

Frequency and genotype distribution of high-risk human papilloma virus types in Karabuk province, Turkey: A hospital based cross-sectional study

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Abstract

Aim: In this cross-sectional study, we aimed to determine the prevalence and genotype distribution of high-risk human papillomavirus (HPV; HR-HPV) infection in patients admitted to the gynecology and obstetrics outpatient clinics of Karabuk University Training and Research Hospital.

Material and Methods: A total of 402 women aged 18–65 years who were admitted to the gynecology and obstetrics Clinic, Karabuk University Training and Research Hospital, between October 2016 and June 2019 were included. The presence of HR-HPV and genotyping of HPV were investigated using real-time polymerase chain reaction in cervical swab samples.

Results: HR-HPV infection was found in 23.9% of women. HR-HPV positivity was detected most commonly in the age group of 20–29 years. Regarding genotype distribution among HPV-positive women, the genotypes that included multiple HR-HPV infections (mixed HR-HPV) were the most common genotype (38.5%), followed by HPV-16 (13.1%) and HPV-52 (9.8%).

Conclusion: Prevalence of HR-HPV infection was found to be high in our region, with the most commonly observed genotypes being those containing mixed HR-HPV. We believe that these results would be helpful during the selection of primary and secondary preventive measures for cervical cancer while planning vaccination and screening programs.

Keywords: HPV; HR- HPV Types; cervical cancer

INTRODUCTION

Human papillomavirus (HPV) is a DNA virus that infects basal epithelial cells. It causes benign and malignant skin and mucosal lesions in anogenital and oropharyngeal regions (including tongue and tonsillar regions) (1). Annually, 570,000 women acquire HPV-related diseases, 50,000 new cases of HPV-related cervical cancer occur, and 250,000 women die owing to cervical cancer worldwide (2). Prevalence of HPV infection is 86%–96.67% in patients with cervical cancer (3,4). Currently, almost all types of cervical cancer are known to occur as a result of cervical changes after exposure to specific oncogenic HPV infection (5). Among >100 known HPV genotypes, 18 are HR-HPVs, whereas the remaining genotypes pose low risk of cervical cancer. HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and 81 cause genital warts and low-grade lesions.

HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are classified as HR-HPVs, whereas genotypes 26, 53 and 66 are classified as possibly HR-HPVs (6). The most common oncogenic HPV genotypes in invasive cervical cancer are HPV 16 (52%–60%), followed by HPV 18 (13%–22%) and HPV 31, 33, 35, 45, 52 and 58 (3,7,8).

Prevalence of HPV infection varies across regions, with different prevalence reported for different regions. The lowest prevalence was found to be <3% in Australia/New Zealand and America, whereas the highest prevalence was 26% in Africa (9). In Turkey, highly variable prevalence, ranging between 2.75% and 33.5%, has been reported through HPV screening programs (10–14).

According to the GLOBOSCAN data published by the World Health Organization's International Agency for Research on Cancer reported in 2018, cervical cancer ranks 4th

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worldwide in terms of incidence and mortality rate in women (9). Cervical cancer is the 11th most common cancer in women in developed countries (9.9/100,000 women) and the 9th most common cause of cancer-related death (3.3/100,000 women), whereas it ranks 2nd in terms of incidence and mortality in developing countries (15). The concurrent use of HPV-based screening programs and HPV vaccine is known to be the most effective preventive measure (16). HPV vaccination programs can help prevent 70%–90% of HPV-related cancer cases (17). In our country, the prevalence of cervical cancer is low, i.e. 4/100,000 women, and this is thought to be associated with the low prevalence of HPV infection. In addition, it is believed that there may be inadequate screening programs or deficient data transfer (18). Determination of the frequency and genotypes of HPV in our region will shed light on the planning of vaccination programmes. In this study, we aimed to determine the prevalence of HR-HPV infection and possibly HR-HPV genotypes in patients admitted to the gynaecology and obstetrics Clinic, Karabük University Training and Research Hospital.

