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Colposcopy results in Smear negative, High-risk HPV positive patients

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Abstract: Cervix cancer is an HPV (Human papillomavirus) related cancer, and HPV positivity is necessary even if there is no cytology abnormality. We aimed to determine the ratios of 13 high-risk HPV types in cases with high-risk HPV positivity without cervical smear pathology referred to our clinic and to determine the relation of HPV types with age, parity, menopausal status, and abnormal histopathological results. Two hundred forty-one cases included in the study, which referred to us because of HPV positivity and colposcopically biopsied between January 2014 to January 2018. HPV prevalences were investigated. The relationship between HPV types and variables such as age, parity, menopausal status examined. The mean age of 241 patients included in the study was 46,1+8,8. The parity average was 2,4+1,1. Sixty-five of the patients (27%) were postmenopausal. Of the 241 HPV-positive patients, 172 (71,4%) had only high-risk HPV viruses. The frequency ranking of HPV types was as follow; 16, 31, 51, 56, 18, 52, 35, 58, 39, 68, 45, 33 and 59. According to the HPV types, the average ages were as follow; 18 (43,6 years), 33 (40,1 years) and 51 (41,9 years) were younger than the average age. 35 (48,7 years), 39 (48,5 years), 52 (49,1 years) and 68 (51,3 years) were older than the average age. 16 (44,9 years), 31 (47,9 years), 45 (44,3 years), 56 (47,3 years), 58 (46,9 years) and 59 (46,7 years) was similar the average age. There was no significant difference between the parities according to HPV types (2 to 2,7). According to the HPV types, the menopausal state was as follows; 39 (50%), 56 (50%) and 68 (53,8%) mostly observed in the postmenopausal period; A small proportion of 33 cases (12,5%) was postmenopausal. The rate of severe dysplasia according to colposcopic biopsy related with HPV types was; 58 (40%), 56 (30,8%), 18 (28%), 45 (27,3%), 31 (26,1%), 39 (25%), 59 (16,7%), 35 (14,3%), 51 (13,8%), 33 (12,5%), 16 (11,8%), 52 (8,3%). The prevalence of HPV types, the age at which they saw, the menopausal status and the potential for the formation of severe dysplasia are highly variable. We think that routine screening programme, colposcopy indications and vaccination program should cover all HPV types according to data.
Keywords: Cervical cancer; HPV positivity; Smear negativity

INTRODUCTION

Various infectious agents, especially Hepatitis B (HBV), Hepatitis C (HCV), Human papillomavirus (HPV) and Helicobacter pylori, account for 23% of the causes of human cancer (WHO Global Cancer Report, 2003). Among HPV infectious agents, it is essential because of the most frequent association with cancer and the most common sexually transmitted disease (Depuydt et al., 2016; Chattzistamatiou et al., 2016; Kim, 2017). HPV is a small double-stranded DNA virus that has been described more than 200 types daily. This difference provides the genetic sequence of the outer capsid protein L1. It has been found that about 40 of this family of viruses go through sexual contact and cause infection in the basal epithelium layer of the genital mucosa in both men and women (CDC HPV report, 2015; Sah et al., 2018). HPV infections heal spontaneously within 1 to 2 years in 70-90% (Cubie, 2013). Persists are known to cause vulvovaginal, penile, anal, head and neck cancers, especially cervical cancer with oncogenic effect (Depuydt et al., 2016; ICO/IARC HPV and Related Diseases Report, 2017).

HPV viruses are divided into two groups as low risk (LR HPV) and high (HR HPV) risk compared to cancer development potentials.

International cancer research agency (IARC) recently identified 25 HR HPV in 2012. These are in turn; 16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, dir (Humans Biological agents, 2012).

Cervical cancer among HPV-related diseases is the fourth most common cancer seen in women worldwide — the second most common cancer in women aged 15 to 44 years. Every year more than 500,000 women are diagnosed, and approximately 265,000 women die from this cancer (ICO/IARC HPV and Related Diseases Report, 2017).

When we work on this information, we try to show the frequency of HPV types in our region. We also investigated the relationship between HPV types and age, menopausal status, and abnormal histopathology results.

MATERIALS AND METHODS

Ordu Provincial Health Directorate and Ordu University Medical Faculty Training and Research Hospital Clinical Practice Ethics Committee approvals obtained (Date: 26/04/2018, Number: 2018-73). My study included 241 patients between January 2014 and January 2018 who underwent colposcopic biopsy with a normal cytology referred to ours due to HPV positivity. Patients with HPV and cytologic examinations made by the Center for Cancer Early Diagnosis Screening and Training (KETEM), a national screening organization. In this organization women aged between 30 and 65 years are invited for HPV based screening by family physicians every five years. Two samples are taken from each woman to enable cytology testing in those found to be HPV positive without the need for a separate visit. The first sample is collected with a brush and transferred to a glass slide for conventional cytology. The second is taken with a different brush and put into 5 ml of Standard Transport Medium for HPV DNA analysis. And the result report is sent to the medical professional to be shared with the patient. The colposcopy examination and biopsy results of the patients were retrospectively scanned and retrieved from the hospital registry system

The 13 high oncogenic HPV types examined by KETEM were as follows; 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68. For women who are HPV positive by Hybrid Capture2 (Qiagen), genotyping performed with the CLART kit (Genomics). HPV results were analyzed, and the prevalences of HPV types subtracted. The relationship between variables such as age, parity, menopausal status and HPV types was examined.

