High-risk Human Papilloma Virus (HPV) infection determined by Hybrid Capture II assay in a Turkish university hospital outpatient clinic

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OBJECTIVE: To determine the frequency of HPV infection using Hybrid Capture II assay and to compare the results with conventional Pap smear for screening cervical neoplasia.
STUDY DESIGN: Between February 2001 and October 2002, 1032 patients admitted for routine Pap smear screening were recruited. Sexually active patients under the age of 60 were recruited for the study. All patients underwent a detailed history, gynecological examination, Hybrid Capture II test, and Pap test. High-risk HPV DNA positive patients were subjected to colposcopy and guided biopsy when indicated.
RESULTS: The mean age of the patients was 36.8±9.3. Forty-one (4.0%) were positive for high risk HPV. Highest rate of infection was observed in patients between ages of 30-34 years (10/182, 5.5%). Colposcopic examination was negative in 24 patients. Of the remaining 17 patients who underwent guided biopsies, 5 had low-grade squamous intraepithelial lesion (LSIL), 1 had high-grade squamous intraepithelial lesion (HSIL), and 11 had chronic infection. HPV testing could identify 3 additional patients with LSIL among Pap test negative group. Age, parity, socioeconomic status, contraceptive method, age of first sexual intercourse, cigarette smoking did not correlate with HPV infection.
CONCLUSION: The first report, to our knowledge, of high-risk HPV infection rate as 4.0% in a Turkish population seems to be in accordance with the previous reports from other countries. Combination of HPV testing and Pap smear improves diagnostic performance for detection of cervical neoplasia.
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Key Words: Human Papilloma virus, Hybrid capture assay, Pap smear, Cervical intraepithelial neoplasia

Epidemiologic and laboratory data have clearly established that HPV infection appears to be an essential step in cervical carcinogenesis. HPV DNA can be identified in the majority of cervical cancer cases and HPV positive women without cervical lesion have increased risk for developing cervical neoplasia.[1] Due to the strength of association between HPV and the development of cervical cancer, HPV DNA testing seems to be promising for both incorporation into cervical cancer screening program and prediction of propagation of preinvasive lesions to invasive cancer.[2,3] Prevalence of genital HPV infection varies depending on the method of diagnosis and characteristics of the study population.[4] HPV infection may be diagnosed clinically by observation of warts, cytologically with Pap test or most sensitively using virologic molecular techniques. Hybrid Capture technology is a nucleic acid hybridization assay with specificity for the genomes of high-risk viral types that are implicated in the pathogenesis of cervical neoplasia.[3]

Since our knowledge about the prevalence and predictors of genital HPV infection in Turkish women is limited due to lack of previous studies, HPV DNA was investigated in a series of outpatient clinic patients using Hybrid Capture II assay and the results of HPV testing were compared with Pap smear for screening cervical neoplasia.

Material and Methods

Between February 2001 and October 2002, 1032 patients admitted to outpatient gynecology clinic were recruited. Sexually active patients younger than 60 years were considered eligible for the study. Patients with a history of hysterectomy or active vaginal bleeding, and virgins were excluded. All patients underwent a detailed history, pelvic examination, cervical swab for HPV test, and Pap smear. Age, parity, socioeconomic status, age of first sexual intercourse, cigarette smoking, contraceptive method, history of genital warts and marital status were recorded. The mean age of the study population is 36.8 years (±9.3). The mean values for gravidity and parity were 3.0 and 2.0, respectively. The mean ages of menarch and first sexual intercourse were 13.5 years (±1.4) and 20.5 years (±4.1), respectively. The chief complaint of the patients was pelvic pain in 273 (26.5%), genital discharge in 266 (25.8%), routine visit in 224 (21.7%), history of vaginal bleeding 181 (17.5%) patients. Of the remaining 88 patients, 31 had pregnancy, 21 had infertility, 16 had perimenopausal complaints, 14 had symptoms related to pelvic relaxation and 6 had urinary symptoms.

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Table 1. Calculated crude odds ratios versus risk factors.

