**Obstetrics;** *Maternal-Fetal Medicine and Perinatology*

# Human Papillomavirus (HPV) and Pregnancy; Prevalence and Diagnostic Methods

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**OBJECTIVE:** To investigate the prevalence of Human Papillomavirus (HPV) and type detection of Human Papillomavirus as the most important causative agent of cervical cancer by PCR in pregnant women applying to Obstetrics and Gynecology outpatient clinic in Gazi Hospital.

**MATERIAL AND METHOD:** Two hundred asymptomatic pregnant women applying to Obstetrics and 102 asymptomatic women applying to Gynecology outpatient clinic were included. HPV DNA was extracted from cervical smear samples by using phenol chloroform isoamyl alcohol and amplified by MY09/11 primers. Specific primers were used for type 16/18 detection.

**RESULTS:** Five (2.5%) of 200 pregnant women were HPV DNA (+), two (1%) of which were type 16,two (1%) of which were type 18 and one(0.5%) of which was type 11.Two(2%) of 102 women in contol group were positive, both of which were type 16.

**CONCLUSION:** HPV prevalence in pregnant women is lower than indicated in the literature. The probable reason may be common monogamy in Turkey and the non-smoking, young patient profile with high social and economic level in our study group. However, asymptomatic but HPV positive patients were directed to clinicians for follow-up and treatment.

**Key Words:** HPV, Pregnancy, PCR

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## Introduction

Human Papillomavirus (HPV), is the most common viral agent of sexually transmitted diseases.[1] Its relationship with genital warts, juvenile laryngeal papillomatosis, cervical cancer and cervical intraepithelial neoplasia is well-document.[2,3] Over a hundred types of HPV have been isolated according to DNA nucleotide sequencing and type 6 and 11 were found to be related with laryngeal papillomatosis, type 16, 18, 31, 33 and 35 were found to be ralated with CIN or cervical cancer.[2] HPV, especially type 16 is reported as an important risk factor for development of cervical dysplasia and cancer and 99.7% of the cervical cancers can be proved to be related to HPV.[4]

It is informed that, HPV infection in pregnant women is more common than in non pregnant women.[5] As a result of increasing steroid hormon levels and interaction of the virus with progesteron, HPV replication may be activated.[5,6] Increasing levels of the virus in cervical tissue may facilitate the spread of the virus to genital organs and amniotic cavity.[5] HPV infection during pregnancy is important since the rapidly enlargement of genital warts causes an obstruction of the labor and juvenile laryngeal papillomatosis and karyotype abnormalities my occur by perinatal transmission.[2,3]

Detection rates of HPV infection by polymerase chain reaction (PCR) have ranged widely between 1% and 20% in newborns of pregnant women without apparent infection in their cervix and between 5% and 72% in women with HPV-related cervical diseases diagnosed during pregnancy.[7] By the ıntroduction of sensitive assays like PCR, it has been shown that subclinical HPV infections are more than supposed[8] and latent HPV infections can be easily detected by a specific and sensitive assay like PCR.

## Material and Method

Two hundred asymptomatic pregnant women applying to Obstetrics and Gynecology outpatient clinic for a routine antenatal control having no lesions related with HPV were included in our study. A hundred and two asymptomatic women applying to our Gynecology outpatient clinic with no lesions in favour of HPV were included as a control group.

Cervical smear samples were collected with sterile swabs

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in sterile tubes containing phosphate buffer saline (PBS) between the 18th-28th weeks of pregnancy. The samples were vortexed and aliquoted in eppendorf tubes and stored at -86ºC until they were studied.

***DNA extraction:*** Tubes were taken from -86°C and centrifuged at 13.000 rpm for 3 minutes and the supernatant was completely removed. Lysis solution containing 20mg/ml proteinase K (20mM (NH4)2SO4, 75mM Tris HCl [pH 8,8] 0,1% Tween 20) was added and stored at 55 °C for 3 h, followed by storing at 95°C for 10 min and DNA extraction by phenol chloroform isoamyl alcohol was performed. For presipitation, pure ethanol including 3M sodium acetate was added and the tubes were stored at -20°C overnight. Following day, samples were mixed with 70% alcohol and dried. After drying, they were incubated on dry block at 55°C for 10 min adding 50 µl sterile deionised water. Incubated samples were stored at -20°C if not used for amplification procedure immediately.

***Amplification of HPV DNA:*** The primers used in this study were the consensus primers for many HPV genotypes and were chosen from L1 location of HPV genome (L1 is associated with major viral capsid protein metabolism) Primers were synthesized by TIB Molbiol (Berlin, Germany).

