INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Investigation of HPV-DNA in Cervical Smear Samples by PCR Methods

Latife Sütcü Kızılkaya[1], Bahadır Feyzioğlu[2], Mehmet Özdemir[2], Bülent Baysal[2], Kazım Gezginç[3], Hacı Hasan Esen[4].

[1]Department Of Medical Microbiology, Konya Beyhekim State Hospital, Konya, Turkey.

[2]Department of Medical Microbiology, Necmettin Erbakan University Meram Faculty of Medicine, Konya, Turkey.

[3]Deparment of Obstetric and Gynecologic, Faculty of Medicine,Necmettin Erbakan University, Konya, Turkey.

[4]Department of Pathology, Necmettin Erbakan University Meram Faculty of Medicine, Konya. Turkey.

Abstract

It was aimed to determine the prevalance of HPV, type distribution and to investigate the relationship between these results and sociodemographic factors. Between May 2010-2011 sexual active in 21-81 age groups 180 patients were included in this study. Cervical swap samples were collected for Pap smear and HPV DNA test during routine gynecological examination. For Pap test, these samples were stained with Papanicolaou and cytological examination were performed. DNA isolation were performed with High Pure Viral Nucleic Asid and amplification with commercial kits. PCR amplicons were hybridized with specific oligonucleotide probes.HPV DNA was detected in 18,9 % (n=34) of cases. The most frequent genotypes were HPV 16 (20.8%), HPV 51 (20.8%), HPV 6 (12.6%), HPV 53 (8.3%) and HPV 52 (8.3%). The marital status and age at the first sexual intercourse of patients were found to be statistically significant with HPV DNA positivity ($p<0.05$). Also, we observed that the prevelance of HPV DNA was increased according to the severity of the cytologic category. The studies that determine HPV prevalance and type distribution in our region might contribute the vaccine trials. Thus, the current vaccines will be insufficient for our region and the vaccines should be redesigned to regional differences.

Key words: Human Papillomavirus, HPV vaccine, PCR, Pap smear test.

INTRODUCTION

Cervical cancer is among prominent health issues important for women's health. Cervical cancer is the second most common type of cancer among women in the world after breast cancer [1, 2]. More than 470 thousand new cervical cancers are diagnosed across the world each year and about 274 thousand deaths related to cervical cancer are reported [1, 2, 3]. On the other hand, the relationship between the development of cervical cancer and Papillomaviruses is clearly established today and studies are being conducted on this relationship. Papillomaviruses are DNA viruses that perform their small, unenvoloped icosahedral symmetry replications in squamous epithelial cells [4, 5]. There are more than 140 types of viruses within the Papillomaviridae family, about 40 of which can infect genital mucosa [1]. They can infect various epithelial

tissues in the entire body including skin, oral cavity, larynx and anogenital tissues [6, 7, 8, 9].

Human papillomavirus (HPV) is an infectious agent that is quite widespread in society and sexually transmitted. Genital HPV infections, especially infections caused by HPV genotype 16 and 18, are the most important risk factors in cervical cancer pathogenes [10, 11]. HPV DNA has been identified in 99.7 % of cervical cancer and precursor lesions [12].

Cervical cancer has become a mostly preventable disease through detection and treatment of precancerous lesions [1, 3]. Treatment options were limited before the screening programs were developed because cervical cancer was generally detected at advanced (terminal) phases when it became symptomatic. However, today, spread of screening programs planned for early diagnosis and treatment of cervical cancer has led to a noticeable decrease in incidence of cervical cancer and related deaths. In recent years, *Human papillomavirus* (HPV) tests have taken their place among new screening techniques and begun to be used together with cytology [1].

Detection of HPV-infected patients, determination of patients infected with HPV types related to cervical cancer and implementation of necessary treatments are important to prevent possible cases of cervical cancer.

The purpose of this study is to identify HPV positiveness and types in cervical swab samples taken during routine gynecological examination from patients who applied to Obstetrics and Gynecology Clinics, and investigate the relationship between HPV DNA and HPV type smear pathologies and risk factors.

