Human Papillomavirus Prevalence and Genotype Distribution among Turkish Women with or Without Cervical Lesion

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Abstract

Context: Human papillomavirus (HPV) infection is the main cause of cervical cancer, but the risk is associated with the various HPV genotypes which may be found in women with or without clinical findings. Aims: We aimed to identify HPV prevalence and genotype distribution in women with or without cervical lesions admitted to Gynaecology and Obstetrics Clinics of one of the largest private hospitals in Istanbul between 2013 and 2017. Subjects and Methods: In the present study, cervical cytobrush samples collected from 2464 women with different cytological conditions, and investigated for the presence of HPV, and the different genotypes. Results were evaluated based on the HPV positivity in different cytological findings, and ages. Furthermore, distribution of high-risk (HR) and low-risk (LR) genotypes in different groups was investigated. Results: Among all participants, 1925 (78.1%) was with the normal cytological condition, 354 (14.4%) with ASC-US; 151 (6.1%) with low-grade squamous intraepithelial lesion (LSIL), and 34 (1.4%) with high-grade squamous intraepithelial lesion (HSIL). Our results showed that 649 out of 2464 patients (26.3%) were positive, and 1815 (73.7%) were negative for the presence of HPV. Among 649 positive patients, 223 (34.3%) were found positive for more than one genotype. HPV 16 was found the most common HR-HPV type in ASC-US and LSIL whereas HPV 18 was the most common in HSIL. HPV 6 was found the most common LR-HPV type in ASC-US and LSIL whereas HPV 11 was the most common in HSIL. 26.9% of women <50 years old, and 22.3% of women ≥50 years old was positive for HPV. The most common HR-HPV genotype was 16 in both groups with (19%) or without (17%) abnormal cytology. Conclusions: We concluded that HPV prevalence and genotype distribution in women with or without clinical findings is an important predictor of cervical cancer.

Keywords: Cervical lesion, genotype, human papillomavirus

INTRODUCTION

Human papillomavirus (HPV) is an infectious agent belonging to the virus family Papillomaviridae, members of which have tropism for cutaneous epithelium and mucosal epithelium.[1] HPV infections are one of the most common sexually-transmitted diseases. Although most infections with HPV cause no symptoms, persistent genital HPV infection can cause cervical cancer in women which is the fourth most common cancer in women, with an estimated 266,000 deaths and 528,000 new cases in 2012.[2,3] Virtually, all cervical cancer cases (99%) are linked to genital infection with HPV, and it is the most common viral infection of the reproductive tract. A large majority (around 85%) of the global burden of cervical cancer occurs in the less developed regions, where it accounts for almost 12% of all female cancers.[3] There are >120 different HPV types, with HPV-16 and HPV-18 having a strong association with cervical cancer. HPV can also cause other types of anogenital cancer, head-and-neck cancers, and genital warts in both men and women.[1] The International Agency for Research on Cancer have classified 12 HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) in Group 1 carcinogens.[4] HPV genotypes have been subdivided into low-risk types (LR-HPV), which are found mainly in genital warts, and high-risk types (HR-HPV), which

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are frequently associated with invasive cervical cancer. There is, however, no consensus concerning the categorisation of many HPV types with low prevalence according to risk. Moreover, the number of putative HR types varies from 13 to 19, and only 11 HPV Types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58) are consistently classified as HR-HPV. Among them, HPV 16 and 18, is the main cause of cervical malignancy, and they have also been found associated with 24% of low-grade lesions, 51% of high-grade lesions and 70% of cervical cancers worldwide. Among all LR-HPV, HPV 6 and 11 are responsible for genital warts >90%, and also rarely found in cervical carcinomas.

Three HPV vaccines are now being marketed in many countries throughout the world-a bivalent, a quadrivalent and a non-avalent vaccine. All three vaccines are highly efficacious in preventing infection with virus Types 16 and 18, which are together responsible for approximately 70% of cervical cancer cases globally. The vaccines are also highly efficacious in preventing precancerous cervical lesions caused by these virus types. The quadrivalent vaccine is also highly efficacious in preventing anogenital warts, a common genital disease which is virtually always caused by infection with HPV Types 6 and 11. The non-avalent provides additional protection against HPV Types 31, 33, 45, 52 and 58.