MY09/MY11 set (5'-CGTCCMARRGGAWACTGATC-3'), (5'-CMCAGGGWCATAAYAATGG-3') was used as a HPV primer set. Amplification procedure was performed by adding 5µl pure DNA to a mixture of 45µl including 100µM from each dNTP(dATP, dCTP, dGTPand dTTP) 100 pmol from each primer and 1 unit of Taq DNA polymerase enzyme (DNA m ltd, Hasts, UK) in 4mM MgCl2, 50mM KCl2 and 10mM Tris HCl [pH 9,0].

A thermal cycler programme in ThermoHybaid (UK) with 35 cycles following; initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 20 sec, annealing at 55°C for 45 seconds and extension at 72°C for 1 min, followed by 7 min of final extension at 72°C was performed. PCR products were analysed on agarose gel stained with ethidium bromide and transluminating band was detected with UV transluminator encompassing 450 bp for HPV.

***Genotyping:***MY09/MY14 (5'-CATACACCTCCAGCACCTAA-3') and MY09/WD74 (5'-GGATGCTGCACCGGCTG A-3') were specific primers used for amplification of HPV 16 and HPV 18 types respectively.

Amplification procedure was performed by adding 5µl pure DNA to a mixture of 95µl including 100µM from each dNTP (dATP, dCTP, dGTPand dTTP) 100 pmol from each primer and 1 unit of Taq DNA polymerase enzyme (DNA mp ltd, Hants UK) in 4mM MgCl2, 50mM KCl2 and 10mM Tris HCl [pH 9,0]. We used the thermal cycler program for HPV genotyping as described above. PCR products were analysed on agarose gel stained with ethidium bromide and transluminating band was detected with UV transluminator encompassing 110 bp for HPV 16 and 140bp for HPV 18.

***DNA Sequencing:*** HPV 16 negative MY09/11 amplicon were sequenced by OpenGene® automated DNA sequencing system and similaritiy percentage of sequences were calculated by GeneObjects® software (Visible Genetics, Canada). Cycle sequencing reactions were done by using Cy5.5 dye terminatior sequencing kit (Amersham Biosciences, USA).

## Results

HPV DNA was positive in five (2.5%) of 200 pregnant women included in the study. Of the positive samples two (1%) were determined as type 16, two (1%) were determined as type 18 and one (0.5%) was determined as type 11.

In control group including 102 women, HPV DNA was positive in two (2%) women both of which were type 16. (Table I).

*Table I: HPV DNA results by PCR in pregnant women and control group*

| | Pregnant (n:200) | Control (n:102) |
|---|---|---|
| HPV DNA (+) | 5 (2.5%) | 2 (2%) |
| HPV 16 | 2 (1%) | 2 (2%) |
| HPV 18 | 2 (1%) | _ |
| HPV 11 | 1 (0.5%) | _ |
| HPV DNA (-) | 195 (97.5%) | 100 (98%) |

## Discussion

There are discordant results in studies investigating HPV prevalence during pregnancy and a ratio between 5%-50 % is pointed out.[9] HPV DNA positivity in asymptomatic pregnant women was found to be 2.5% in our study where Smith et al, [10] Gajewska et al[11] and Takakuwa et al[12] found 29%, 26% and 12.5% positivity respectively in pregnant women taking routine obstetric care. HPV type 16 was found as 1% in our study where the DNA of HPV type 16 was detected as 13.3% by Gajewska et al.[11]

Besides studies demonstrating an increase in HPV infection during pregnancy,[13,14,15] there are also studies demonstrating no change or a decrease.[9,16,17] There is not a statistically significant difference between pregnant women and control group in our study.

Significant difference in the results of studies investigating HPV prevalence may be related with the characteristics of the chosen patients or the diagnostic methods, the clinical feature of the pregnant woman and the collection time of the sample. [11,18] Besides this, age, smoking, low social and economic income level, presence of other sexually transmitted diseases may be a cause of different results.[19]

Maternal age may be a factor changing the prevalence of HPV infection. Eppel et al.[3] in their study, showed that HPV infection prevalence decreases as maternal age increases and Takakuwa et al [12] found that there is a statistically significant difference of positivity rate between pregnant women younger than 25 years old (22.6%) and equal to or older than 25 years old (11.3%). If we had the chance to study more patients, we could make a decision about the relationship between maternal age and HPV infection.