MATERIAL AND METHOD

180 patients who *applied to Selcuk University Meram Medical Faculty Hospital and to Konya Dr. Faruk Sükan Obstetrics and Children's Hospital Gynecology and Obstetrics Polyclinics between the dates* May 2010 and 2011 were included in our study. Patients between the ages of 21 and 81 who were sexually active participated in the study. Patients with past history of hysterectomy, cervical stenosis or pregnancy were not included in the study. Information was recorded about age, marital status, age of first sexual intercourse, number of partners, cigarette use, condom use and use of oral contraceptives of the patients in the study group.

Our study was supported by Scientific Research Projects Coordination Office with project number 10102006, and approved by the Ethical Board decision number 2009/050 dated 25.11.2009.

Sample Collection

Cervical samples were taken during routine gynecological examination for detection of Pap smear and HPV DNA from patients who applied to Obstetrics and Gynecology Polyclinics and were eligible for our study. Before the samples were taken, the patients were informed about how the samples would be taken and their consent was obtained. For Pap smear test, swab sample taken with a cervical brush was spread on microscope slide and fixed and then brought to the Pathology Laboratory of our hospital for analysis. The samples were stained with Papanicolaou (Pap) stain and evaluated on the basis of Bethesda 2001 terminology system.

Swab samples taken from the endocervical canal and the whole transformation area were put on Stuart's transport medium (agar) (Copan, Brescia, Italy) and after they were taken to the Microbiology Laboratory of our hospital for detection of HPV DNA, they were kept at -20 °C until the study began.

DNA isolation

First, DNA isolation was performed in order to determine and type HPV DNA. High Pure Viral Nucleic Acid kit (Roche Diagnostics, Mannheim, Germany) was used for DNA isolation.

For amplification, INNO-LIPA HPV Genotyping Extra Amp (INNOGENETICS N. V., Belgium), which targets 28 different HPV gene areas and works on the principle of reverse hybridization, was used. It was intended to reproduce the 65 bp genome of HPV in the best protected L1 gene area by using short PCR fragment 10 (SPF 10) primers marked with biotin. At the same time, a pair of primers reproducing human HLA-DPB1 gene was used to control the sample quality and extraction while HPV6 genome was used as positive control. Following amplification, hibridization of PRC amplicons to specific catcher oligonucleotide props in nitrocellulose band was performed. After necessary washing was performed in accordance with the suggestions of the manufacturing firm, chromogenic subtract was added and formation of chromogenic band on nitrocellulose band was observed. Evaulations were made by taking 28 lines and 4 control lines indicating specific DNA sequence on a membraneous strip involving props attached to one another in the form of paralel lines. Assessment of the results was conducted by eye using INNO-LIPA HPV Genotyping Extra Reading Card.

The Statistical Method: The data were entered in the Statistical Program for Social Sciences (SPSS, version 13.0)package program. X^2 analysis was used for comparison. $P< 0.05$ was taken as the significance level. Descriptive findings were expressed in average, ± standard deviation and percentages.

RESULTS

180 patients who applied to Selcuk University Meram Medical Faculty Hospital and to Konya Dr. Faruk Sükan Obstetrics and Children's Hospital Gynecology and Obstetrics Polyclinics between the dates May 2010 and 2011 were included in our study. 7 (3,9 %) of the patients, who were aged 21-81 and had an average age of 38,6±10,3, were at or below age 24, 59 (32,8 %) were between 25 and 34, 66 (36,7 %) were between 35 and 44, 37 (20,6 %) were between 45 and 54 and 11 (6,1 %) were 55 or above.

While HPV DNA was identified in 18.9 % (n=34) of the samples taken from the patients, HPV DNA was negative in 81.1 % (n:146) of them. Presence of HPV DNA was at the highest in the 55 and above age group with a rate of 27.3 % whereas the lowest rate was in the 25-34 age group with a rate of 13.6 %. On the other hand, there was not a statistically significant difference between age groups in terms of HPV DNA positiveness (p>0.05) (Table-1). The most commonly identified HPV types were type 16 (20.8 %) and type 51 (20.8 %), followed by type 6 (12.6 %), type 53 (8.3 %) and type 52 (8.3 %). On the other hand, infection with more than one HPV type was observed in 8 patients aged above 30 (Table-2, Table-3).