MATERIAL and METHODS

Study participants and design

This cross-sectional study was approved by the Non-Interventional Clinical Research Ethics Committee of Karabük University. A total of 402 women aged 18–65 years who were admitted to the gynaecology and obstetrics outpatient clinics of Karabük University Training and Research Hospital, between October 2016 and June 2019 were included. With a 95% confidence interval for detecting an average of 10% HPV-positive cases, 402 women were included in the study population. The inclusion criterion was being a sexually active woman aged 18–65 years. The exclusion criteria included the following: women who were pregnant, had a history of hysterectomy or cervical conisation, provided refusal, undergoing menstruation, having undergone vaginal treatment up to 3 days ago, had engaged in sexual activity within the last 24 h and with mental or physical insufficiencies (Figure 1). Cervical swab samples taken using a cervical smear brush (Medbar, Izmir, Turkey) from the endocervical area of patients who presented to the polyclinic with gynaecological complaints or for routine gynaecological check during a routine pelvic examination were placed in a special liquid collection medium (US Surepath, Becton Dickinson, Sparks, MD, USA) and sent to the microbiology laboratory.

HPV DNA isolation and genotyping

DNA isolation from cervical swab samples was performed with the Magnesia 1 Automatic isolation device (Anatolia Geneworks, Istanbul, Turkey) using the Magnesia 202 Viral DNA/RNA Isolation Kit in accordance with the manufacturer's recommendations. Subsequently, HPV genotype was determined with the Montania 484 device using the Bosphore HPV Genotyping High-risk Kit 1 (Anatolia Geneworks) by real-time polymerase chain reaction. In total, 14 HR-HPV genotypes can be identified and differentiated with this kit: HPV 16, 18, 31, 33, 35, 39,

45, 51, 52, 56, 58, 59, 66 and 68. The analytical sensitivity of the kit is 1×10^3 IU/ml. Fluorescence detection was performed using FAM, HEX, Texas RED and Cy5 filters.

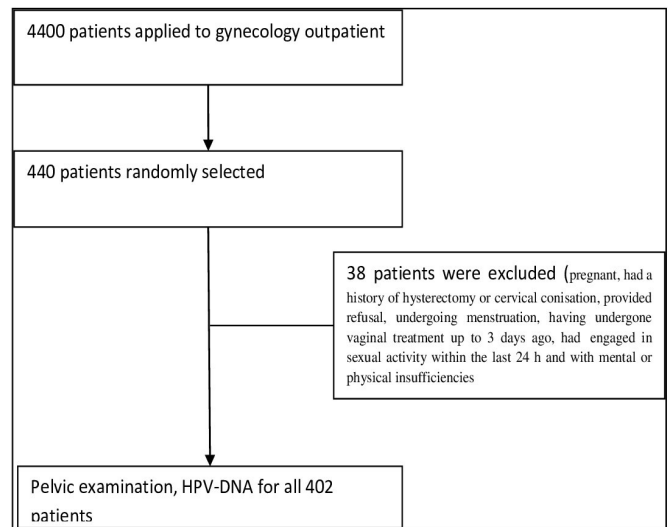


Figure 1. Patient selection and evaluation algorithm

Statistical analysis

The data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, US). Kolmogorov–Smirnov test was performed to determine whether the variables were normally distributed. Continuous variables are expressed as mean \pm standard deviation or median (min–max), and categorical variables are expressed as numbers or percentages, if applicable. The Pearson's chi-square test was performed. Two-tailed p-values of <0.05 were considered to indicate statistical significance.

RESULTS

HR-HPV positivity was detected in 91 (23.9%) of the 402 women who underwent HPV screening. The average age of all participants was 39.47 (18–65) years. The HPV-positive group was significantly younger than the HPV-negative group (35.58 ± 9.84 vs. 39.6 ± 10.5 years, $p = 0.001$).

When we classified HR-HPV positivity according to age, the highest positivity was observed in the age group of 20–29 years (30.8%). Although it was also high in the age group of 30–39 years (27.6%), it was less common in the age group of 40–49 years (17.8%) and the least common in the age group of 50–59 years (9.8%), with a relatively high prevalence in the age group of >60 years (21.4%; Table 1).

Single-genotype HR-HPV positivity was detected in 61.5% ($n = 56$) in the HPV-positive group. The most common genotype among the HR-HPV DNA-positive ($n = 91$) samples was mixed HR-HPV [38.5% ($n = 35$)] with multiple HR-HPV infections. HPV-16 was the second most common genotype [13.1% ($n = 12$)], followed by HPV-52 [9.8% ($n=9$)]. When mixed HR-HPV samples were considered, the proportion of HPV-16 among positive

samples reached 26.3% (n = 24). The proportion of HPV-52 increased to 15.3% when samples containing >1 HPV genotype were included (n = 13). The genotype distribution of HR-HPV DNA-positive samples is shown in Table 2.