In this study, we aimed to identify HPV prevalence and genotype distribution in women with or without cervical lesions admitted to Gynaecology and Obstetrics Clinics of one of the largest private hospital in Istanbul between 2013 and 2017.

Subjects and Methods

Sample collection
Cervical cytobrush samples collected from 2464 women admitted to gynaecology polyclinic of our hospital. The samples were collected with cervical brushes (Cervix-Brush) and stored in ThinPrep PreservCyt transport medium (Hologic, Inc., Marlborough, MA, USA). The study was approved by the Ethics Committee and written informed consent was received from each participant. Flowchart of the study is shown in Figure 1.

Screening and genotyping
HPV DNA was extracted from a 250 μl aliquot of sample, using the AmpliLute liquid media extraction kit (Roche Diagnostics GmbH, Mannheim Germany) with the QIAvac 24 Plus vacuum system (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The DNA extracts were then stored at −20°C until amplification. All samples were tested for the presence of HPV DNA, using the PGMY09/11 L1 consensus primers, and genotyped using the reverse-line blot method with the linear array (LA) HPV Genotyping Test (Roche Diagnostics GmbH, Mannheim Germany) according to the manufacturer’s instructions. To detect the presence of HPV DNA, biotinylated PGMY09/11 consensus primers were used, and 450-bp region of the L1 gene were amplified. Polymerase chain reaction (PCR) was performed with GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) using the following procedure; denaturation at 95°C for 9 min, then 40 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, 5 min terminal extension at 72°C, and a hold step at 4°C.

The LA HPV Genotyping Test was used by labelling the PCR products with biotin to determine the HPV genotype distribution. The LA test amplifies the target HPV DNA for 37 HPV genotypes including 24 LR HPV types (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108) and 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). HPV-52 may be found positive only in the absence of HPV types 33, 35 and 58 due to the combined probe used for these four types in the LA assay. A volume of 50 μl DNA extract were amplified using the LA HPV Genotyping test following the instructions of the manufacturer. This assay includes β-globin amplification in the same PCR reaction as a control for cell adequacy, extraction and amplification. After hybridization; a blue coloured complex precipitates at the probe positions where hybridisation occurs. Then, the LA HPV genotyping strip is read visually by comparing the pattern of blue lines on the test reference guide. Obtained test strips were interpreted and photographed for future reference.

Data analysis
All data analyses were performed using SPSS 20.0 programme (IBM, Corp Armonk, NY, USA). The correlation between the variables such as HPV-positive and HPV-negative genotyping status and Pap test results was evaluated using the Spearman’s rho correlation coefficients.

Results

Demographics and cytology results
There were 2464 samples included in this study, 1925 (78.1%) was with the normal cytological condition, 354 (14.4%) with...
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ASC-US; 151 (6.1%) with low-grade squamous intraepithelial lesion (LSIL), and 34 (1.4%) with high-grade squamous intraepithelial lesion (HSIL). Ages of the participants were ranging from 20 to 80 years. Mean age was 37.09 ± 9.88 years. Results of cervical examinations and age distributions are shown in Table 1.

**Single or multiple human papillomavirus genotype infection status**

HPV genotyping test showed that 649 out of 2464 patients (26.3%) were positive, and 1815 (73.7%) were negative for the presence of HPV. HPV positivity was found most commonly in the age group of 30–39 years. Among 649 positive patients, 223 (34.3%) were found positive for more than one genotype [Table 2]. Distribution of single or multiple HPV genotype infections is also shown in Table 2. Totally, we detected 441 HR and 606 LR-HPV genotypes in 649 positive samples. Among 426 (17.29%) women infected by a single HPV genotype, 232 were found positive for HR and 194 for LR genotypes. Furthermore, among 223 women infected by multiple HPV genotype, 136, 40, 19, 15 and 13 women were found infected by 2, 3, 4, 5 and 6 different HPV genotypes, respectively [Table 3].