According to the findings of the cytological evaluation of the samples, there was a distribution of 92 (51,1 %) normal, 71 (39,4 %) ASCUS (Atypical Squamous Cells of Undetermined Significance), 4 (2,2 %) AGUS (Atypical Glandular Cells of Undetermined Significance), 3 (1,7 %) ASCH (Atypical Squamous Cells, Cannot Rule Out High-Grade Squamous Intra-epithelial Lesion), 2 (1,1 %) LSIL (Low-grade Squamous Intraepithelial Lesion), 2 (1,1 %) HSIL (High-grade Squamous Intraepithelial Lesion) (Table-4). 95 % of the patients who were included in the study (n=171) were married, whereas 5 % (n=9) were single or widowed (Table-5). A statistically significant correlation was determined between the marital status of the patients and HPV DNA positiveness (p<0.05). HPV DNA positiveness was higher among those that were single or widowed than those that were married. The average first sexual experience age of the patients was 20,7±3,5. The first sexual experience age of 5 patients (2,8 %) was 15 or below, while the first sexual experience age of 65 patients (36,1 %) was between 16 and 19, and the first sexual experience age of 110 patients (61,1 %) was 20 and above 20. A statistically significant relationship was found between the patients' first sexual experience age and HPV DNA positiveness (p<0.05). Those whose first sexual experience age was 19 or below were more HPV DNA positive than those aged 20 or above (Table-6). In the sexual life anamnesis, 172 patients (95,6 %) had 1 partner, 7 patients (3,9 %) had 2 partners while 1 patient (0,6 %) had 3 partners (Table-7). There was not a statistically significant relationship between the patients' number of partners and HPV DNA positiveness (p>0.05).

15,6 % of the patients (n=28) smoked whereas 84.4 % of them (n=152) did not smoke (Table-8) and no statistically significant relationship was found between smoking and HPV DNA positiveness (p>0.05). It was determined that 16.7 % (n=30) of the patients used condom as a means of birth control while 6.1 % (n=11) of them used oral contraceptives (Table-9). It was found that HPV DNA positiveness was higher in patients who did not use condom than in those who used condom but this difference was not statistically significant (p>0.05). Likewise, a statistically significant relationship was not found between the use of oral contraceptives and HPV DNA positiveness (p>0.05) (Table-10). On the other hand, HPV DNA positiveness was determined in 2 (33.3 %) of the 6 patients who were included in our population and whose genital condyloma were identified.

DISCUSSION

Human papillomavirus causes widespread genital infections and this is an asymptomatic infection in young and sexually active people. The link of lower genital system malignencies to HPV infection especially to cervical cancer has been established in many molecular, experimental, epidemiologic and clinical researches [13]. According to the data of the Ministry of Health belonging to the years 2006-2008, the incidence of cervical cancer in Turkey is about 4.4-4.8/100.000 and ranks 10th among cancers in terms of prevalence [14].

Likewise, according to the data of Globocan (The World Health Organization Cancer Research Unit), cervical cancer comes 7th in deaths caused by cancer among women in our country [15].

In addition to screening programs aimed at determining people carrying cervical cancer risk and conducting appropriate clinical follow-up, investigation of presence of HPV infection in individuals and determination of the type of the HPV agent are of critical importance in developing strategies against this problem [16,17]. Due to the fact that the agent can not be isolated in diagnosing HPV infections and serological tests are not adequately sensitive methods aimed at determining viral nucleic acid are effective and valid approaches for a conclusive diagnosis [1,18]. The HPV DNA analyis, which is used for this purpose, appears to be a golden standard but the fact that it is a difficult and expensive technique often causes other molecular techniques to be preferred [18,19]. In our study, the LIPA method, which is based on the principle of

reproducing L1 gene target area through PCR and its reverse hybridization on strip, was used. HPV DNA was identified in 34 (18,9 %) of the 180 patients, of whom 92 had normal cytology, 71 had ASCUS, 4 had AGUS, 3 had ASC-H, 2 had LSIL, 2 had HSIL and 6 had condyloma.

