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Original Article

Should Women Between the Ages of 25 and 30 Get Tested for HPV?

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ABSTRACT

Objectives: The aim of this study is to discuss whether performing the human papillomavirus test on women aged 25-30 in Turkey has any effect on preventing cervical cancer. It is aimed to reevaluate the screening program.

Methods: A total of 400 patients between the ages of 25-30 who had the Papanicolaou smear and the human papillomavirus test were included in our study. Pap smear and the human papillomavirus test were performed again on the patients with a positive human papillomavirus test for high-risk types in accordance with the screening program. Demographical and clinical characteristics of the patients were recorded. The incidence of human papillomavirus test positivity with a high risk among patients aged 25-30, regression, and persistence ratios were calculated.

Results: The incidence of human papillomavirus test positivity with a high risk among patients aged 25 to 30 was found to be 7%. Human papillomavirus persistence ratio was 17.6% and the regression ratio was 82.4%. Among patients with a positive high-risk human papillomavirus test between the ages of 25 and 30, human papillomavirus 16 was found in 47.1% of the patients. For one of our patients with a persistent human papillomavirus 16 positivity, conization was performed after the cervical biopsy.

Conclusion: We believe that human papillomavirus, which plays an important role in the etiology of cervical cancer, should be screened from the age of 25. This way, we can catch and treat precursor lesions of cervical cancer at earlier ages and lower the incidence and mortality of cervical cancer.

Keywords: Cervical cancer, human papillomavirus test, Papanicolaou smear test, screening program, young women

INTRODUCTION

Cervical cancer is the fourth most common cancer among women worldwide.[1] Most of the cases are seen in developing countries.[2] The low incidence and mortality of cervical cancer in developed countries depend on screening programs and human papillomavirus (HPV) vaccination programs.

Almost all cases of cervical cancer are caused by HPV infections. High-risk types of HPV can be counted as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Human papillomavirus 16 is seen in almost 50% of the patients and HPV 18 is seen in 20%.[3] In addition, 19% of cervical cancer is suspected to be caused by HPV types 31, 33, 45, 52, and 58.[4]

It takes 10-20 years for HPV infections to progress into invasive carcinoma. This duration helps us catch the disease during the premalignant stage when treatment is most effective. Premalignant cervical lesions are asymptomatic and they can only be detected with appropriate screening tests. The aim of the screening of cervical cancer is to find the high-grade lesions in asymptomatic women, to treat them, and to prevent them from progressing into invasive carcinoma.[5]

Since 2014, the Papanicolaou (Pap) smear test and the HPV test are used for the screening of cervical cancer in our country. The cytological evaluation of the cervical Pap smear and simultaneous HPV DNA study of the same sample is defined as the "co-test." Today, the co-test is the most widely accepted screening method for women over the age of 30. American Society of Colposcopy and Cervical Pathology and the American College of Obstetricians and Gynecologists recommend that screening be done for women between the ages of 30 and 65 with co-testing once every 5 years.[6-8] Because HPV infections are mostly transient in patients under the age of 30, co-testing is not recommended for this age group as it has low persistence and it might increase the false-positive rate of the test. For that reason, screening with only cytology once every 3 years is recommended for women aged 21-30.[9]

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The Cobas HPV test is the only Food and Drug Administration (FDA) approved test for primary HPV screening in women over the age of 25. It has perfect reproducibility with solid and automatically end-to-end encrypted procedures.[10]

There is no clinical study comparing mortality rates between various screening strategies. However, a microsimulation model for US Preventive Services Task Force found out that screening strategies including the HPV test correlated less with cervical cancer mortality when compared to screening strategies with the Pap smear test.[11]

The aim of our study is to evaluate the persistence and regression ratios of high-risk HPV in women aged 25-30 in our country. We also aim to discuss the effect of performing HPV test for women aged 25-30 in Turkey on preventing cervical cancer, to ensure further evaluation of the screening program, hence contributing to the literature.

