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Original Research

Human Papilloma Virus Frequency and Genotypes; Evaluation of the 4879 Screenings Made with Polymerase Chain Reaction and Chip Array Between 2001 and 2019 in Istanbul

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Abstract

Objectives: The aim of this study is the documentation of human papilloma virus (HPV) frequency and types seen in the city of Istanbul, Turkey, as well as evaluation of the relationship between these subtypes and cytological and pathological diagnoses.

Methods: 4879 cases were studied in our molecular pathology department between 2001 and 2019 in Istanbul. Between 2001 and 2010, 1692 cases were screened for HPV 6, 11, 16, 18, 31, and 33 by conventional hybridization and polymerase chain reaction (PCR). Since 2011, up to 49 HPV typing has been performed for 3187 cases with chip array. The cases were referred to the pathology center and the hospital pathology department by clinicians for screening before HPV vaccination and on the observation of precancerous changes and koilocyts in cytological-histopathological evaluations.

Results: In this study, the frequency of HPV was found to be 10.8% (527 HPV-positive cases). Among these, 348 cases were high-risk groups, whether or not they were previously associated with a low-risk group. When we look at the distribution of the cases according to the high-risk HPV types, HPV 16 is the most common type. The frequencies of occurrence of other HPV types are as following: HPV–16: 41.7%, HPV–31: 11.7%, HPV–52: 7.9%, HPV–51: 7.1%, HPV–33: 6.9%, HPV–45: 6.5%, HPV–18: 6.3%, HPV–39: 6.1%, and HPV–58: 5.8%. It was further found that multiple infections were 28% of high grade squamous intraepithelial lesion cases. HPV frequency was 38% and 72%, respectively, in cases with cytologically or histopathological precancerous, low-grade squamous intraepithelial lesion, and HSIL changes. As a final note, HPV was detected in 9 of 10 cases with cervical cancer (90%). Only 1 adenocarcinoma case detected in the series was a double infection with HPV types 18 and 45.

Conclusion: HPV 16 was the most common type found in this study. It is followed by types 31, 52, 51, 33, 45, 18, 39, and 58, respectively. The most common association observed in double infections was between HPV 16 and 58. It was also observed that the incidence of HPV in the city of Istanbul, Turkey, was similar to other developed countries. As a final note, in addition to screening tests, PCR and chip array studies should be conducted and the community should be informed about preventive medicine and the importance of condom use.

Keywords: Cervical cancer; chip array; epidemiology; genotype; human papilloma virus; polymerase chain reaction.

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Human papilloma virus (HPV) has an important place in the etiopathogenesis of cervical cancer.[1,2] Cytological and histopathological cervical precancerous and cancer lesions and HPV types detected in invasive cancer cases were evaluated and reported in studies conducted in various countries. However, HPV can cause head and neck

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cancers as well as penile cancers in men.[3,4] The virus has double-stranded deoxyribonucleic acid (DNA) which is enveloped by a protein capsule. The first affection of the virus is proliferation of the squamous epithelium with acanthosis and papillomatosis. Epithelial cells may show irregular perinuclear halo, nuclear pleomorphism, binucleation, and cytoplasmic enveloping. Absence of koilocytes does not exclude the HPV infection. Molecular methods including hybridization, polymerase chain reaction (PCR), and hybrid capture are the accurate method to detect the virus DNA. The presence and type of virus before HPV vaccination have also gained importance.[5]

Since 2001, the molecular pathology department has been established at the Sitonet Cyto-Pathology Center. In this department, 4879 HPV screenings were carried out until between 2001 and 2019, including 421 cases from Okan University Hospital in between 2017 and 2019. In this study, besides documenting the HPV types seen in some parts of our society, the relationship between these subtypes and cytological and pathological diagnoses was also evaluated.