It is estimated that 6.2 million new cases infected with high-risk HPV occur in America. In time, the virus is eliminated in many of the infected individuals without exhibiting any symptoms. The prevalence of women infected with HPV varies between 2 % and 44 % in the world [10, 11]. In our country, HPV prevalence and types were investigated in a study conducted by Dursun et al. on 403 women who applied to gynecology clinics. The study found the total HPV prevalence as 23.1 % while the prevalence was determined to be 36 % in patients whose pap smear test results were abnormal (22 % ASCUS, 51 % LSIL, 60 % HSIL) [20]. In another study that was conducted in 2006, presence and types of HPV were investigated, using the PCR method, in patients in whom only the atypical form of the virus was identified as a result of a routine cytological examination. A total of 35 cases were included in the study, of which 14 were ASCUS, 1 was AGUS, 3 were ASC-H, 5 were HSIL, 7 were LSIL, 4 were LSIL+ possible HSIL, and 1 was atypical cell of unknown nature. In conclusion, a high level of HPV DNA positiveness was obtained in 28 (80 %) of the 35 samples, whereas viral DNA could not be determined in 7 individuals (20 %) consisting of ASCUS and ASC-H cases [16]. In another study where presence of HPV DNA was investigated in 50 cervical tissue samples diagnosed with cervical cancer and CIN, the virus was found to be positive in 35 samples (70 %) [21]. Our study found the total HPV prevalence as 18.9 %. The prevalence we obtained from the patient group which we included in our study was acquired from a population that involved patients who exhibited both normal and abnormal findings in terms of Pap smear results. Therefore, the values we obtained were lower than those obtained from groups that were narrowed through atypical cervical tissue samples and this was interpreted to be an expected result.

Attempts are being made to provide protection against cervical cancer through screening programs. However, given the prevalence of the disease, it is a fact that HPV vaccines are also of substantial importance. Today, two bivalent and quadrivalent HPV vaccines developed by two companies have obtained licences. Both vaccines target the HPV type 16 and type 18, which are the most common and high risk types, but the quadrivalent one additionally targets prophylaxis for type 6 and type 11 (3, 22, 23). In our study, type 16 (20.8 %) and type 51 (20.8 %), type 6 (12.6 %), type 53 (8.3 %) and type 52 (8.3 %) were the most common types respectively. Unlike what is expected in the light of studies conducted so far and the relevant literature, HPV type 18 was found only in one patient (0.6 %) together with other HPV types. Therefore, this leads one to think that especially expectations of vaccine effectiveness and vaccine target type strategies should be revised seriously.

In our study, it was determined that 8 patients had been infected with more than one HPV type and all of these patients were above the age of 30. It is quite interesting that the rate of older people who were infected with more than one type of HPV was quite high in comparison to younger patients. Longer periods of exposure and reactivation of the viral infection can explain this situation. Moreover, a parallel distribution between HPV presence and smear type was not observed in these patients.

Although some studies have stated that HPV prevalence is at the highest between the ages of 20 and 24 and there is a sharp decrease after age 25 [24], there are other studies indicating that HPV prevalence reaches a peak in those below age 25, decreases between the ages of 35 and 54 but reaches a second peak after age 55 [1, 25]. A study which investigated high-risk HPV prevalence for our study determined HPV prevalence to be at the highest in the 30-34 age group [26]. On the other hand, when the age-specific prevalence of HPV was investigated in our study, unlike these studies, a peak was not found in younger or middle ages and it was determined that prevalence increased with age and reached the highest level at age 55 and above (27.3 %). The sample group can be evaluated in terms of the age and frequency of first exposure in the context of the social and cultural features of the population involving our patient group. The most prominent risk factors for genital HPV infection include the number of sexual partners throughout one's life, the age of first sexual experience and the sexual behaviors of the male partner. Genital HPV infection prevalence is highest among sexually active young women. The findings of our study are also in support of this situation. While the prevalence of HPV is 60 % in patients whose age of first sexual experience is 15 or below, this figure falls to 14.5 % in those aged 20 or above [3, 12, 27].