**Human papillomavirus genotype status**

There was a significant correlation between HPV genotyping status and Pap smear results (rs: 0.771, P < 0.05) [Table 4]. The rate of single HR-HPV-positive cases among normal Pap smears negative for intraepithelial lesions/malignancy (NILM) was 3.9% (75/1925), whereas the rate among those with ASC-US, LSIL and HSIL was 27.7% (98/354), 23.8% (36/151) and 67.6% (23/34), respectively. HPV 16 was the most common type in NILM, ASC-US and LSIL groups, whereas the HPV 18 was the most common in HSIL group. The rate of single LR-HPV-positive cases among normal Pap smears NILM was 3.8% (73/1925), whereas the rate among those with ASC-US, LSIL and HSIL was 21.8% (77/354), 21.9% (33/151) and 32.4% (11/34), respectively. HPV 6 was the most common type in NILM, ASC-US and LSIL groups, whereas HPV 11 was the most common in HSIL group.

Distribution of all HR-HPV genotypes in different biopsy results is shown in Table 5. HPV 16 was found the most common type in ASC-US and LSIL samples, whereas HPV 18 was the most common in HSIL samples. Also, the distribution of all LR-HPV genotypes in different biopsy results is shown in Table 6. HPV 6 was found the most common type in ASC-US

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### Table 1: Confirmed biopsy results in different age groups n (%)

| Ages     | Sample (n) | NILM | ASC-US | LSIL | HSIL |
|----------|------------|------|--------|------|------|
| 20-29    | 467 (18.95)| 384 (15.58)| 55 (2.23)| 23 (0.93)| 5 (0.2)|
| 30-39    | 1246 (50.57)| 971 (39.41)| 178 (7.22)| 70 (2.84)| 27 (1.1)|
| 40-49    | 469 (19.03)| 354 (14.37)| 80 (3.25)| 34 (1.38)| 1 (0.04)|
| 50-59    | 175 (7.1)| 137 (5.56)| 25 (1.01)| 13 (0.53)| 0 |
| 60-69    | 78 (3.17)| 67 (2.72)| 6 (0.24)| 4 (0.16)| 1 (0.04)|
| >70      | 29 (1.18)| 12 (0.49)| 10 (0.41)| 7 (0.28)| 0 |
| Total    | 2464 (100)| 1925 (78.13)| 354 (14.37)| 151 (6.13)| 34 (1.38)|

NILM: Negative for intraepithelial lesion or malignancy, ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion

### Table 2: Distribution of human papillomavirus types in different age groups

| Ages     | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | >70 | Total |
|----------|-------|-------|-------|-------|-------|-----|-------|
| Patients (n) | 467   | 1246  | 469   | 175   | 78    | 29  | 2464  |
| HPV negative | 345   | 909   | 342   | 133   | 62    | 24  | 1815  |
| HPV positive | 122   | 337   | 127   | 42    | 16    | 5   | 649   |
| Single type | 86    | 224   | 75    | 27    | 11    | 3   | 426   |
| HPV positive | 36    | 113   | 52    | 15    | 5     | 2   | 223   |

HPV: Human papillomavirus

### Table 3: HPV positive genotype

| HPV positive genotype | Patients (%) |
|-----------------------|-------------|
| Single positive       | 426 (17.3)  |
| HR                    | 232 (9.4)   |
| LR                    | 194 (7.9)   |
| Multiple positive     | 223 (9.1)   |
| 2 HPV genotype        | 136 (5.5)   |
| HR + LR               | 95 (3.9)    |
| LR + LR               | 34 (1.4)    |
| HR + HR               | 7 (0.3)     |
| 3 HPV genotype        | 40 (1.6)    |
| HR + 2LR              | 32 (1.3)    |
| 2 HR + LR             | 6 (0.2)     |
| 3 HR                  | 2 (0.1)     |
| 4 HPV genotype        | 19 (0.8)    |
| 1 HR + 3 LR           | 18 (0.7)    |
| 2 HR + 2 LR           | 1 (0.04)    |
| 5 HPV genotype        | 15 (0.6)    |
| 1 HR + 4 LR           | 15 (0.6)    |
| 6 HPV genotype        | 13 (0.5)    |
| 1 HR + 5 LR           | 11 (0.5)    |
| 2 HR + 4 LR           | 2 (0.1)     |
| Total HPV positivity  | 649 (26.3)  |