Methods

Between 2001 and 2019, 4879 samples were sent to our center in Fulya, Istanbul in a liquid-based cytology (Thin-Prep) collection container for HPV testing. The age of the patients for these cases was in the range of 16–67. Thin layer cervical smears were prepared with the Thin-Prep 2000 semi-automatic processor and the remaining sample was used for HPV screening and typing. Since 2008, cytological evaluation has been carried out by scanning the cytological material both manually and with the help of a computerized system called Integrated Imager. Histopathological samples were analyzed by staining the sections prepared after classical tissue followed-up with hematoxylin-eosin. The material for HPV studies was obtained from the cytological residue sample, the paraffin block or the pool in which the two were used together.

Two different methods were used in HPV screening and typing in this study. Classical hybridization and PCR technique were applied in 1692 cases between 2001 and 2010. Following the application of phenol and chloroform from the sample remaining in the collection container, DNA was extracted, and the isolation was performed by centrifuging with NaCl at a speed of 13,000 rpm for 20 min. Density with purity optimization was achieved by measuring the isolated DNA in the spectrophotometer. The southern blot hybridization and PCR techniques were applied to DNA samples. In PCR, primers specific to each type of HPV (Consensus primers), manus primers (My 09/11), and type-specific primers were amplified and compared.[6] After PCR, agarose gel was prepared and HPV-positive bands that progress in electrophoresis were stained with cybergin and examined under fluorescent light. In this period, 6 and 11 were screened as low risk types of HPV and HPV 16, 18, 31, and 33 were classified as high-risk types. Between 2011 and 2019, typing was performed with chip array HPV kits in 3187 cases. Following DNA isolation, PCR-specific to the HPV genome regions was studied using the CLART HPV kit that was hybridized to contain 49 different types and read with the Genomica Clinical Reader.

HPV types were obtained between the years 2001 and 2010 were grouped into two different groups as "High Risk" and "Low Risk."

a. High-risk group: HPV 16, 18, 31, 33
b. Low-risk group: HPV 6, 11.

HPV types obtained between the years 2011 and 2019 were grouped into four different groups as "high risk," "possible high risk," "low risk," and "risk assessment not yet known;"

a. Low-risk group: HPV 6, 11, 40, 42, 43, 44, 54, 61, 62, 70, 71, 72, 81, 83, 84, 85, 89
b. High-risk group: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
c. Possible high-risk group: HPV 26, 53, 73, 82
d. Risk assessment not yet known group: HPV 34, 64, 67, 69, 74, 86, 87, 97, 101, 102, 103, 106, 150, 151.

In this study, further analysis and reporting were conducted for only "high-risk" and "low-risk group". Further analysis and reporting for "Possible high-risk" and "Risk assessment not yet known" groups will be evaluated and reported in another study in the future.

Results

HPV types have been increasingly determined in the past 30 years, but it has taken time to create commercial kits that will show these types in routine laboratory applications. For this reason, while six subtypes were studied in the first 10 years in this study, with the arrival of technological possibilities, 49 types were evaluated after 2011. In the previous group, 68.4% of 197 positive cases were found as high risk and 33.6% as low risk. The frequency of HPV type 16 in this group was 40.2%. In the latter group, 75.6% of 375 positive cases were high risk and 30.4% low risk. The frequency of HPV type 16 was found to be 43.3%.

In this study, the overall (including both groups) frequency of HPV was found to be 10.8% (527 HPV-positive cases). Among these, 348 cases are high risk groups, whether or not they were associated with a low-risk group. When we look at the distribution of the cases according to the high-risk HPV types, HPV 16 is the most common type. The

frequency of HPV types were as follows HPV-16: 41.7%, HPV-31: 11.7%, HPV-52: 0.9%, HPV-51: 7.1%, HPV-33: 6.9%, HPV-45: 6.5%, HPV-18: 6.3%, HPV-39: 6.1%, and HPV-58: 5.8% (Table 1).