Asymptomatic individuals infected with the virus play a significant part in the spread of the virus [10,11]. Asymptomatic porters serve as a reservory and can be potential vectors in the contagion of the virus. In our study, on the other hand, a statistically significant relationship could not be found between the number of partners and HPV positiveness. A large majority of the patients who were included in our study had only

one sexual partner (172 patients/ 95.6 %). There were only 8 patients who had two or more sexual partners (4.4 %). HPV DNA was positive in 18 % of the patients who had one partner (31 patients) whereas positive results were obtained in 2 of the 7 patients who had two partners (28.6 %) and one patient who had 3 partners.

Although young age and increasing number of sexual partners are major risk factors in the acquisition of HPV infections, other sexually transmitted infections, smoking, immunesuppression and the use of oral contraceptives are among other risk factors. As estrogen stimulates antibody response and cellular immune system, it plays an important part in the early stages of infections. Data concerning the effect of hormone therapy on the incidence of HPV-related cervical lesions exhibit variation [28]. In a study conducted in the USA on 444 university students, a positive and a statistically significant relationship was found between the use of oral contraceptives (the average total control period was 41.2 months) and HPV infections [29]. Moreno et al.[30] reported that when patients who never used oral contraceptives and patients who used them for less than 5 years were compared, there was no risk increase in cervical cancer whereas there was a three-fold increase in cancer risk of patients who used oral contraceptives for 5-9 years or more than 10 years. Information about oral contraceptive use was obtained from the patients who participated in our study but no clear information could be obtained about the total period of use, and patients could not be monitored for longer periods. Therefore, cumulative effect of oral contraceptives could not be investigated. Only 11 of the 180 patients who participated in our study reported using oral contraceptives and only in 2 of them (18.2 %) HPV DNA was positive. On the other hand, positive results were obtained in 18.9 % of the patients who did not use oral contraceptives. According to these results, no statistically significant relationship was observed between the use of oral contraceptives and HPV.

Many case control studies that have been conducted have shown a significant relationship between smoking and cervical cancer [31]. Smoking exerts mutagenic effects on cervical tissues and causes decreases in Langerhans cells, which are major components of the cellular immune reponse of the cervix [32]. 28 patients (15.6 %) who participated in our study were smokers. HPV DNA was positive in 25 % of them. HPV DNA was positive in 17.8 % of the 152 patients who did not smoke.

Cervical cancer evolves from precancerous lesions defined as squamous intraepithelial lesion (SIL) or cervical intraepithelial neoplasia (CIN) [33]. Cytopathic effects can be seen in cells due to HPV infection. Precancerous lesions are grouped on the basis of atypical changes in the epithelium into low grade (SIL; LSIL) and high grade (SIL; HSIL) squamous intraepithelial lesions [1]. In our study, 2 patients were LSIL and 2 were HSIL. The patients who were HSIL had type 16 and type 33. While one of the patients who had LSIL had type 51, no positive result was obtained from the other.

In another study conducted by Kahn et al. in America, the relationship between the high-risk HPV infection and socio-democratic factors was investigated. It was reported that HPV prevalence was higher in patients who were economically poor and had low educational level. Moreover, the rate of HPV infection was lower in married women than in unmarried ones [34]. In parallel to this, our study also demonstrates that being widowed or single carried a high risk in terms of HPV infection and a statistically relationship was found between HPV positiveness and the marital status of the patients.

Sexual activity is a primary risk factor in the spread of HPV infection. It is thought that the use of condoms, which is effective in preventing other sexually transmitted infections, is partially effective against HPV infections. Even permanent condom use can provide only 60 % protection because contagion is still possible through skin contact in the genital area [3, 10]. However, a study conducted by Hogewoning et al. in 2003 indicated that condom use led to a reduction in lesions in women who had cervical precancerous lesions [35]. A study conducted in Mexico on 237 female university students reported that there was a 3.8 fold increase in high-risk HPV prevalence in those who did not always use condoms and had more than one sexual partner [36]. A large majority of the patients included in our study used contraception methods other than condom (%83.3). However, it was observed that HPV DNA positiveness was lower in patients who used condoms than those who did not, but the difference between the results was not statistically significant (HPV prevalence was 6.7 % in the group that used condom whereas it was 21.3 % in the group that did not use condoms).