METHODS

A total of 400 patients aged 25-30 who were incidentally tested for HPV after presenting to Dicle University Obstetrics and Gynecology Clinic for the Pap smear test and patients who were referred from other hospitals to receive the HPV test between 2015 and 2018 were included in our retrospective study. For our study, approval no. 277 was received from Dicle University Ethics Committee on December 5, 2019.

Human papillomavirus test results were evaluated one by one and the high-risk positive ones were determined (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, and HPV-68). Patients with hysterectomy, cervical cancer, radiotherapy or chemotherapy histories, and immunosuppressive diseases were left out. From the patients included in our study, the ones with past abnormal cytology results in their Pap smear and/or cervical lesions were left out. Healthy women were included in the study. Human papillomavirus negative and high-risk positive ones were determined, and then a control HPV test and a Pap smear were performed in accordance with the screening program in patients that were high-risk positive. Women that had the control HPV test were over the age of 30 in accordance with the criteria of our study, and at least 2 years had passed since their first HPV test.[12] According to the control HPV and the Pap smear results, the diagnostic algorithm was employed, cervical biopsy through colposcopy and conization were performed when necessary and the results were noted down. Pap smear and HPV test results of patients between 25 and 30 and patients over the age of 30 were noted down and evaluated. As to the patients aged 25 to 30, regression and persistence ratios of the Pap smear and HPV test results and high-risk HPV positivity incidence were calculated. Persistent HPV infection was defined as 2 consecutive positive HPV tests at least 12 months apart.[12] Demographical characteristics of the patients, the time between the HPV tests (in years), age at first coitus, marital status, smoking status, previous HPV vaccination, history of cervical cancer within the family, and the methods of contraception that they used were acquired either from the hospital's information management system or through phone calls with the patients.

Main Points

- Cervical cancer is the fourth most common cancer among women worldwide.
- Almost all cases of cervical cancer are caused by human papillomavirus infections.
- Cervical cancer can be prevented with regular and effective screening programs.
- Cervical cancer screening programs should be revised at certain intervals for each country.

Our hospital has been performing HPV tests since 2015. The Cobas test is the chosen test for HPV. A Pap smear test is performed as liquid-based cytology. Sample taken from the cervix with a brush is placed inside of the SurePath vial and both the Pap smear and the HPV test are done as a co-test using the same sample. The sample is taken by a gynecology specialist working at our clinic. During the testing, it was made sure that the patient had no history of coitus within the last 48 hours and that the patients were not in their menstruation period. As to the Cobas HPV testing, a sample preparation module was employed to prepare the master mix and to aliquot it, and then conduct the sample addition.[13] For this purpose, 25 µL of sample was added to 25 mL of master mix in a 96-well polymerase chain reaction plate.[13] The plate was sealed by manual means and taken to the z480 real-time amplification and detection module of the Cobas 4800 system in line with the manufacturer's protocol, making use of spectrally unique fluorescent dyes to mark TaqMan probes for HPV16, HPV18, and 12 other high-risk human papillomavirus (HR HPV) genotypes.[13] The assay aims at the 14 high-risk types included in the Cobas assay and also HPV6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39(82 subtype), and 89 (CP6108).[13] The results of the Pap smear were reported in accordance with the 2001 Bethesda System.[14] The results were reported as follows: High-grade squamous intraepithelial lesion (HGSIL), atypical squamous cells cannot rule out HGSIL (ASCH), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LGSIL), atypical squamous cells of undetermined significance (ASCUS), and negative for intraepithelial lesion or malignancy. As a routine at our clinic, patients that are HPV 16 or 18 positive, or the ones with an ASCUS and higher smear result with HR HPV positivity are given information about cervical biopsy through colposcopy. Consent is taken from the patients that accept the procedure and then the procedure is performed.[11] A second co-test after 12 months is recommended for patients with a normal Pap smear with an HR HPV positivity.[11] For patients with an HGSIL biopsy result from the colposcopy, conization is recommended.

Statistics were evaluated by means of the Statistical Package for Social Sciences (SPSS) 21 (SPSS, Inc., Chicago, Ill, USA) package program. Percentage, frequency, arithmetic mean and standard deviation were used for the analysis of the data.

