

# Screening for and Genotyping of Human Papilloma Virus in Prostitutes

## Seks İşçilerinde Human Papilloma Virüs Varlığı ve Tiplerinin Araştırılması

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**ABSTRACT Objective:** The human papilloma virus (HPV) causes various infectious clinical pictures and anogenital cancers, and it is recognized as the primary etiological agent of cervical cancer worldwide. In this study, we aimed to demonstrate the presence of HPV in prostitutes and to identify its genotypes in cervical specimens. **Material and Methods:** This study included 137 commercial sex workers from Ankara brothels who were referred to the Ankara Municipality Hospital, Department of Venereal Diseases from September 3-7, 2007 for routine examinations and who volunteered to participate. After obtaining informed consent from the participants, one-on-one interviews were conducted to obtain sociodemographic data. Cervical specimens were collected from each subject in order to investigate HPV genotypes by DNA extraction. **Results:** The tests for HPV DNA were positive in 53 specimens following the extraction of DNA from swab specimens collected from the prostitutes. Seven cases had genotypes associated with the low-risk cancer group, one had genotypes affiliated with the undetermined risk group, and 38 had genotypes related to the high-risk group. In addition, genotypes associated with more than one risk group were detected in seven other study participants. Four subjects had genotypes related to both the undetermined indefinite and high-risk groups, two had genotypes connected with the low and high-risk groups, and one had genotypes from all three risk groups. Eighty-four participants tested negative for HPV DNA. **Conclusion:** Women working in brothels are at risk for venereal diseases. This should be considered to protect both their own health and public health. It is noteworthy that the genotypes of HPV identified in the prostitutes were predominately of the high-risk cancerous type. Therefore, information regarding HPV should be distributed to women working in the sex industry, and preventive measures should be implemented to protect them from acquiring HPV.

**Key Words:** Sexually transmitted diseases; community health planning

**ÖZET Amaç:** İnsan papillomavirüsleri [Human Papilloma Virüsü (HPV)] çeşitli enfeksiyöz tablolara ve anogenital kanserlere neden olmaktadır. HPV bütün dünyada servikal kanserin primer etiyolojik etkeni olarak kabul edilmektedir. Bu çalışmadaki amaç, genelev kadınlarında, servikal örnekler kullanılarak, HPV varlığını ve genotiplerini ortaya koymaktır. **Gereç ve Yöntemler:** 3-7 Eylül 2007 tarihleri arasında Ankara Belediye Hastanesi Zührevi Hastalıklar Bölümü'ne rutin muayeneleri yapılmak üzere başvuran Ankara Genelevleri'nde çalışan 137 genelev kadını çalışmaya gönüllü olarak katılmıştır. Aydınlatılmış onamları alındıktan sonra çalışma grubu ile sosyodemografik veriler ile ilgili bilgi edinmek amacıyla birebir görüşme yapılmıştır. Olgulardan servikal sürüntü örnekleri alınarak bu örneklerde 21 tür HPV DNA'sının genotipi araştırılmıştır. **Bulgular:** Genelev kadınlarından alınan servikal sürüntü örneklerinde DNA ekstraksiyonları yapılmış ve örneklerin 53'ünde HPV DNA pozitif bulunmuştur. Bu örneklerden 7 olguda kanser riski düşük, birinde kanser riski belirsiz ve 38'inde kanser riski yüksek genotipler görülürken, 7'sinde değişik risk gruplarına ait genotipler birlikte tespit edilmiştir. Değişik risk gruplarının birlikte görüldüğü örneklerden 4'ünde riski belirsiz ve yüksek, 2'sinde düşük ve yüksek genotipler, birinde ise her 3 risk grubuna ait genotipler tespit edilmiştir. Seksen dört katılımcıya ait sürüntü örneklerinde herhangi bir HPV tipine ait DNA elde edilmemiştir. **Sonuç:** Genelevde çalışan kadınlar cinsel yolla bulaşan hastalıklar açısından risk altındadır. Hem kendi sağlıklarının hem de toplum sağlığının korunması için bu konunun üzerinde hassasiyetle durulmalıdır. Genelev kadınlarında tespit edilen HPV genotiplerinin, özellikle kanser yapıcı etkileri yüksek risk taşıyan tiplerde olması dikkat çekicidir. Bu nedenle genelevde çalışan kadınlar için cinsel yolla bulaşan hastalıklar konusunda bilgilendirici eğitim faaliyetleri düzenlenmeli, koruyucu hizmetler geliştirilmelidir.