HPV prevalence and its relationship with demographic characteristics have been investigated in Turkey and in other countries and differences have been observed in these studies. Differences in the age range of the participants and smear characteristics, tests use of differentially sensitive tests, geographical and cultural features of countries, and effectiveness of systematic screening programs in developed countries and HPV vaccines can be cited among the reasons for differences in HPV prevalence values.

Our study reveals preliminary information about HPV prevalence and distribution of its types in

Konya and its environs, and contributes to shedding light on the epidemiology of HPV infections in our country. Thus, it will be possible to demonstrate the distribution of regional high-risk HPV types and information of this kind will be used in vaccine studies.

Table 1
HPV DNA positiveness according to age groups

	HPV DNA positive		**HPV DNA negative**		**Total**	
Age groups	n (number)	% (percentage)	n (number)	% (percentage)	n (number)	% (percentage)
24 and below	1	14,3	6	85,7	7	3,9
25-34	8	13,6	51	86,4	59	32,8
35–44	14	21,2	52	78,8	66	36,7
45-54	8	21,6	29	78,4	37	20,6
55 and above	3	27,3	8	72,7	11	6,1
Total	34	18,9	146	81,1	180	100

p=0.716

Table 2
Distribution of HPV types

HPV type	**n (number)**	**% (percentage)**
16	10	20.8
51	10	20.8
6	6	12.6
52	4	8.3
53	4	8.3
33	3	6.3
31	2	4.1
45	2	4.1
66	2	4.1
18	1	2.1
11	1	2.1
44	1	2.1
56	1	2.1
82	1	2.1

Table 3
Patients infected with more than one HPV type

Types	**Sample number**
16, 18, 51, 45 1	1
31, 33, 51, 53 1	1
6, 52, 53 1	1
31, 33, 44 1	1
51, 56 1	1
53, 82 1	1
6, 16 1	1
6, 66 1	1

Table 4
HPV DNA positiveness according to smear results

	HPV DNA positive		HPV DNA negative		Total	
Smear result	n (number)	% (percentage)	n (number)	% (percentage)	n (number)	% (percentage)
Normal	15	16.3	77	83.7	92	51,1
ASCUS	12	16.9	59	83.1	71	39,4
AGUS	1	25	3	75	4	2,2
ASC-H	1	33.3	2	66.7	3	1,7
LSIL	1	50	1	50	2	1,1
HSIL	2	100	0	0	2	1,1

Table 5
HPV DNA positiveness according to patients' marital status

	HPV DNA positive		HPV DNA negative		Total	
Marital Status	n (number)	% (percentage)	N (number)	% (percentage)	n (number)	% (percentage)
Married	29	17	142	83	171	95
Single/Widowed	5	55,6	4	44,4	9	5
Total	34	18,9	146	81,1	180	100

x^2 value= 8.31, p=0.004

Table 6
HPV DNA positiveness according patients' first sexual experience age

	HPV DNA positive		HPV DNA negative		Total	
Age of first sexual experience	n (number)	% (percentage)	n (number)	% (percentage)	n (number)	% (percentage)
15 and below	3	60	2	40	5	2,8
16-19	15	23.1	50	76.9	65	36,1
20 and above	16	14.5	94	85.5	110	61,1
Total	34	18.9	146	81.1	180	100

x^2 value= 7.61, p=0.022

Table 7
HPV DNA positiveness according to patients' number of partners

	HPV DNA positive		HPV DNA negative		Total	
Number of partners	n number)	% (percentage)	n (number)	% (percentage)	n (number)	% (percentage)
1	31	18	141	82	172	95,6
2	2	28,6	5	71,4	7	3,9
3	1	100	0	0	1	0,6
Total	34	18,9	146	81,1	180	100