Table 1. HPV positivity rates with ages

Variables	HPV negative N (%)	HPV positive N (%)	Total N (%)	P value
Age groups (years)				0.031
19-29	63 (69.2)	28 (30.8)	91 (22.7)	
30-39	84 (72.4)	32 (27.6)	116 (29.2)	
40-49	111 (82.2)	24 (17.8)	135 (34)	
50-59	37 (90.2)	4 (9.8)	41 (10.3)	
>60	11 (78.6)	3 (21.4)	14 (3.5)	
Total	311 (77.36)	91 (13.93)	402 (100)	

Table 2. Distributions of HR-HPV types in the specimens

HR-HPV Type	n	Percentage (%)
Multiple high-risk genotypes	35	38.5
HPV 16	12	13.1
HPV 52	9	9.8
HPV 59	6	6.5
HPV 56	4	4.3
HPV 33	4	4.3
HPV 39	4	4.3
HPV 66	3	3.2
HPV 51	2	2.1
HPV 18	2	2.1
HPV 31	2	2.1
HPV 58	2	2.1
HPV 68	2	2.1
HPV 36	1	1.0
Total	91	100

DISCUSSION

Although the incidence of cervical cancer varies considerably worldwide, 85% of cases are found in low- and middle-income countries (29). A rapid reduction in the prevalence of cervical cancer has been observed as a result of community-based cervical cancer screening programs in most European countries, Australia/New Zealand and North America. Since it is known that almost all of the

cervical cancer is the result of HPV infection, knowing the regional prevalence and types of HPV is crucial in terms of therapeutic management. In this study, the prevalence of HPV infection was found to be 23.9% (91/402). In our country, through screening programs, its prevalence has been reported to be 2.75% in Zonguldak by Oz (14), 4% in Ankara by Tuncer (13), 5.6% in Balikesir by Taskin (11), 25% in Ankara by Dursun (10), and 33.5% in Ankara by Aydogan (12). When the prevalence of HPV infection in our region was compared with its national prevalence, we found them to be similar. The global prevalence and spread of HPV infection vary across different regions owing to social, geographical and cultural differences and diagnostic method-related differences. While the lowest global prevalence is <3% in Australia/New Zealand and America, the highest prevalence is 26% in Africa (9). Prevalence of HPV infection was reported to be 11.7% in 1,016,719 women with normal cervical cytology in a meta-analysis conducted in Brazil by Bruni (19), 26.8% through self-sampling by 1,921 women in the USA by Dunne (20) and 22.3% in 12,816 samples in China by Wang (21). In a comprehensive meta-analysis, in which 78 studies from various regions of the world were examined and 157,879 samples with normal cervical cytology were evaluated, the prevalence of HPV infection was found to be 10.4% worldwide, with the highest prevalence in West Africa (31.6%) and lowest prevalence in Southwest Asia (6.2%) (22). In the abovementioned study, the average prevalence of HPV infection was reported to be 10% in developed countries and 15.5% in underdeveloped countries (22). In our study, although the results were similar to those of the abovementioned meta-analysis, a higher prevalence of HPV infection was obtained than the global prevalence. However, cervical cytology test was not performed during patient selection in our study, and patients were usually admitted to our outpatient clinic for any complaint. Because women with normal cervical cytology without complaints were included in the abovementioned studies, the comparison may only be considered relative (19,22).

Unlike in other studies, the most common genotype detected among HR-HPV DNA-positive samples in the present study was mixed HR-HPV (38.46%, n = 35). HPV-16 was the second most common genotype [13.18%, (n = 12)], followed by HPV-52 (9.8%). Notably, HPV-16 positivity has often ranked 1st in some country-wide studies (10,12,13). Similarly, the proportion of HPV-16 in HR-HPV DNA-positive samples reached 26.3% (n = 24) when mixed HR-HPV samples were included in our study. In contrast, Taskin reported that the most common genotypes in Balikesir were HR-HPV genotypes other than HPV-16 and 18 (11). The prevalence of HPV-18 infection, the second most common cause of cervical cancer, was found to be 2.19% in our study and was lower than that reported for other HR-HPV genotypes. Globally, Bruni reported HPV-6 to be the most common genotype in the low-risk HPV group and HPV-16, 18, 31, 52 and 58 in the HR-HPV group (19). Francesca et al. found HPV-16 and 31 to be the most common genotypes in Europe and Latin America, and they reported that these genotypes were

less common in North America and Asia (23). Further, in a meta-analysis, De Sanjosé reported that HPV-16 and 18 were the most common HPV genotypes (24). Unlike these, Wang reported HPV-18 as the most common HPV genotype in China (18), and Dunne reported that non-HPV-16 genotypes were the most common genotypes in the USA (20). HPV-16 and 18 cause 50%–60% and 10%–12% cases of cervical cancers, respectively (25). Therefore, we believe that paying more attention to these genotypes during screening programs would be more beneficial in terms of cost as well as follow-up and treatment decisions.