RESULTS

A total of 400 patients aged 25-30 who fit the inclusion criteria and who received both Pap smear and HPV test between 2015 and 2018 were included in the study. The incidence of high-risk HPV positivity was calculated as 7% (28) for these patients. Additionally, 17 of the high-risk HPV-positive patients wanted to receive control HPV and Pap smear tests. The remaining 11 patients either did not want to get retested or could not be reached (Figure 1).

The mean age of HPV high-risk positive patients was found to be 31.7 ± 0.7, age at first coitus was 20.7 ± 3.5 and the interval between the 2 HPV tests was 4.7 ± 2.1 years (Table 1). Two of the patients were single (11.8%), 2 of them had an HPV vaccine after testing positive for the first time (11.8%), 5 of them were smokers (29.4%), and 8 of them used condoms as their protection method (47.2%) (Tables 1 and 2).

Among high-risk HPV-positive patients aged 25-30 that we were able to reach, 8 patients had HPV 16 (47.1%), 8 patients had HR HPV (47.1%), and 1 patient was positive for both HPV 16 and HR (5.8%). The results of the Pap smear came as ASCUS or higher lesions for 13 patients (76.5%) (Figure 2).

Among patients over the age of 30, 14 patients (82.4%) were HPV negative, 2 patients were HR HPV positive (11.8%) and 1 patient was HPV 16 positive (5.8%). Pap smear results were reported as

Figure 1. Flow chart of the study.