p=0.09

Table 8
HPV DNA positiveness according to patients' smoking habit

	HPV DNA positive		HPV DNA negative		Total	
Smoking	n (number)	% (percentage)	n (number)	% (percentage)	n (number)	% (percentage)
Yes	7	25	21	75	28	15,6
No	27	17,8	125	82,2	152	84,4
Total	34	18,9	146	81,1	180	100

p=0.369

Table 9
HPV DNA positiveness according to patients' condom use

	HPV DNA positive		HPV DNA negative		Total	
Condom use	n (number)	% (percentage)	n (number)	% (percentage)	n (number)	% (percentage)
Yes	2	6,7	28	93,3	30	16,7
No	32	21,3	118	78,7	150	83,3
Total	34	18,9	146	81,1	180	100

p=0.061

Table 10
HPV DNA positiveness according to patients' oral cantraceptive use

	HPV DNA positive		HPV DNA negative		Total	
Oral contraceptive use	n (number)	% (percentage)	N (number)	% (percentage)	n (number)	% (percentage)
Yes	2	18,2	9	81,8	11	6,1
No	32	18,9	137	81,1	169	93,9
Total	34	18,9	146	81,1	180	100

p=0.951

REFERENCES

1. Ağaçfidan A, Erturan Z. Human papillomaviruses. XXXIII Turkish Microbiology Congress, Congress Book, Bodrum, Turkey, 21-25 October 2008: 313-337.
2. Longworth MS, Laimins LA. Pathogenesis of Human Papillomaviruses in differentiating epithelia. Microbiology and Molecular Biology Reviews 2004; 68:362-372.
3. Wang KL. Human papillomavirus and vaccination in cervical cancer. Taiwan J Obstet Gynecol 2007;46(4):352-362.
4. Tuncer S. Human Papillomaviruses. In: Ustaçelebi Ş. Basic and Clinical Microbiology, Ankara: Güneş publishing house, 1999:797-802.
5. Duensing S, Münger K. Mechanisms of genomic instability in human cancer: Insights from studies with Human papillomavirus oncoproteins. Int J Cancer 2004; 109:157-162.
6. Anaya-Saavedra G, Ramirez-Amador V, Irigoyen- Camacho ME, Garcia-Cuellar CM, Guido-Jimenez M, Mendez-Martinez R, et al. High association of Human papillomavirus infection with oral cancer. Archives of Medical Research 2008;39:189-197.
7. Syrjanen S. Human papillomavirus (HPV) in head and neck cancer. Journal of Clinical Virology 2005;32:59-66.
8. Gillison ML. Human Papillomavirus- related diseases: Oropharynx cancers and potential