Variable	Total number of Women	Number of HPV positive women	%	RR (95% CI)
Age (year)				
<25	125	5	4.0	1.31 (0.30-5.60)
25-29	109	5	4.6	1.51 (0.35-6.48)
30-34	182	10	5.5	1.82 (0.49-6.78)
35-39	241	9	3.7	1.22 (0.32-4.59)
40-44	163	4	2.5	0.79 (0.17-3.60)
45-49	115	5	4.3	1.42 (0.33-6.12)
>49	97	3	3.1	1
Parity				
Nullipara	151	11	7.3	1
Multipara	881	30	3.4	0.47 (0.23-0.95)
Socioeconomic status				
Good	135	8	5.9	1.60 (0.72-3.56)
Medium	790	30	3.8	1
Poor	107	3	2.8	0.73 (0.22-2.44)
Marital status				
Married	980	33	3.4	1
Widowed	32	2	6.3	1.91 (0.44-8.34)
Single	20	6	30	12.30 (4.45-34.02)*
Age of first sexual intercourse				
<16	69	2	2.9	0.72 (0.16-3.22)
16-18	291	13	4.4	1.10 (0.51-2.4)
18-21	326	12	3.6	0.91 (0.42-2.00)
>21	346	14	4.0	1
Cigarette smoking				
Yes	253	12	3.7	1
No	779	29	4.7	1.29 (0.65-2.56)
Contraceptive method				
None	487	19	3.9	1
Intrauterine device	187	7	3.7	0.96 (0.40-2.32)
Periodic abstinence/Calendar	146	5	3.4	0.87 (0.32-2.38)
Condom	83	5	6.0	1.58 (0.57-4.35)
Tubal ligation	77	2	2.6	0.66 (0.15-2.88)
Oral contraceptive	45	2	4.4	1.15 (0.26-5.08)
Other	7	1	14.3	4.11 (0.47-35.82)
History of genital warts				
Yes	18	5	3.7	1
No	1014	36	30.8	10.45 (3.54-30.88)*

* 95% Confidence intervals not including 1

Cervical samples were studied for 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) using Hybrid Capture II assay (Digene Corp., USA). Specimens with a relative light unit/positive control value equal or greater than 1.0 were considered positive. The cytologic samples were collected using an Ayre spatula and a brush. Conventional Pap smears from the participants of this study were evaluated using the Bethesda system (2001). While 999 (96.8%) of the Pap smear specimens were categorized as satisfactory for evaluation, 33 (3.2%) had limited specimen adequacy due to lack of endocervical cells (n=22), or obscuration of epithelial cells partially by blood (n=6) or inflammatory cells (n=5).

All patients were prospectively followed for HPV and Pap test results. Patients with abnormal cytology or HPV positivity underwent colposcopic examination and guided biopsy when indicated.

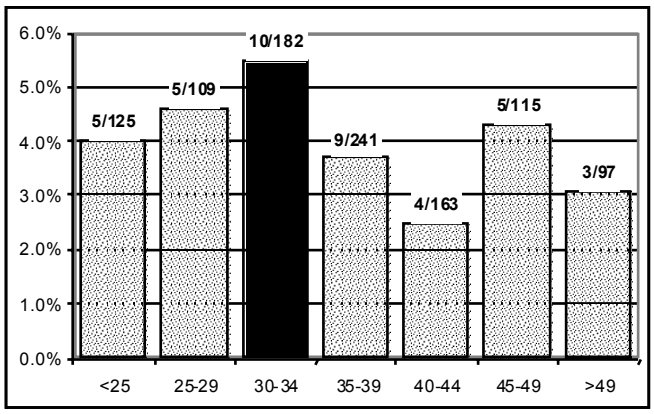

Figure 1. HPV DNA positivity versus age groups

Results

Of the 1032 patients, 41 (4.0%) were found to be positive for HPV DNA and the highest rate of infection was observed in 30-34 age group (Fig. 1). Age, parity, socioeconomic status, age of first sexual intercourse, cigarette smoking and contraceptive method did not correlate significantly with frequency of high-risk HPV infection (Table 1). Only history of genital warts and marital status were found to be a significant predictor of high-risk HPV infection. Of the 41 HPV positive patients, 24 (58.5%) had no evidence of cervical neoplasia at colposcopy. In the remaining 17 (41.5%) patients, guided biopsies revealed chronic infection in 11 (26.8%), LSIL in 5 (12.2%), and HSIL in 1 (2.4%) case (Fig. 2)