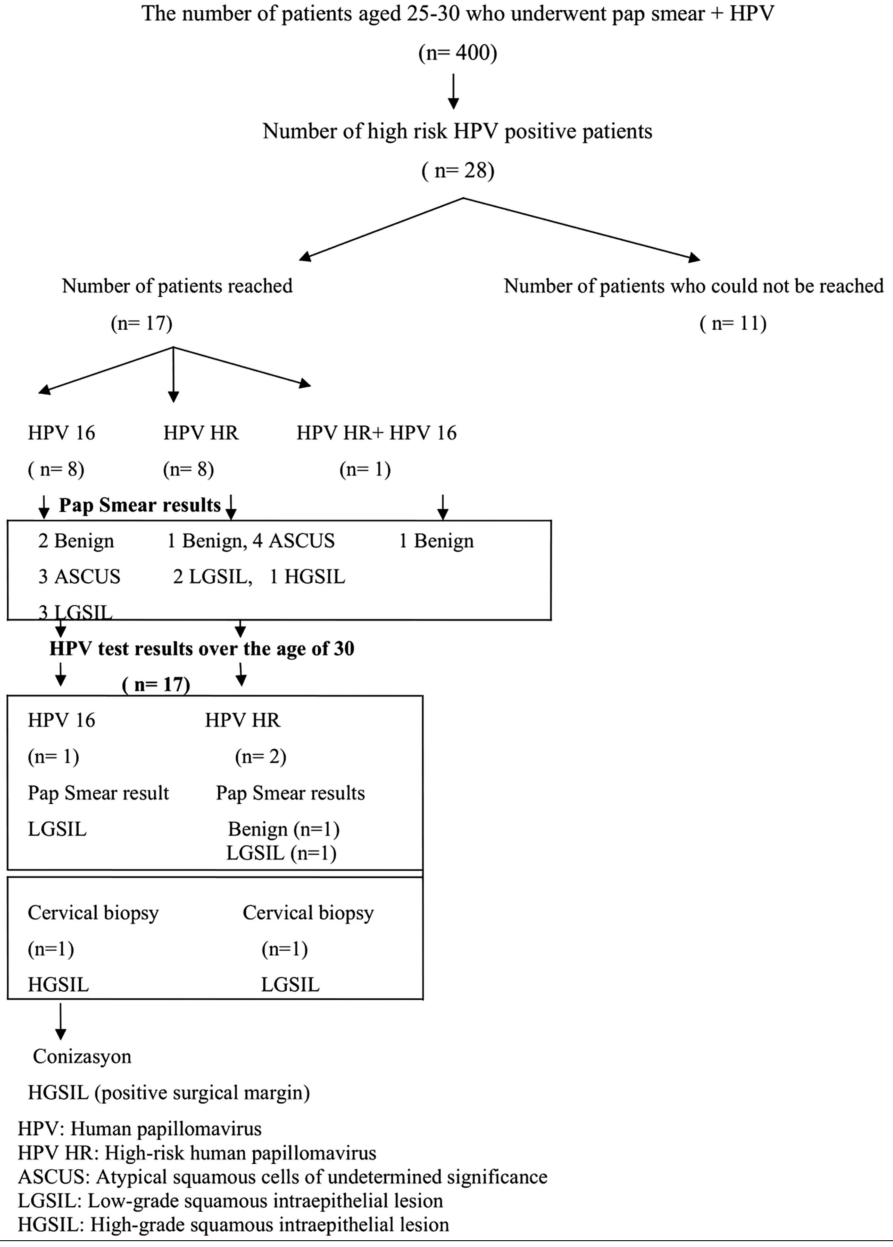


Table 1. Evaluation of Demographic and Clinical Data

	Mean ± SD	(Min–Max)
Age	31.7 ± 0.7	(31–33)
Gravidity	1.9 ± 1.7	(0–5)
Parity	1.7 ± 1.7	(0–5)
Number of living children	1.6 ± 1.6	(0–4)
Abortion	0.2 ± 0.7	(0–3)
The time between the HPV tests (in years)	4.7 ± 2.1	(2–5)
Age at first coitus	20.7 ± 3.5	(16–28)

Data are presented as mean ± SD (min–max).
HPV, human papillomavirus; SD, standard deviation.

Table 2. Evaluation of Clinical Data

		n	Percentage
Marital status	Married	15	88.2
	Single	2	11.8
Smoking status	Yes	5	29.4
	No	12	70.6
HPV vaccination	Yes	2	11.8
	No	15	88.2
History of cervical cancer within the family	Yes	0	0
	No	17	100
The methods of contraception	No	7	41.2
	Condom	8	47.2
	Intrauterine device	1	5.8
	Depoprogesterone	1	5.8

Data are presented as percentage.
HPV, human papillomavirus.

ASCUS or higher for 3 patients (17.6%). Two of these (11.8%) were ASCUS patients between the ages of 25 and 30, and one of these (5.8%) was a patient with a past Pap smear result that was reported as benign (Figure 3). None of the patients that were included in our study tested positive for HPV 18.

When high-risk HPV positivity results of the patients aged 25-30 and of the patients over the age of 30 were evaluated (Figure 4), the persistence ratio of HPV was calculated as 17.6% (3) and the regression ratio was found to be 82.4% (14). As to the Pap smear results (ASCUS and higher lesions), the regression ratio was 84.6% (11) and the persistence ratio was calculated as 15.4% (2).

Of 17 patients aged 25-30, 8 had cervical biopsy through colposcopy. Either LGSIL or HGSIL was found in 75% of them. Control HPV tests and Pap smears taken from these patients were reported as normal cervical epithelium. Three patients over the age of 30 (17.6%) had indications for cervical biopsy through colposcopy, and they accordingly received the procedure. One of these patients' results was reported as normal cervical epithelium, another had LGSIL, and the third patient had HGSIL. The patient that had HGSIL was given information and she went through the conization procedure. The result was reported as HGSIL and a positive surgical margin. This case was a single patient with persistent HPV 16.

DISCUSSION

It is widely accepted that almost all cervical cancer is caused by HPV. In our country, screening with HPV is not recommended for women under the age of 30 within the screening program

Figure 2. Human papillomavirus test and pap smear results between the ages of 25-30.