HR: High-risk, LR: Low-risk, HPV: Human papillomavirus

### Table 4: Correlation between human papillomavirus positive/negative genotyping and Pap Smear results

| HPV genotype | Pap Smear | Rs* |
|--------------|-----------|-----|
| Negative     | 1737 (70.5)| 0.771|
| Positive     | 188 (7.62) | 26.33|
| Total        | 1925 (78.12)| 2464 (100) |

*Rs: Spearman correlation value was (P<0.05), **ASC-US, HSIL and LSIL results considered as a Pap Smear positive. ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, HPV: Human papillomavirus
**Table 5: Distribution of all high-risk-human papillomavirus types in different biopsy results**

| HR-HPV (positive) | Total, \( n(\%)^* \) | NILM | ASC-US | LSIL | HSIL |
|-------------------|------------------------|------|--------|------|------|
| 16                | 74 (7.07)              | 37   | 26     | 7    | 4    |
| 18                | 30 (2.87)              | 16   | 5      | 4    | 5    |
| 31                | 34 (3.25)              | 19   | 8      | 4    | 3    |
| 33                | 31 (2.96)              | 15   | 7      | 7    | 2    |
| 35                | 22 (2.1)               | 11   | 8      | 2    | 1    |
| 39                | 30 (2.87)              | 17   | 6      | 5    | 2    |
| 45                | 25 (2.39)              | 17   | 7      | 1    | 0    |
| 51                | 14 (1.34)              | 6    | 5      | 2    | 1    |
| 52                | 43 (4.11)              | 23   | 16     | 3    | 1    |
| 56                | 36 (3.44)              | 18   | 12     | 6    | 0    |
| 58                | 27 (2.58)              | 16   | 9      | 2    | 0    |
| 59                | 20 (1.91)              | 10   | 8      | 1    | 1    |
| 66                | 21 (2.01)              | 11   | 6      | 2    | 2    |
| 68                | 34 (3.25)              | 16   | 14     | 3    | 1    |
| Total             | 441 (42.12)            | 232  | 137    | 49   | 23   |

*Percentages were calculated in 1047 HPV types. NILM: Negative for intraepithelial lesion or malignancy, ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, HR: High-risk, HPV: Human papillomavirus

**Table 6: Distribution of all low-risk-human papillomavirus types in different biopsy results**

| LR-HPV (positive) | Total, \( n(\%)^* \) | NILM | ASC-US | LSIL | HSIL |
|-------------------|------------------------|------|--------|------|------|
| 6                 | 92 (8.79)              | 44   | 36     | 12   | 0    |
| 11                | 73 (6.97)              | 33   | 26     | 11   | 3    |
| 26                | 6 (0.57)               | 0    | 1      | 4    | 1    |
| 40                | 0                      | 0    | 0      | 0    | 0    |
| 42                | 10 (0.96)              | 5    | 3      | 2    | 0    |
| 53                | 33 (3.15)              | 16   | 12     | 4    | 1    |
| 54                | 44 (4.2)               | 22   | 18     | 3    | 1    |
| 55                | 38 (3.63)              | 16   | 19     | 2    | 1    |
| 61                | 42 (4.01)              | 21   | 14     | 7    | 0    |
| 62                | 38 (3.63)              | 19   | 13     | 6    | 0    |
| 64                | 0                      | 0    | 0      | 0    | 0    |
| 67                | 28 (2.67)              | 14   | 8      | 6    | 0    |
| 69                | 14 (1.34)              | 11   | 2      | 1    | 0    |
| 70                | 32 (3.06)              | 15   | 8      | 8    | 1    |
| 71                | 5 (0.48)               | 0    | 2      | 3    | 0    |
| 72                | 7 (0.67)               | 0    | 6      | 1    | 0    |
| 73                | 22 (2.1)               | 6    | 7      | 7    | 2    |
| 81                | 26 (2.48)              | 11   | 12     | 3    | 0    |
| 82                | 24 (2.29)              | 6    | 8      | 9    | 1    |
| 83                | 4 (0.38)               | 0    | 3      | 1    | 0    |
| 84                | 33 (3.15)              | 11   | 11     | 11   | 0    |
| IS39              | 35 (3.34)              | 16   | 16     | 3    | 0    |
| CP6108            | 0                      | 0    | 0      | 0    | 0    |
| Total             | 606 (57.88)            | 276  | 217    | 102  | 11   |