In progressing cervical cancer, cervical preinvasive lesions are significant. According to the classic classification of cervical preinvasive lesions; colposcopic biopsy results classified as Normal, CIN I, CIN II, CIN III, and CIS (cervical carcinoma in situ). CIN II and advanced cases (which have high risk potential for cervical cancer) were evaluated as severe dysplasia. The rates of severe dysplasia of HPV types investigated.

All data analyses were done by SPSS 20.0. One Way ANOVA and T-Test were used due to group number to analyze data consist with independent measurements showing normal distribution. Pearson Correlation Test was used to determine the relation¬ship between the groups. To analyze the variations that do not distribute normally, Kruskal Wallis and Mann-Whitney U tests used. Spearman Correlation Test was used to determine relationship between the groups. Data in a categorical structure analyzed by Chi square test. A p-value <0.05 was accepted as significant.

RESULTS AND DISCUSSION

The mean age of 241 patients included in the study was 46.1 + 8.8. The parity average was 2.4 + 1.1. Sixty-five of the patients (27%) were postmenopausal. Of the 241 HPV-positive patients, 172 (71.4%) had single, 48 (19.9%) had two, 15 (6.2%) had three, 5 (2.1%) had four, 1 (0.4%) had five high-risk HPV viruses were detected. (Table 1 and 2).

Table 1. The General Information

The mean age	46.1 $\pm$ 8.8
The parity average	2.4 $\pm$ 1.1
The postmenopausal ratio	27% (65/241)

Table 2. High-risk HPV Distribution

One high-risk HPV	172 patients	71.4%
Two high-risk HPV	48 patients	19.9%
Three high-risk HPV	15 patients	6.2%
Four high-risk HPV	5 patients	2.1%
Five high-risk HPV	1 patient	0.4%

Table 3. The frequency ranking and the mean ages of HPV types (According to the incidence)

16	93	44.9
31	46	47.9
51	29	41.9 (younger)
56	26	47.3
18	25	43.6
52	24	49.1 (older)
35	21	48.7
58	20	46.9
39	16	48.5
68	13	51.3 (older)
45	11	44.3
33	8	40.1 (younger)
59	6	46.7

Table 4. The rate of detection of severe dysplasia after colposcopic biopsy (According to the severity)

58	40%
56	30.8%
18	28%
45	27.3%
31	26.1%
39	25%
68	23.1%
59	16.7%
35	14.3%
51	13.8%
33	12.5%
16	11.8%
52	8.3%
total	27.8%

The frequency ranking of HPV types was as follows; 16 (93 patients), 31 (46 patients), 51 (29 patients), 56 (26 patients), 18 (25 patients), 52 (24 patients), 35 (21 patients), 58 (20 patients), 39 (16 patients), 68 (13 patients), 45 (11 patients), 33 (8 patients), and 59 patients (6 patients).

According to the HPV types, the mean age was as follows; 33 (40.1) and 51 (41.9) were younger than the average age. 52 (49,1) and 68 (51,3) were older than the average age. 16 (44.9), 18 (43.6), 31 (47.9), 35 (48,7), 39 (48,5), 45 (44.3), 56 (47.3), 58 (46.9) and 59 (46.7) was observed like the average age. (Table 3). There was no significant difference between the parties according to HPV types (2 to 2.7). ($p>0.05$).

According to the HPV types, the menopausal state was as follows. 39 (50%), 56 (50%) and 68 (53.8%) were observed in the postmenopausal period; A small proportion of 33 cases (12.5%) were postmenopausal.

The rate of detection of severe dysplasia after colposcopic biopsy was 67/241 (27,8%). The rates of severe dysplasia according to HPV types were as follows; 58 (40%), 56 (30.8%), 18 (28%), 45 (27.3%), 31 (26.1%), 39 (25%), 68 (23.1), 59 (16,7%), 35 (14,3%), 51 (13,8%), 33 (12,5%), 16 (11,8%), 52 (8,3%). (Table 4).

The HPV DNA testing now has shown as the primary screening program by many organizations, notably the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC). In cervical cancer screening, many high-income countries such as Norway, the Netherlands and Australia now use HPV DNA testing in cancer screening programs instead of conventional cervical smear screening (Anttila et al.,2015; Huh et al., 2015). Turkey cervical cancer screening program based on data from the population screening HPV DNA test screening 5 - 6 points increase reported to provided. It has also been shown that this program has additional advantages, such as less human workload, faster results, less need for sampling, and fewer hospital visits (Gultekin et al., 2018).

The prevalence of HPV in the world is highly variable compared to geographical regions. The HPV frequency in the community is around 10% (1.4% - 25.6%). Also, 95% to 100% of patients with cervical cancer have a close relationship with the virus and cancer (Walboomers et al., 1999; Basu et al., 2011; Kirschner et al., 2013).