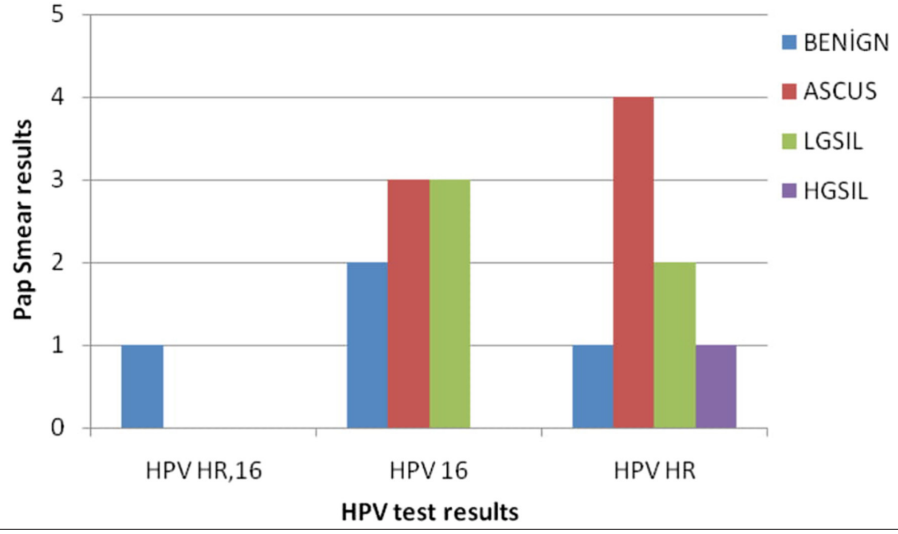


Figure 3. Human papillomavirus test and pap smear results over the age of 30.

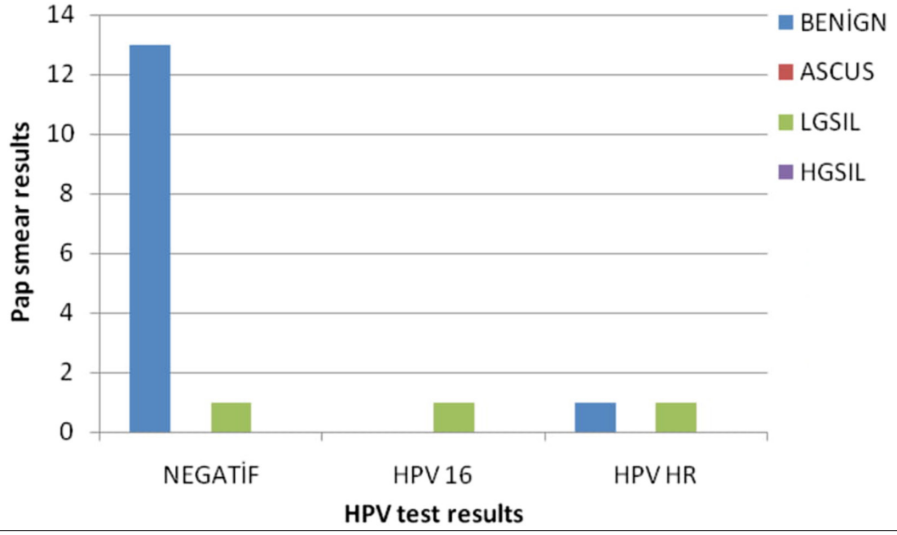
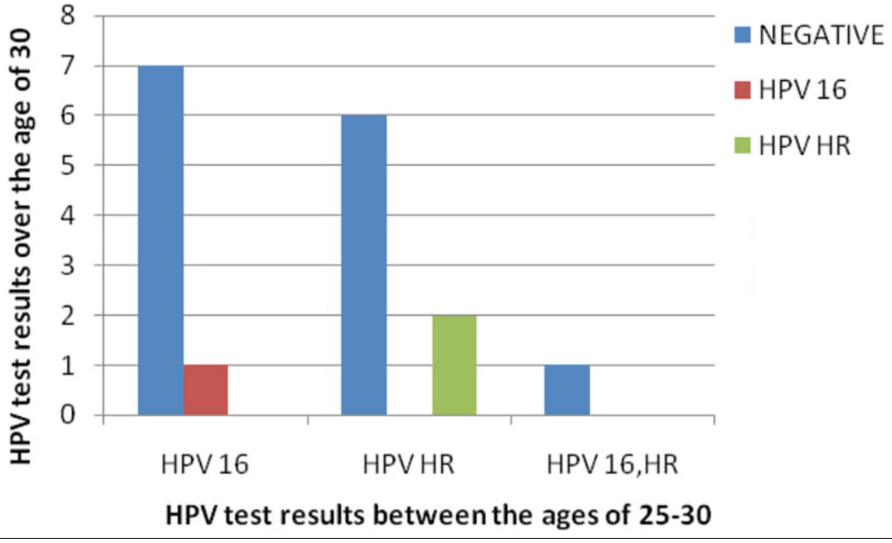