It is important to note that multiple infections are 28% of high grade squamous intraepithelial lesion (HSIL) cases. HPV frequency is 38% and 72%, respectively, in cases with cytologically or histopathological precancerous low-grade squamous intraepithelial lesion (LSIL) and HGSIL changes. HPV was detected in 9 of 10 cases with cervical cancer (90%). In 8 HPV-positive squamous cell carcinomas (SCC), double infection was observed in one, and triple infection in another (25%). In the case of SCC with double infection, HPV type 16 and type 58, in triple infection HPV type 16, 39, and 52 were found together. Only one adenocarcinoma case detected in the series is a double infection with HPV types 18 and 45 (Table 2).

Discussion

It has been reported in various publications that genital HPV infection is highly contagious, and that clinical-subclinical HPV infection includes 10–14% of the sexually active population.[7-10] On the other hand, diagnosis by detecting cytological morphological changes associated with HPV infection is relatively limited, with a sensitivity of 25–35% and a specificity of 65–80%.[11-13] In international literature, the frequency of HPV in cervical squamous cancers is 95% and above.[7-10] The total HPV frequency detected in this series was 10.8%, slightly below the internationally reported average rate of 12%.[1-3,7-10] Double, triple, and multiple HPV types, which we have started to see more recently, were found to be 2.7%, 1.8%, and finally 0.9%, respectively (Table 3), and these values were (0.3–0.8%) less common.[1-3,5-10]

In accordance with the literature, the most common type detected was HPV 16.[1-3,5-10,14] Top-4 identified within high-risk types have been reported as: types 16, 18, 45, and31 in Northern Ireland; types 16, 31, 18, and 45 in Canada; and types 16, 52, 58, and 33 in China. According to the European Union (EU) joint study, the most common HPV types were types 16, 33, and 31 in HGSIL lesions and 16, 18, 45, and 33 in cervical carcinomas.[7-10,15] In this study, the decreasing order of frequency of oncogenic types is 16, 32, 18, and 52.

The frequency of HPV in the 20–24 age range in the United States of America (USA) is 44.8% and the most common oncogenic HPV type is 16.[9] In this study, the highest HPV frequency was found in the patient range up to the age of 25. In this age group, the rate of high-risk types was 20.5%, and the rate of low-risk types was 8.4% (Fig. 1). In the USA statistics, HPV 62 was detected as the non-oncogenic type.[16] In this study, the frequency order of non-oncogenic types

Table 1. High-risk HPV types prevalence

	%
HPV 16	41.7
HPV 31	11.7
HPV 52	7.9
HPV 51	7.1
HPV 33	6.9
HPV 45	6.5
HPV 18	6.3
HPV 39	6.1
HPV 58	5.8

HPV: Human papilloma virus.

Table 2. HPV types at malignant cervical lesions

SCC 1	HPV 18
SCC 2	HPV 51
SCC 3	HPV negative
SCC 4	HPV 16 and 18
SCC 5	HPV 16
SCC 6	HPV 39
SCC 7	HPV 16, 39, and 52
SCC 8	HPV 16
SCC 9	HPV 31
ADENO CA	HPV 18, 45

SCC: Squamous cell carcinomas; HPV: Human papilloma virus.

Table 3. Multiple HPV infection significance In 4987 tests

	% (n)
Double infection	2.7 (135)
Triple infection	1.8 (90)
Multiple infection	0.9 (49)

HPV: Human papilloma virus.

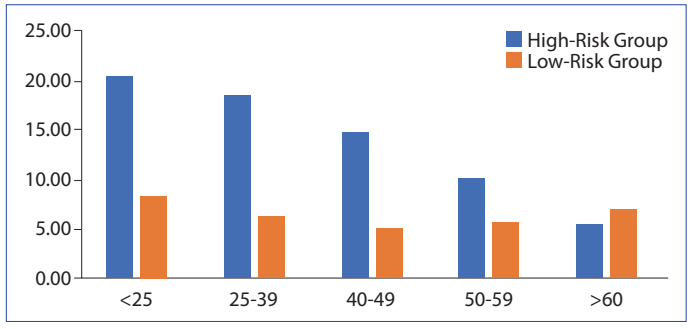

Figure 1. High- and low-risk type human papilloma virus infection distribution according to age groups.

was determined as HPV 6, 54, and 11. A high frequency of type 62 has not been observed in this population.