**Anahtar Kelimeler:** Cinsel yolla bulaşan hastalıklar; toplum sağlığı planlaması

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The human papilloma virus (HPV) is of particular importance regarding sexually transmitted infections (STIs) because it is closely associated with various infectious clinical pictures as well as anogenital cancers.<sup>1</sup> It is also recognized as the primary etiological agent of cervical cancer.<sup>2</sup> In addition, it has been reported to play a role in the formation of verruca vulgaris (simple warts), verruca plana (flat warts), oral infections, respiratory papillomatous, mucosal lesions, and epidermodysplasia verruciformis (anogenital warts).<sup>1</sup>

Contact other than full sexual intercourse also plays a role in transmission. Various studies have revealed that the risk for HPV transmission is higher for those who are sexually active compared to other sexually transmitted viral infections such as human immunodeficiency virus (HIV) or herpes simplex virus type 2 (HSV-2).<sup>3</sup> The use of condoms reportedly does not provide full protection.<sup>3,4</sup>

Sexual intercourse with an infected partner is the initial step of the epidemiological chain for HPV transmission. Besides peno-vaginal intercourse, other sex practices such as peno-anal intercourse, digital-vaginal sex, oral sex, and the use of sex toys can also promote HPV transmission. In addition, perinatal transmission is a rare occurrence.<sup>5</sup> A suppressed immune system leaves the body more susceptible to all microorganisms in the infected patients. In fact, the risk for genital HPV infection is higher among people infected with HIV because having one STI increases the risk of contracting others.<sup>6</sup> Other STIs often accompany HPV infection. Therefore, physicians and clinicians should be aware that an STI might be accompanied by HPV.<sup>1</sup>

Because of the limited number of studies on this subject in Turkey, there are uncertainties regarding the incidence of HPV infection, best treatment options, and recommendations for appropriate measures to be taken to avoid this virus. The objective of this study was to demonstrate the incidence and genotypes of HPV in cervical samples obtained from prostitutes.

## MATERIAL AND METHODS

Approval for this study was granted by the Ethics Committee of Gazi University and permission was also obtained from the Ankara Metropolitan Municipality. A hundred and thirty-seven registered prostitutes were referred to the Ankara Metropolitan Hospital, Department of Venereal Diseases for routine examination from September 3-7, 2007. They voluntarily participated in the study and gave their informed consent. Cervical swab specimens were collected after all participants answered a five-item questionnaire and the samples were then analyzed at the Hacettepe University pathology laboratory. Twenty cases who presented to the gynecology outpatient clinic with a normal cytology were used as the control group.

### QUESTIONNAIRE INTERVIEW

One-on-one interviews were conducted in order to obtain sociodemographic data. The participants were interviewed about their educational status, years of experience as a prostitute, frequency of condom usage, and any current health problems.

### CERVICAL SPECIMEN COLLECTION

The cervical specimens were collected with Dacron swabs during the gynecological examination. After placing the vaginal speculum, the swab was inserted 1-1.5 cm into the cervix uteri while avoiding the vaginal walls. It was withdrawn after full rotation 3-5 times in the opposite direction. Afterwards, the specimens were placed in a tube and were stored at -80°C.

### HPV DETECTION

Human papilloma virus DNA was extracted using phenol-chloroform, which was resuspended in 50 µL of ultra pure distilled water and was kept at -30°C. The quality and integrity of the sample DNA for polymerase chain reaction (PCR) was confirmed with the amplification of a 185 base-pair region of the housekeeping genes (glyceraldehyde 3-phosphate dehydrogenase). Any specimens with negative internal control amplification were excluded.

**PCR**

The PCR was performed with 20 µL reaction mixture consisting of a 10× PCR buffer, 25 mM magnesium chloride (MgCl<sub>2</sub>), 200 µM deoxynucleotide triphosphate, 2 U Taq polymerase, 10 pmol of primer, and 2 µL template DNA. The protocol was performed by preheating the sample at 94°C for five minutes, denaturation with 35 cycles at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 35 seconds. Finally, an extension was carried out again at 72°C for five minutes. The products were defined with electrophoresis in 2.0% agarose gel stained with ethidium bromide. A negative and a positive control were run for every PCR test to check for possible contamination and accuracy.

**HPV GENOARRAY TEST**

HybriBio’s HPV GenoArray Test kit (HybriBio Ltd., Wanchai, Hong Kong), which uses flow-through hybridization technology, and DNA amplification were used to simultaneously identify 21 HPV genotypes: HPV-6, -11, -16, -18, -31, -33, -35, -39, -42, -43, -44, -45, -51, -52, -53, -56, -58, -59, -66, -68, -81, and CP8304. A gene chip with a nylon membrane on which type-specific oligonucleotide probes had been immobilized was used in the test, and the outcomes were identified by colorimetric changes on the chip under direct visualization. The test was carried out in accordance with the manufacturer’s instructions.