There are incoherent results in studies demonstrating the presence of HPV infection during pregnancy week. While some of them demonstrate an increase in the advanced weeks of pregnancy,[15] others demonstrate no relationship between HPV infection and pregnancy weeks.[9,12] Since we collected samples between the 18th-28th weeks (avarage of 24th weeks) of pregnancy, we cannot make a decision about advanced pregnancy weeks.

One of the most important risk factors in HPV infection is number of partners[20] We could not have the chance of questioning number of sexual partners of pregnant women because of social reasons.

The transmission of HPV infection to fetal compartments is not well-known. In utero transmission could be caused either by ascending infection from an infected birth canal or hematogenously, via the placenta.[21] Ascendent spread from vulva, cervix, infection of oocyte or zygote before or after implantation or latent infection of the sperm may be the ways of transmission.[6,22] Also vertical transmission by the aspiration of cervical or vaginal secretions may cause perinatal infection [23,24] Demonstration of a high prevalence of latent HPV infection in asymptomatic women by using sensitive assays in epidemiological studies makes us think that many newborns have a risk of oropharyngeal and genital HPV infection [25]

The possibility of perinatal transmission may be related with the viral load in infected cells or other risk factors such as smoking and low levels of economic income. Authors suggest that perinatal HPV transmission occurred and that newborns are at higher risk of exposure to HPV with vaginal delivery compared to cesarean section. and C/S delivery is considered to be protective for perinatal infection [26] The detection of HPV DNA in amniotic fluid and peripheral blood [5] shows that the virus can pass the placental barrier and infect the fetus. We preferred vaginal delivery in women who did not have indications for C/S. Of the five positive cases, three had vaginal delivery and two had C/S section.

During the follow-up of HPV positive group, there was not difference with the HPV negative group in delivery week and delivery weight. And there was not a congenital or neonatal abnormality in five HPV positive cases although HPV infection may cause placental dysfunction and is associated with adverse pregnancy outcomes, including spontaneous preterm delivery.[27]

Vertical transmission of HPV infection is between 0%-80% in literature.[25] In HPV positive cases, none of the nasopharyngeal swab samples of the neonates were positive by PCR in favour of HPV in our study (%0).

The focal point of our study was to investigate latent HPV infection in asymptomatic pregnant women with no abnormal smear or genital lesions and to investigate whether it causes a risk in our population since it is thought to be related with maternal and fetal complications.

Our results show that HPV prevalence in our study is lower than that reported in literature. Infection was not found in neonates of the HPV (+) women. The probable reasons of this result may be related with the chosen group; nonsmoking, young women with high social and economical income level and no lesions. And also since monogamy is common in our country and sexual experience before marriage is limited, the sexual transmission of HPV infection is rare.

## Human Papillomavirus (HPV) ve Gebelik; Prevalans ve Tanı Yöntemleri

**AMAÇ:** Gazi Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum polikliniğine başvuran gebelerde serviks kanseri etiyolojisinde çok önemli rolü olan Human papillomavirus (HPV)'un Polimeraz Zincir Reaksiyonu (PCR) yöntemiyle tespiti ve tip tayini amaçlanmıştır.

**GEREÇ ve YÖNTEM:** Rutin kontrol için Gazi Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum polikliniğine başvuran asemptomatik 200 gebe ve 102 kontrol hastasından alınan sürüntü örneklerinden fenol kloroform izoamilalkol yöntemiyle DNA izolasyonu yapılmış ve MY 09/11 primerleri ile HPV DNA amplifikasyonu yapılmıştır. Tip 16 ve 18 tayini için özgül primerler kullanılmıştır.

**BULGULAR:** Çalışmaya alınan 200 gebenin beşinde (%2.5) HPV DNA pozitif bulunmuştur. Pozitif örneklerin ikisi (%1) tip 16, ikisi (%1) tip 18, biri (%0.5) tip 11 olarak tespit edilmiştir. Yüziki kişilik kontrol grubunda iki (%2) hastada HPV DNA pozitif bulunmuştur. Pozitif örneklerin her ikisi de tip 16 olarak tespit edilmiştir

**SONUÇ:** Sonuçlarımız literatürdeki oranlara göre düşüktür. Bunun nedeni Türkiye'de tek eşliliğin yaygın olması ve hasta grubumuzun yüksek sosyoekonomik düzeye sahip sigara içmeyen kadınlardan oluşması olabilir. Bununla birlikte, asemptomatik olduğu halde virus tesbit edilen hastalar tedavi ve takip açısından yönlendirilmişlerdir.

**Anahtar Kelimeler:** HPV, Gebelik, PCR

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