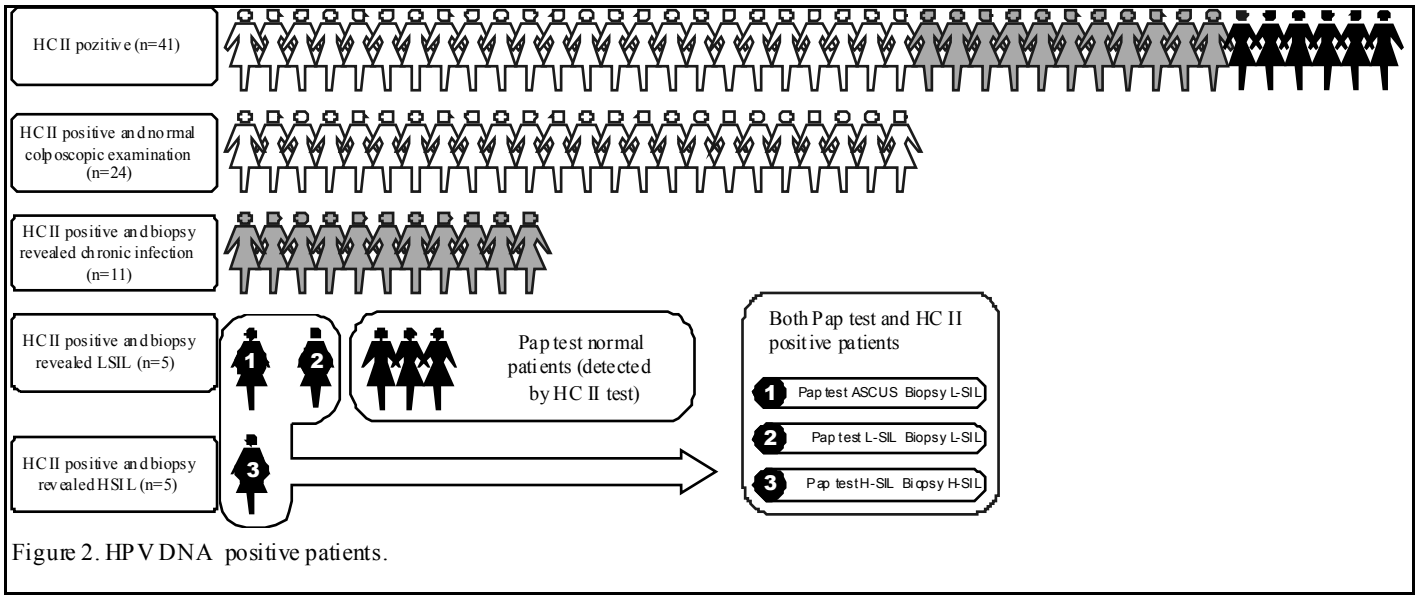

Figure 2. HPV DNA positive patients.

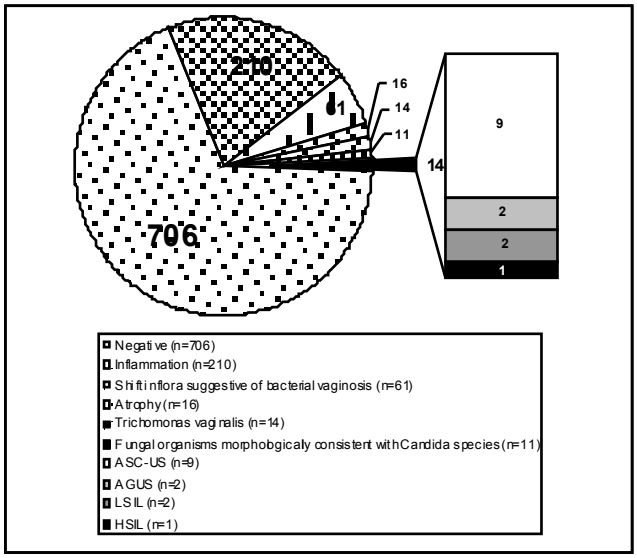

Figure 3. Pap test results.

Relative risks with 95% confidence intervals for the Hybrid Capture II positivity were calculated by using logistic regression model (SPSS 10.0, SPSS Inc.).

Epithelial cell abnormalities were observed at Pap smears of 14 (1.4%) patients (Fig. 3). Cytological diagnoses were atypical squamous cell of undetermined significance (ASC-US) in 9, atypical glandular cells of undetermined significance (AGUS) in 2, LSIL in 2 and HSIL in 1 patient. While final histological diagnoses were LSIL in 2 and HSIL in 1 patient, the other patients had no evidence of cervical neoplasia at colposcopy.

Evidence of infection at Pap smear was observed in 92 patients (Figure 3). Pap smear revealed shift in flora suggestive of bacterial vaginosis in 61 (5.9%), trichomonas vaginalis in 14 (1.4%), and fungal organism morphologically consistent with Candida species in 11 (1.1%) patients. None of the 41 HPV positive patients had koilocytosis at conventional Pap smear.