**STATISTICAL ANALYSIS**

The data were analyzed using the SPSS version 15.0 for Windows software program (SPSS Inc., Chicago, Illinois, USA), and descriptive statistics were used in the evaluations. Mean±standard deviation, number (n) and percentage (%) of cases were presented as descriptive values.

**RESULTS**

A hundred and thirty-seven women working in Ankara brothels were examined at the Ankara Metropolitan Hospital, Department of Venereal Diseases. The age distribution of the study group was given in Table 1.

The years of experience as a prostitute for the participants was presented in Table 2.

The women included in the study had education levels between zero and 15 years and the average number of years of education was 6.57±3.60.

The frequency of condom usage was shown in Table 3.

The participants were questioned about their current health conditions during the interviews, and 130 reported no complaints. On the other

**TABLE 1: Age distribution.**

Age range*	Case numbers	%
21-25	1	0.7
26-30	20	14.6
31-35	33	24.1
36-40	26	19
41-45	22	16.1
46-50	18	13.1
>51	17	12.4
Total	137	100

\* Mean: 39.9±9.31.

**TABLE 2: Working years as a prostitute.**

Year of experience as a prostitute (range)*	Case numbers	%
<1	2	1.5
1-5	25	18.2
6-10	35	25.6
11-15	32	23.4
16-20	25	18.2
>21	18	13.1
Total	137	100

\* Mean:11.9±7.34

**TABLE 3: Frequency of condom usage.**

Frequency of condom usage	Case numbers	%
Always	68	49.6
Sometimes	39	28.5
Often	29	21.2
Never	1	0.7
Total	137	100

hand, some participants complained of vaginal discharge (2.2%), vaginal pruritus (1.5%), and pain (1.5%).

Fifty-three of the prostitutes tested positive for HPV DNA (38.6%) and 84 tested negative. HPV genotype distribution were shown in Table 4.

Of these specimens, the genotypes of the low-risk cancer group were found in seven cases. One subject had genotype with undetermined risk and 38 cases had genotypes associated with the high-risk group. In addition, seven participants had genotypes affiliated with more than one risk group, with four having genotypes of the undetermined and high-risk groups, two having genotypes of the low and high-risk groups, and one having genotypes of all three groups.

There was no significant correlation between HPV prevalence and age ( $p=0.174$ ), education ( $p=0.217$ ), frequency of condom use ( $p=0.315$ ) and years of experience as a prostitute ( $p=0.104$ ).

## DISCUSSION

Anogenital cancers other than HPV cervical cancer are associated with vulvar, vaginal, anal, and

penile cancers as well as genital warts and respiratory papillomatous lesions.<sup>7,8</sup> Each type of HPV leads to clinical problems and the genital types are classified as low or high risk according to their oncogenic potential. Human papilloma virus 6 and 11 are commonly found in genital warts, whereas HPV 16, 18, 31 and 45 are mostly involved in the development of invasive anogenital cancer.<sup>9,10</sup> In previous studies, high-risk HPV was found in 99% of cervical cancers.<sup>11,12</sup> Additionally, more than 90% of genital warts are caused by HPV 6 and 11, and HPV has been detected in 70% of anal cancers.<sup>8</sup> Furthermore, HPV is more prevalent among young women and the rate decreases with age. Many HPV infections occur immediately after sexual activity begins.<sup>11</sup>

The majority of the women included in this study were between the ages of 26 and 50. In other studies that have focused on prostitutes, similar age-related results were reported.<sup>13,14</sup> The average number of years of education was 6.5 and 16 (11%) participants stated they had received no education at all. This rate was higher than in similar studies from Turkey.<sup>13,15</sup> We can attribute this difference to the higher level of education of women who have recently started to work as prostitutes. Nonetheless, 90 (65.5%) participants in our study had education levels of five years or less. A low level of education may be a significant factor that increases the risk for STIs. In a study by Chakraborty et al. in India, 84% of prostitutes were reported to be uneducated and this rate was 63% in the study by Pickering et al. in Gambia.<sup>16,17</sup>

Sixty-eight women (47%) responded “always” when asked about how often they used a condom. This low rate may be attributed to unawareness and the reluctant and oppressive behavior of the customers. The condom usage rate was 18% in the study by Öcal and Börekçi in a Mersin brothel, and it was 85.8% and 91.8% for two different time periods in a study by Pathom et al. in Thailand.<sup>15,18</sup> It is remarkable that our results point to such a low rate of condom usage and it suggests that sociocultural levels and the lack of sexual education may be important factors contributing to this low rate.