In the present study, the highest HPV positivity was found in the age group of 20–29 years (30.8%). We found that it was least common in the age group of 50–59 years (9.8%) and increased in women aged >60 years (21.4%). Similarly, Dursun and Hasbek reported that the prevalence of HPV infection was the highest in patients aged <30 years (10,13). In a study involving women aged >25 years in Italy, Francesca et al. reported that the prevalence was high in the age groups of 25–34 and 35–44 years (23). Dunne reported that it was most common in the age group of 14–24 years in the USA (20). It was most common in women aged <25 years in a meta-analysis conducted by De Sanjosé, followed by in women aged 45–54 years and then in women aged >54 years (24). Francesca et al. also observed that there was a second peak in Chile, Colombia and Mexico at an advanced age (24). Wang identified the 1st and largest peak of HPV infection in women aged <20 years in China, and there was a 2nd but small peak in women aged >60 years (21). These results are consistent with our results. In poor countries such as Nigeria and India, similar or high prevalence of HPV infection has been reported for all ages (23). HPV infection is often considered acquired in the initial years following sexual intercourse and is possibly cleared from the body later in life. For this reason, providing information about sexual intercourse and preventive measures, particularly at a young age, is of primary importance. It is believed that there are two reasons why HPV infection shows a peak again in middle age. A diminished immune system as a result of hormonal changes after menopause may reactivate latent HPV (26), and/or it may be related to changes in sexual orientation and partners of middle-aged women (27). Althoff argued that geographical variation can be partially explained not only by menopause-related hormonal pattern indicators such as body mass index and ethnicity but also by age at this second peak (26). It appears that the distribution of HPV genotypes by age also varies according to population and geographical regions. The burden of pre-cancerous lesions and persistent HPV infections can be particularly reduced with the help of screening programs. In addition, lesion removal is believed to have a direct antigenic effect, which may provide protection against subsequent HPV infections (28). For instance, this second peak in middle age was found to be weakened in regions with effective screening programs such as Europe and North America (19).

However, because of the lack of effective screening programs and changes in sexual behavior, an increased risk of persistent HPV infection has been reported and a

rapid increase was noted in early mortality rates in women with cervical cancer born during 1940–1950 in East Asia and Central Asia including the Soviet Union (9). Currently, the most common screening tests used include the pap test and HPV DNA test (9). Cervical cytology screening should not be performed alone as a screening test because of its relatively low sensitivity (15,22). Recently, HPV vaccination program is the only approach used for primary prevention (30). HPV vaccination program can prevent 70%–90% of HPV-related cancers (17). As a result of the widespread use of HPV vaccination protocols, a 71% decrease in the prevalence of HPV infection in the age group of 14–19 years and 61% decrease in the age group of 20–24 years in the United States as well as a 38% decrease in Australia were reported owing to vaccination for HPV-6, 11, 16 and 18 infections (31). Both bivalent and quadrivalent vaccines currently used worldwide, including in our country, are effective against HPV-16 and -18 infections (30). When we evaluated the results of our region, the most common types were mixed HR-HPV genotypes followed by HPV-16. HPV-18, which is present in vaccines, infection was found to have a very low prevalence. We believe that being informed about the distribution of HPV genotypes in our region would guide vaccine selection. A higher number of comprehensive and regional studies are needed to demonstrate the efficacy of these vaccines in the long term and in a broad population.

One of the limitations of our study is that the results may not clearly reflect general population data because of the limited number of patients. Owing to the retrospective nature of this study and deficiencies in terms of documentation, the findings could not be confirmed using cervical cytology results.

CONCLUSION

In conclusion, although HPV positivity in our region was similar to those reported previously in Turkey and globally, the difference was that in our study, the most common genotype was mixed HR-HPV, followed by HPV-16. Regarding vaccination programs, we believe that the presence of multiple genotypes should be considered and primary prevention against cervical cancer should be performed using necessary guidance in vaccine selection. In addition, reduced incidence and mortality rate of cervical cancer can be expected as a result of early diagnosis and treatment with cervical cytology via the widespread use of screening programs.

Competing interests: The authors declare that they have no competing interest.

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