While Pap smear identified 3 patients with histologically confirmed cervical intraepithelial neoplasia in the study group, HPV testing resulted in diagnoses of 6 cases. Thus, HPV DNA testing could identify 3 additional patients with LSIL among Pap test negative group (Figure 2).

Table 2. HPV prevalence studies

	N	Method	High-risk	Low-risk	Total	Notes
Borg et al[5] (Australia)	377	Dot blot	-	-	7%	Women attending sexually transmitted disease clinic, without a history of genital warts
Hallam et al.[6] (England)	131	PCR and Dot blot	-	-	53% (PCR) 7% (Dot blot)	Unselected women attending family planning unit
Clavel et al.[7] (France)	1518	HC II	18%	-	24.3%	Results of 1647 Pap smears from 1518 unselected women
Kiviat et al.[8] (USA)	500	DNA probes	-	-	24%	Consecutive patients attending sexually transmitted disease clinic
Kornya et al.[9] (Hungary)	1121	HC II	17.5%	-	-	30-55 years old women with normal Pap test
Melchers et al.[10] (Holland)	1290	Dot spot		1%	-	Regularly screened women aged 30-55 years
Molano et al.[11] (Colombia)	1859	PCR	9%	3.1%	14.8%	Women selected by interview in Bogota
Mugica et al.[12] (Bask)	1178	Slot-blot hibridizasyon	-	-	17%	Low risk women with normal cytology
Haeley et al.[13] (Canada)	1290	HC II	26%	-	-	13-79 years old Nanavuts in Northern Canada
Sellors et al.[14] (Canada)	955	HC II and PCR		12.7%		15-49 years old randomly selected women
Alexandrova et al.[15] (Russia)	309	PCR	16%	7%	29%	Pap test normal reproductive age women attending gynecology clinic without any evidence of infection
Becker et al.[16] (New Mexico)	1603	Dot blot			9% (overall) 5% (women with normal cytology)	Randomly selected 1603 women
Denny et al.[17] (Cape Town, South Africa)	2944	HC I	16.2%	-	-	2944 patients aged 35-65 years
Kulasingam et al.[18] (Washington, USA)	4358	PCR and HC II	18.3% (PCR) 28.4% (HC II)	3.9% (PCR)		4358 aged 18-50 yeasr consecutive women admitted to Planned Parenthood clinics
Current study (Turkey)	1032	HC II	%4.0	-	-	Sexually active consecutive women attending gynecology clinic, aged younger than 60 years

Discussion

High-risk HPV positivity determined by different molecular techniques was reported between 3% and 26% in the literature (Table 2). Significant variations in the prevalence rates are thought to be due to social and demographic risk factors in study groups and higher figures were reported from high-risk populations. The first report, to our knowledge, of high-risk HPV infection detected by HC II assay as 4% in a Turkish population seems to be in accordance with previous reports from other countries.[5-18] Current study is a single point prevalence study based on an unselected group of hospital patients. Since the study group is not at high risk for sexually transmitted diseases, current prevalence figure for HPV is naturally within the low range of the previous reports. Although may be evaluated to have drawbacks for representing general population, current study may show that high-risk HPV infection is a significant gynecologic problem of Turkish women. Of the epidemiological factors studied, only history of genital warts and single marital status were found to be a significant predictor of high-risk HPV infection. However, since these factors constitute a small percentage of the study group, it seems rational to sc-

reen all subjects with HPV test instead of a selective approach based on determination high-risk group.

Over the years, Pap smear has proved to be one of the most successful methods of cancer detection available and the implantation of cytologic screening program has brought a major reduction in the incidence and mortality of cervical cancer.[19] However, the procedure does have inherent limitations that compromise the sensitivity of its results. The sensitivity of Pap test ranged from 57% to 66% in the literature.[20] Recently, a number of new technologies including liquid-based preparations, computer-assisted screening and HPV testing have been developed to improve the detection of cervical neoplasia.[21] Since the HPV has been clearly established as the primary cause of cervical cancer in most cases, early detection of HPV infection may identify women who are at high risk for cervical cancer, and they will benefit most from surveillance for cytological abnormalities. Pap smear can not be considered to be sensitive enough to detect HPV infection, since high-risk HPV positive patients were missed at conventional cytology in this series. Hybrid capture II is the latest refinement of HPV tests and has been described as having enhanced sensitivity of about 90% or greater with detection of 13 different high-risk types of HPV.[18] HPV detection by Hybrid Capture II assay detected additional cases of cervical neoplasia among cytologically negative patients in this series. This finding supports a promising future of combination of HPV testing and Pap smear to improve performance of screening for cervical neoplasia.

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