**TABLE 4:** Distribution of HPV genotypes (n=53).

HPV Type	Case numbers	%
16*	15	28.3
18*	20	38
31*	10	19
33*	4	7.6
35*	3	5.7
39*	2	3.8
45*	1	1.9
52*	4	7.6
58*	5	9.4
59*	1	1.9
68*	2	3.8
53**	6	11.4
CP8304**	3	5.7
6***	3	5.7
44***	2	3.8

\* High-Risk Types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82

\*\*Indefinite risk types: 26, 53, 66, CP8304

\*\*\*Low risk types: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81,10

No vaginal complaints were reported by 130 (95%) prostitutes when they were questioned in the interview. This may be attributed to the routine follow-ups of the prostitutes and self-use of antibiotics as well as underreporting due to concerns that the existence of a disease might result in a deficiency or loss of work. Furthermore, asymptomatic progress related to HPV infections may also be a factor.

In this study, HPV was identified in 53 (38.6%) of the 137 prostitutes. Sexual habits of a population may account for the difference in HPV positivity rate. For example, in previous studies, the rate of HPV DNA was 20.5% in Scotland, 3.9% in Thailand, 3% in Spain, and 2.5% in northern Greece.<sup>16,19-21</sup> In addition, rates of higher than 30% have been reported in many prevalence studies such as the study by Tachezy et al. involving Czech women, with an HPV positivity rate of (44%) among 389 women.<sup>22-25</sup>

Another study that investigated HPV DNA by PCR reported a rate of 43.8% among female sex workers in Bulgaria, whereas it was 38.9% in China and 66.8% in Peru.<sup>26-28</sup> Previous studies from Turkey suggested an HPV DNA positivity rate between 8.7% and 21.6%.<sup>29-31</sup> The rate in our study was higher than the average rate in Turkey, which may be attributed to the uniform composition of the study group including only prostitutes. We determined that a high rate of HPV in prostitutes not only leads to a greater risk for genito-urinary cancers, but it also increases the possibility of other public health problems associated with sexual relationships.

In our study, seven (13.7%) cases were found to have the low-risk genotype of HPV and 45 (84%) had the high-risk genotype. Infections caused by the oncogenic types of HPV account for 50-75% of all HPV infections. The rate of the high-risk cancerous HPV genotype in our cohort was 84%. Batmaz et al. identified the rate of high squamous intraepithelial lesions (HSIL) in Turkey as 66.6%, and Cuschieri et al. detected high-risk HPV types in 76.6% of the patients who tested positive for DNA.<sup>2,32</sup> In our study, the high rate of oncogenic types of HPV indicates that it deserves more attention.

The study by Batmaz et al. also reported that the most common HPV genotypes were type 16 at a rate of 18.6% and type 18 at a rate of 10%.<sup>2</sup> Studies investigating the prevalence of HPV in sex workers reported that types 16 and 18 were also the most common HPV genotypes.<sup>28,33</sup> Moreover, the study by Li et al. revealed a similar distribution of HPV genotypes.<sup>27</sup>

Although, prostitutes are routinely examined for HIV and other STIs in centers located in big cities, the existence and types of HPV have not been previously analyzed in Turkey. The results of this study suggest that HPV is a health problem transmitted by infected people to both prostitutes and the general population.

There was no significant correlation between the existence of HPV and condom usage, education level, or years of experience as a prostitute. Although it is difficult to interpret this finding, underreporting and the high rate of low-level education may have contributed to this insignificance. Epidemiological data has limitations since HPV infections may have subclinical progression and the infections are transient.<sup>9,10</sup> Batmaz et al. suggested that the risk of HPV infection was higher for women who had a high number of sexual partners and for those who used contraceptives and tobacco.<sup>2</sup> The rate was also higher for women with herpes simplex virus (HSV) and vulvar warts.<sup>34</sup>

## CONCLUSION

Epidemiological studies are needed in our country to monitor the incidence and prevalence of HPV.

HPV prevalence in prostitutes in our study was higher than the average rate for the general population. It is noteworthy that the HPV genotypes found in the prostitutes were high-risk types with carcinogenic effects. Therefore, training programs should be planned to raise awareness among prostitutes on STIs and the importance of using condoms.

Women working in brothels are also at risk for venereal diseases. This should be considered to protect both their health and public health. HPV vaccination of prostitutes before they begin to work

may be a good preventive measure. Since HPV infection may be asymptomatic, the addition of routine screening for HPV may also be beneficial for early diagnosis. In addition, HPV infection in sex

workers should be reassessed by legislators and be classified as an occupational disease and their working conditions should be evaluated to lower their risk for diseases associated with their lifestyle.

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