In China, the frequency of HPV was 15.6% in the normal population, 20.5% in Central China (cities), and 14.5% in heterosexual men and 59.9%, which is approximately 4 times more probable in homosexual men. Chinese study reports HPV types 31, 33, 52, and 58 as morbid high-risk types.[15]

If the studies conducted in our country are to be summarized; in a study by Ergüney et al., HPV positivity was reported as 80% in 35 precancerous cases. About 79% of them are from the high-risk group and 14% are from the low-risk group.[17] In the study conducted by Seçkin et al.,[18] it was reported that only three positives (2.2%) were found in 134 consecutive patients who had gynecological problems. Safi et al.[19] reported HPV positivity in only two patients (3.3%) in the vaginal discharge samples of 60 cases in their PCR-screening. In the study performed by Özçelik et al.,[20] 230 patients with low-risk group lesions were found to be positive in 14 cases (6.1%) in HPV scanning. When classified according to age groups, it was reported that HPV positivity was 5.9% under the age of 45, while it was 7.7% over the age of 45, and a different result was obtained from the general literature and this study. In the study performed by Onan et al, using real-time PCR, HPV 16, and 18 types were studied in 94 patients with histopathological precancerous lesions and half of the cases (50%) were defined as cervical intraepithelial neoplasia (CIN) I, 27 cases (29%) as CIN II, and finally 20 cases as CIN III. In patients with these histopathological diagnoses, HPV 16 and/or HPV 18 positivity is 4.2%, 14.8%, and 45%, respectively.[21]

In the study conducted in Ireland, LSIL and HSIL were evaluated together and the frequency in these cases was reported as 64.3%.[7] In the Canada study result, positivity was found as high as 99.2% in HSIL cases.[8] In the EU joint study, high values such as 98.5% in HSIL and 91.8 in invasive cancer were reported.[10] In this study, HPV positivity was found to be 38% higher in LGSIL cases and 72% in HGSIL (high-grade) cases. The closest results to our study were reported on 10.805 cases in the Shenzhen region in China and HPV positivity was 36.4% in LSIL and 82% in HSIL.[22]

High-risk HPV was observed in 8 of 9 squamous carcinomas and one adenocarcinoma (90%) found in the series. One adenocarcinoma case is a double infection with HPV 18 and 45. HPV 18 has been reported frequently in adenocarcinomas.[8]

Vaccination has not reached the sufficient application rate yet, and it was found to be 3.4% in a study in the United States.[23] Despite vaccination programs, The Centre for Cervical Cancer Prevention in Sweden reported[24] that the frequency of HPV positivity, which was 9.7/100.000 in 2006, increased to 11.7/100.000 in 2015.

Conclusion

This study is the largest known HPV study of types and frequency in Istanbul, Turkey, and the total HPV frequency detected is 10.8%. Double infection cases were found to be 2.7%. The dominant type is HPV 16 in the high-risk group and HPV 11 in the low-risk group. While the frequency of HPV is 38% in cases with a cyto-histopathological diagnosis of LSIL, it is 72% in HSIL diagnoses. Eight of nine cases diagnosed with cervical squamous carcinoma were HPV-positive (89%). These results show that the incidence of HPV in Turkey is not much different than in other developed countries. In addition to screening tests, PCR and chip array studies should also be used as additional diagnostic methods in clinical necessity.

Disclosures

Ethics Committee Approval: The study was approved by the Okan University Ethics Committee, 23.12.2020/130.

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Conflict of Interest: None declared.

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