In Turkey, there are many studies demonstrating the HPV prevalence (2% - 25%). In a retrospective analysis of 6388 patients who referred to member centers for the Turkish gynecologic oncology group, the HPV positivity rate was 25%. The high prevalence in this study can be attributed to the fact that the centers participating in the study are referee oncologic centers (Dursun et al., 2013). 30 - a million women are screened from age 65 for Turkey, according to the results of cervical cancer screening and HPV positivity in our country was 3.5% (Walboomers et al., 1999).

On the other hand, common HPV types also vary from region to region. The 5 most common types of HPV were 16, 53, 52, 18, 39; In Europe 16, 18, 31, 33, 58; In Asia there are 16, 52, 58, 18, 56 (Barut et. al., 2018). Turkey study also showed that Turkey's peculiar distribution; 16 (20,7%), 51 (10,8%), 31 (8,7%), 52 (7,1%), 56 (5,7%). For example, the uncommon type observed in other regions 51 to the second common in Turkey. Such as having a high oncogenic Type 16 18 Turkey ranks seventh in the study. Also common in North America, Asia and type 52 and type 31, which is still in the top five in Europe is often seen in Turkey shows that we are a mosaic of countries regarding HPV (Walboomers et al., 1999).

In our study, the first five HPV types were as follows; 16 (38,5%), 31 (19,1%), 51 (12%), 56 (10,8%) and 18 (10,4%). We found some differences sailed close to Turkey as well as the general data of our study data. Prevalence of Turkey was 12.6% higher than other studies because the data in Turkey (13 HR HPV types external olds) HPVs not directed by us. In addition, depending on the type of high referral rates by 16 family physicians and 18 types, we found extremely high data rates compared to Turkey. We also had our differences in the method of operation with Turkey. Turkey abnormal cytology 19.1% of HPV-positive patients in the studies of HPV positivity we left off work to show the independent effects of abnormal cytology. Finally, we found that type 31 was higher than type 51 when we were working on a possible regional difference.

In our study, we found that HPV 16 and 18 positivity observed in 46.9% of patients.

However, we believe that 16 and 18 non-HR HPV ratios are quite high, and 16 and 18 of the potential for severe dysplasia considered.
The incidence of single / multiple HPV prevalence, such as HPV prevalence, was also found at quite different rates in studies. In a large-scale Chinese study, 79% of the cases had a uniform infection rate, while a recent study from our country yielded multiple infection rates of 59% (Barut et al., 2018; Tao et al., 2018). In our study, the odd infection rate was more than 71.4%.

According to HPV types, 18, 33, 51 observed at younger ages than mean age; 35, 39, 52, 68 were older than the average age. On the other hand, there was no significant difference between the parities according to HPV types (2 to 2.7). Also, more than half of 39, 56 and 68 cases observed in the postmenopausal period; A relatively small proportion of 33 cases (12.5%) were postmenopausal.

The HPV test scans the conventional smear. One million women screened by Turkey in 3499 women working colposcopy directed in 1869 (53.4%) observed in any cervical smear abnormalities. However, colposcopic biopsy results showed 708 CIN I (20,2%), 285 CIN II (8,1%), 436 CIN III (12,4%) and 85 cancer (2,4%). The most important outcome of the study was the ability to skip 45.9% of CIN III and advanced cases with conventional smear scanning (Walboomers et al., 1999). The incidence of severe dysplasia in Turkey operating in the HPV positivity in our study, while 22.9% of patients with pathology cervical smear to exclude Although we rate rose to 27.8%.

In our study, we also performed a review of the species. The rate of severe dysplasia after colposcopic biopsy was according to HPV types. 58 (40%), 56 (30.8%), 18 (28%), 45 (27.3%), 31 (26.1%), 39 (25% 59 (16,7%), 35 (14,3%), 51 (13,8%), 33 (12,5%), 16 (11,8%), 52 (8,3%). Although we observed very little severe dysplasia in Type 16 positivity, we think it is very important because of the most common type of HPV.

It is known that HPV screening, as well as studies on HPV vaccines, are continuing rapidly. HPV 16 and 18 were shown to be responsible for 70% of cervical cancer. And it is said that HPV 31, 33, 45, 52 and 58 are an additional 22% of cancer cases. And in our study we showed that HPV 58, 45 (due to the high tendency to dysplasia) and 31, 51, 56 (due to frequent occurrence) deserves more attention; like HPV 16 and 18. Vaccine studies are carried out in this framework. Cervarix® (16,18) and Gardasil® (6,11,16,18) are the first vaccines and protect against only 46.9% of HPV types in our cases. Finally, Gardasil 9® (6,11,16,18,31,33,45,52,58) has been applied to the market and is protective against 84.6% of the HPV types in our study.

We think that vaccine programs should develop rapidly and produce new generations of vaccines containing only the most common types in European countries - inclusive low and middle-income countries where deaths from the entire world - more often cervical cancer deaths - are more common.

CONCLUSION

Although reflex cytology suggested in the literature with HPV 16-18 non-HR HPV positivity, we advocate the necessity of direct colposcopy from these cytologic follow-ups.

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