Figure 4. Human papillomavirus test results for 25-30 years old and over 30 years old.



which had its last update in 2014.[6-8] In our study, we evaluated the HPV test results of women aged 25-30 and also of women over the age of 30 with at least 2 years interval from their last test together with the Pap smear results to analyze the HPV incidence, regression, and persistence ratios. It was our wish to blaze a trail for further studies for the HPV screening to be performed from the age of 25 in our country to reduce the mortality and the incidence of cervical cancer.

In a study conducted by Veldhuijzen et al. the incidence of high-risk HPV-positive patients between the ages of 24 and 28 was found to be 8.6%. It was stated that as patients got older, the incidence and prevalence decreased. High-risk HPV incidence between the ages of 24 and 60 was reported as 3.13%. The most common types were found as HPV 16 (1%), HPV 31 (0.54%), HPV 51 (0.46%), HPV 18 (0.41%), and HPV 56 (0.29%).[15] In their study, Castle et al performed HPV and Pap smear tests for patients over the age of 25. They found 10% HPV 16/18 positivity and 6% abnormal cytology. Performing the HPV test as a co-test or performing it as a primary testing method was found to be reliable and cost-effective in cervical cancer screening.[16] In our study, we calculated the high-risk HPV positivity incidence in women aged 25-30 as 7%, which is comparable with the literature. Moreover, 76.5% of these HPV-positive patients had ASCUS or higher lesions as their Pap smear results. Conde-Ferráez et al had 76.1% of women under the age of 32 and 43.7% of women under the age of 25 in their study. Even though HPV infections are common and mostly transient around these ages, some studies have shown that after the infection, the incidence of high-risk lesions is higher after a very short while and cervical cancer mortality

in young women also increases; thus, they have emphasized the importance of screening at younger ages.[17] In our study, 1 of 17 patients (5.8%) was found to have a high-risk lesion with a positive surgical margin after conization. This case was a patient who did not want to get the cervical biopsy between the ages of 25 and 30 and had persistent HPV 16 after the age of 30, and the conization was performed when she was over the age of 30, 2 years after the first HPV test. The lesion might not have progressed this much had we performed the cervical biopsy between the ages of 25 and 30. For that reason, we believe that extensive research is necessary within this field. Sasieni et al also reported in their study that the increasing cervical cancer rate in the United Kingdom in 2009 did not correlate with the fact that patients aged 20-24 were not being tested under the screening program; however, HPV did play a part.[18] That is the reason why we believe that it is important to perform screening tests on younger patients and that it should start even from the age of 25 as recommended by the FDA, also as stated in these studies.

In a study performed by You et al.[19] the HPV test was performed on patients between the ages of 21 and 65 who had abnormal Pap smear results, and the age groups with the highest risk were found to be women between the ages of 26 and 30, and between the ages of 51 and 55. Fındık et al stated that the incidence of HPV between the ages of 30 and 65 in Turkey was 3.16%, and it was most common among women aged 30-40 with 39%. Furthermore, 18.3% of HPV-positive cases had cytological abnormalities and it was most commonly seen in women aged 30-40 with 43%. As a result, it was stated that HPV-positive women were found to have more cervical intraepithelial lesions.[20] Because HPV is not routinely tested in Turkey before the age of 30, we believe that this is the reason why HPV is most commonly seen in women aged 30-40 and that this information might change if the screening starts to include women under 30. Unlike the aforementioned studies, in our study, women that were high-risk HPV positive at 25-30 years of age were tested for HPV and Pap smear when they were over 30. We calculated the persistence ratio of HPV as 17.6% and the regression ratio as 82.4%. For the Pap smear results (as ASCUS and higher lesions) the regression ratio was 84.6% and the persistence ratio was 15.4%. We deeply believe that these ratios should be taken into consideration and further studies should be conducted to support our findings.

