV prevalence was 5.4% in our region. High-risk or oncogenic HPV types can cause certain cancers, including anogenital and oropharyngeal cancers, in both men and women. Among HPV-related cancers, cervical cancer is unique because it was found that HPV may be detected by PCR in virtually all cervical cancer patients related to persistent infection with HPV [10]. After detecting the HPV-DNA with PCR, cervical HPV-DNA detection screening policies became important and studies were executed for developing HPV vaccines [11], because decreasing the frequency of cervical cancer requires preventing, diagnosing, and treating HPV infection.
Detection of the prevalent HPV genotypes in certain areas of the world has become vitally important due to their different oncogenic potential. In a study published by Sanjose et al. [12], overall HPV prevalence in women with normal cervical cytology was reported to be 10.4% on average, ranging from 6.2% to 31.6%. In Turkey, studies have reported lower prevalences for overall HPV than the worldwide overall HPV prevalence stated by Sanjose et al. [13-16]. On the other hand, there are also higher rates for overall HPV positivity in Turkey ranging between 16.5% and 25.7% [17-22].

Cohort studies show that persistent HR-HPV infections lead to high risk for cervical neoplasia. Due to this close relationship, we identified HR-HPV by Xpert HPV genotyping test in our study. According to our results, HR-HPV positivity was 5.4%. In Turkey, similar lower rates have been reported previously. Ozcan et al. studied 501 women and reported 4.2% positivity for HR-HPV by testing with hybrid capture 2 [23]. Akcali et al. [16] used PCR and found that HR-HPV positivity was 5%, similar to our results. Eren et al. [20] studied HPV positivity in Istanbul they found a 12% positivity rate for HR-HPV. The HR-HPV prevalence rate differs from region to region: it was reported as 7.5% in Europe [24], 31.2% in states of the former Soviet Union [25], and 13% in Russia [26]. Rates reported from Turkey are lower than those of other regions.

HR-HPV positivity is a significant risk factor for abnormal cytological findings according to our results. HR-HPV positivity in women with abnormal Pap smears was 46.7% in this study. However, a previous report also from Turkey found HR-HPV prevalence to be 62.8% by PCR in 35 patients with abnormal cytology evaluated with liquid-based cytological screening [27]. In another study from Turkey, HR-HPV positivity in women with abnormal cytology was 19% [25].

In a study from Izmir, located in western Turkey and adjacent to Balikesir, HPV positivity was reported to be 32.1% women with normal cytology referred for cervical cancer screening. When the smears with pathological cytology were included, HPV positivity increased to 38.9%. HR-HPV positivity was 74.3% in women with normal cytology. These rates are higher than ours. We detected HR-HPV in 5.2% of women with normal cytology. This difference can be attributed to the lack of risk factors for HPV infection in our study group, because smoking habit, number of sexual partners, and history of sexually transmitted diseases were associated with HPV infection [28]. The low incidence of HPV infection has been thought to be due to the low number of sexual partners because of religious beliefs, and social and cultural rules [17].

Although previous reports mention an increase in HPV prevalence with oral contraceptive use and a decrease with barrier method use, we did not observe an association between HR-HPV positivity and contraceptive method [19,23,29]. Moreover, age, smoking, history of PID and condyloma, and number of sexual partners were also not associated with HR-HPV prevalence. Factors affecting HR-HPV positivity were the number of voluntary abortions and age at first sexual intercourse in our study (Table 6). In the literature, HPV prevalence was reported to be correlated with the number of births. Kasap et al. [28] reported a positive correlation, while Demir et al. [19] reported a negative correlation. However, according to our review of the literature, there are no data about abortions. We showed that the risk of HR-HPV increased with the number of abortions. This is probably because of cervical trauma caused by multiple trauma due to recurrent pregnancy terminations.

According to some studies, HPV type 16 is the most frequent type in cervical atypical changes [12, 16, 17, 19, 21]. We found multiple HR-HPV types including HPV 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68 as the most frequent type, at a rate of 2.65%, followed by type 16, at a rate of 0.5%. However, other prevalence studies from Kayseri report HPV type 18 as the most frequent type [30]. We did not detect HPV 18 in our specimens. Eren et al. [20] and Akcali et al. [16] also detected very low HPV 18 rates in Turkey. In a study from Mersin, HPV type 66 was the most common [31]. Distribution of individual HPV genotypes varies across geographic areas and ethnic groups. These differences in HPV types among regions ought to be considered when vaccines are developed. Multiple HR-HPV was the most common type in our study. Niesssen et al. [32] found that the risk of having multiple HR-HPV types increased with the number of sexual partners, but we did not detect a correlation. Akcali et al. [16] showed a significantly high rate of multiple HPV infection in women with single partners, like in our study.

The International Biological Study on Cervical Cancer study group stated that 92.9% of tumors on average contained HPV-DNA, with a range of 75–100% [33]. Sharifah et al. [34] detected high-risk HPV-DNA in 95% of patients with abnormal cervical cytology, in 100% of patients with cervical cancer, and in 92% of cervical intraepithelial lesions. Kasap et al. [28] reported HPV-DNA in 78.3% of women with cervical cancer and 76.9% of women with HGSIL. Abnormal cervical cytology was observed in 30% of HPV-DNA positive cases and in 5.4% of HPV-DNA negative cases; 73.9% of their patients with cervical cancer and 71.2% of those with ASCUS, ASC-H, LGSIL, and HGSIL were high-risk HPV-DNA positive cases. In our study, abnormal cervical cytology was observed in 53.8% of HR-HPV positive cases and in 4.2% of HR-HPV negative cases. HR-HPV was positive in 16.7%, 80%, and 50% of the ASCUS, LSIL, and HGSIL, respectively. One woman with ASC-H was negative for HR-HPV and one woman whose smear result showed squamous cell carcinoma was positive for HR-HPV.

HPV detection methods rely on the detection of viral nucleic acids in the infected tissue because HPV cannot be cultured. The most commonly used tests are based on direct hybridization or DNA-based amplification techniques. Among DNA tests, Hybrid Capture-II is widely used assay. The analytical sensitivity of Hybrid Capture-II is lower than PCR. Cross-reactivity resulting in false-negative and false-positive results are its major limitations. PCR is currently the most sensitive detection technique [20,35,36]. Because of this, Xpert HPV genotyping test (Cepheid, USA) which is a PCR technique was used for the detection and typing HPV in the present study.

Conclusion

According to our results, 7.4% of patients had abnormal cervical cytology and 5.4% of all patients had HR-HPV infection. HPV 16 and 18 were also not detected as the most common types in our population. Further studies with larger groups will shed more light regarding the prevalence of the different HPV types.
in our region and guide local vaccination programs.

**Competing interests**

The authors declare that they have no competing interests.

**References**


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