implications for adolescent HPV vaccination. Journal of Adolescent Health 2008;43:52-60.
9. Corbalan-Velez R, Ruiz-Macia JA, Brufau C, Carapeto FJ. Cutaneous squamous cell carcinoma and human papillomavirus. Actas Dermosifiliogr 2007;98:583-593.
10. Stanley M. Pathology and epidemiology of HPV infection in females. Gynecologic Oncology 2010;117:5-10.
11. Trottier H, Franco EL. The epidemiology of genital Human papillomavirus infections. Vaccine 2006;24:4-15.
12. Franco EL, Duarte-Franco E, Ferenczy A. Cervical cancer: epidemiology, prevention and the role of Human papillomavirus infection. CMAJ 2001;164(7):1017-25.
13. Rota S, Biri A, Bozdayı G, Dinç B, Güner H. Farklı hasta gruplarında servikal biyopsi ve sürüntü örneklerinde PCR ile genital Human papillomavirus araştırılması ve tip tayini. Türk Mikrobiol Cem Derg 2004;34:185-189.
14. http://www.sağlik.gov.tr/TR/belge/1-13438/sağlık istatistikleri yıllığı 2010.pdf (last date of access 17.8.2011)
15. http://globocan.iarc.fr (last date of access 17.8.2011)
16. Ergünay K, Misirlioğlu M, Firat P, Tuncer ZS, Tuncer S, Yildiz I, Ustaçelebi. S. [Detection and typing of human papilloma virus by polymerase chain reaction and hybridization assay in cervical samples with cytological abnormalities]. Mikrobiyol Bul. 2008 Apr;42(2):273-82
17. Akhan SE. The Epidemiology of Cervical Cancer and HPV Serotypes in Turkey. ANKEM Derg 2007;21(2):96-98.
18. Molijn A, Kleter B, Quint W, Doorn LJ. Molecular diagnosis of Human papillomavirus (HPV) infections. Journal of Clinical Virology 2005;32:43-51.
19. Feng Q, Cherne S, Winer RL, Balasubramanian A, Lee SK, Hawes SE, et al. Development and evaluation of a liquid based microarray assay for genotyping genital Human Papillomaviruses. Jorunal of Clinical Microbiology 2009;47(3):547-553.
20. Dursun P, Senger SS, Arslan H, Kuşçu E, Ayhan A. Human papillomavirus prevalance and types among Turkish women at a gynecology outpatient unit. BMC Infectious Diseases 2009;9(191):1-6.
21. Yavuzer D, Karadayı N, Erdağı A, Salepçi T, Baloğlu H, Dabak R. HPV typing with PCR in cervical cancerous and precancerous lesions. Kartal Eğitim ve Araştırma Hastanesi Tıp Dergisi 2009;(1):1-6.
22. Campo MS, Roden RBS. Papillomavirus prophylactic vaccines: Established successes, new approaches. Journal of Virology 2010;84(3):1214-1220.
23. Thomison J, Thomas LK, Shroyer KR. Human papillomavirus: molecular and cytologic/histologic aspects related to cervical intraepithelial neoplasia and carcinoma. Human Pahology 2008;39:154-166.
24. Woodman CBJ, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Cancer 2007;7:11-22.
25. Baseman JG, Koutsky LA. The epidemiology of Human papillomavirus infections. Journal of Clinical Virology 2005;32:16-24.
26. Tuncer ZS, Başaran M, Ustaçelebi Ş, Mocan-Kuzey G. High risk Human papillomavirus infection (HPV) determined by hybrid capture II assay in a Turkish university hospital outpatient clinic. Gynecol Obstet Reprod Med 2006;12:129-134.
27. Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, et al. Prevalance of HPV infection among females in the United States. JAMA 2007; 297:813-819.
28. Frega A, Scardamaglia P, Piazze J, Cerekja A, Pacchiarotti A, Verrico M, et al. Oral contraceptives and clinical recurrence of Human papillomavirus lesions and cervical intraepithelial neoplasia following treatment. International Journal of Gynecology and Obstetrics 2008;100:175-178.
29. Mohllajee AP, Curtis KM, Martins SL, Peterson HB. Hormonal contraceptive use and risk of sexually transmitted infections. Contraception 2006;73:154-165.
30. Moreno V, Bosch FX, Munoz N, Meijer CJLM, Shah KV, Wallboomers JMM, et al. Effect of oral contraseptives on risk of cervical cancer in women with Human papillomavirus infection. Lancet 2002;359:1085-1192.
31. Castellsague X, Munoz N. Cofactors in Human papillomavirus carcinogenesis-role of parity, oral contraseptives and tobacco smoking. Journal of the National Cancer İnstitute Monographs 2003;31:20-28.
32. Giuliano AR, Sedjo RL, Roe DJ, Harris R, Baldwin S, Papenfuss MR, et al. Clearance of oncogenic Human papillomavirus (HPV) infection: effect of smoking (United States). Cancer causes and Control 2002;13:839-846.
33. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370:890-907.
34. Kahn JA, Lan D, Kahn RS. Sociodemographic factors associated with high risk Human

papillomavirus infection. Obstet Gynecol 2007;110:87-95.

35. Ault KA. Epidemiology and natural history of Human papillomavirus infections in the female genital tract. Infectious Diseases in Obstetrics and Gynecology 2006:1-5.

36. Sanchez-Aleman MA, Uribe-Salas FJ, Lazcano-Ponce EC, Conde-Glez CJ. Human papillomavirus incidence and risk factors among Mexican female college students. Sexually Transmitted Diseases 2011;38(4):275-278.