Testing for HPV under the age of 30 is not included within the screening program due to the fact that it might cause unnecessary colposcopies and overtreatment. When we review the literature, we see that Felix et al compared primary HPV with Pap smear in women aged 25 to 30 and co-testing with primary HPV in women aged 30-70 with regards to clinical and economic advantages. It was stated that primary HPV testing in women aged 25-30 had a minimal effect on diagnosing cancer and reducing mortality, and it was not cost-effective when compared with cytology. They found that co-testing 3 years apart between the ages of 30 and 70 was more cost-effective and prevented cancer even more compared to primary HPV testing.[21] In a study conducted by Bhatla et al. it was mentioned that most countries started to perform primary HPV tests in cervical cancer screening for women over the age of 25. However, in their study, they found that more colposcopies were performed after primary HPV tests compared to the patients that either had the co-test or just cytology.[22] We suggest that in our country, the co-test currently being performed for women over the age of 30 should be performed for women from the age of 25. Even though this might increase the costs of colposcopies, we still stand by this suggestion and believe that it will be indirectly more cost-effective because it is known that HPV is more sensitive when it comes to preventing cervical cancer or detecting precancerous lesions.

In our study, age at first coitus being 20.7, 29.4% of patients being smokers, 41.2% having completely unprotected sexual intercourse, and just 2 of the patients getting the HPV vaccine after testing positive for HPV show us that risk factors for cervical cancer are present in our country. That is the reason why we believe that in addition to further improving the screening program, women in their reproductive ages should be educated about HPV and vaccination to prevent cervical cancer.

One of the limitations of our study is that we were not able to reach all of the patients that tested high-risk positive for HPV, because 11 of them were hesitant to get the control test. In addition, we were not able to determine the positive and negative predictive values of HPV because it was not ethical to perform colposcopy on every patient included in the study. Besides, as we only included the patients that applied to our hospital, the number of positive patients was limited. Another limitation of our study is that because we acquired the patient information either from the patient files or by phone calls for some questions, we had to depend on the answers given by the patients. The strength of our study is that all of our patients were otherwise healthy, and because the HPV test was done in a random manner, we were able to include patients with different socio-cultural backgrounds. Also during our literature review, we did not come across any past study that evaluated HPV test results of women between the ages of 25 and 30 in Turkey, which can also be seen as another strength of our study.

CONCLUSION

Papanicolaou smear test has been the chosen method for screening since the 1960s and the last update to the screening program in Turkey was done in 2014. However, cervical cancer still creates a big health risk within our society. For that reason, the screening program should be revised at certain intervals in accordance with the risk factors present in the society. We believe that screening for HPV, which has an important role in cervical cancer etiology, could be reconsidered for potential coverage of women from the age of 25. That way, precursor lesions of cervical cancer can be caught at earlier ages and the incidence and the mortality of cervical cancer can be reduced.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Dicle University (Date: December 5, 2019, Decision no: 277).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – R.G., E.A., O.P.B., U.A., M.S.I., T.G.; Design – R.G., E.A., M.S.I.; Supervision – R.G., E.A., U.A., M.S.I.; Funding – R.G., O.P.B.; Materials – R.G., U.A.; Data Collection and/or Processing – R.G., O.P.B., U.A.; Analysis and/or Interpretation – R.G., E.A., U.A., M.S.I., T.G.; Literature Review – R.G., T.G.; Writing – R.G., M.S.I.; Critical Review – R.G., E.A., O.P.B., U.A., M.S.I., T.G.

Declaration of Interests: The authors declare that they have no competing interest.

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