*Percentages were calculated in 1047 HPV types. NILM: Negative for intraepithelial lesion or malignancy, ASC-US: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, LR: Low-risk, HPV: Human papillomavirus

**Discussion**

As the etiologic cause of cervical cancer, HPV has been studied in many ways including its prevalence and genotype distribution. The results of relative studies were varied because of different regions, races, age groups, and different methodologies or assays used.[20] Initial results of population-based Cervical Cancer Screening Programme of Turkey has recently showed that the prevalence of HPV infection was found 3.5%. Furthermore, it was stated that the most common HPV genotype was 16, followed by 51, 31, 52 and 18. Among the 37,515 HPV-positive cases in this large screening study, cytological abnormality rate was found 19.1%. Among HPV-positive cases, 16,962 cases had HPV 16 or 18 or other oncogenic HPV types with abnormal cytology (>ASC-US).[11] Overall, the percentage of HPV positivity in Turkish Screening Programme was lower than our study is related with the women involved in our study were already suspected for a gynaecologic condition instead of the population. However, HPV 16 was the most common HR genotype in both of the studies. In our study, differently, this was followed by 52, 56, 31 and 68. Furthermore, cytological abnormality rate among the 441 h-HPV-positive cases was 47.4% (209/441) which is quite higher than the Turkish Screening Programme. According to similar epidemiological studies from Turkey, HPV prevalence was ranging from 2% to 30% depending on the number of patients, analysed HPV types and the diagnostic method.[12-19] Our results were also similar with most of these studies.

Bruni et al.[20] showed that the estimated crude and adjusted HPV prevalences among women with normal cytological findings worldwide were 7.2% and 11.7%, respectively. Sub-Saharan African regions (24.0%), Latin America and the Caribbean (16.1%), Eastern Europe (14.2%), and South-Eastern Asia (14.0%) were reported as the regions with the highest prevalences.[20] In our study, 508 out of 1925 women (26.4%) with normal cytological findings detected as positive for the presence of HPV. While 12.1% of these women were positive for HR-HPV, 14.3% were positive for LR-HPV genotypes. The most common HR-HPV genotype was 16 followed by 52 and 31; the most common LR-HPV genotypes were 6 and 11 in women with normal cytological findings.

Distribution of HPV positivity among different age groups was similar in our study. 26.9% of women <50 years old and 22.3% of women ≥50 years old was positive. HPV positivity in 20–29, 30–39, and 40–49 age groups were 26.1%, 27% and 27.1%, respectively. Among the women <50 years old, most common HR-HPV genotype was 16 in both groups with (19%) or without (17%) abnormal cytology. In the largest study in
Turkey, HR-HPV genotypes, HPV 16 was the most common genotype in all age groups similarity to our study.[11]

**Conclusions**

HPV prevalence and genotype distribution restricted to women admitted to gynaecology clinics with or without abnormal cytological findings may be an appropriate indicator when population-based sampling is not available or feasible. Furthermore, our study showed that HR-HPV types are not rare in women with normal cytological findings which is an important indicator of the importance of screening programmes. This may lead similar countries with low screening rates to create new programmes.

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**Conflicts of interest**

There are no conflicts of interest.

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