Original Article Acta Med Eur. 2024;6(2):20-23

Acta Medica Europa

ISSN Online: 2996-1211 doi: 10.5281/zenodo.10848854

Evaluation of Pap-Smear Tests in Accordance with the Results of HPV-DNA and Colposcopical Examinations Performed in Bülent Ecevit University Hospital in the Year of 2020

Gunter Dilsiz [1]

[1] Department of Famly Medicine, Bülent Ecevit University, Medical Faculty, Zonguldak, Türkiye

Article Info

Received: 13 March 2024

Revised: 21 March 2024

Accepted: 21 March 2024

Published: 21 March 2024

Keywords:

Cancer, cervix uteri, gynecology, Pap smear, HPV, screening, vaccines, colposcopy.

Corresponding author:

Gunter Dilsiz.

Department of Obstetrics and Gynecology, Bülent Ecevit University, Medical Faculty, Zonguldak, Türkiye.

ORCID: 0000-0002-5683-1976

diba95@yahoo.com

ABSTRACT

Assessment of the results of Pap smear tests and comparison with colposcopic biopsy and HPV-DNA test results performed in our facility. Pap smear tests of overall 1079 patients were examined and compared out of1332 patients aged between 30-65 years that applied to the Gynecology out-patient clinic of Bülent Ecevit University hospital during 2020. 986 out of 1079 Pap-smear tests were found to be negative, 76 ASC-US, 3 AG-US,1ASC-H, 9 LISL, 3 HSIL and 2 carcinomas respectively. When compared with similar studies ASC-US rates were found to be higher and this can be attributed to utilization of conventional cytological testing in our hospital. Eighty (80) patients that had Pap-smear results with ASC-US, AG-US, and ASC-H had 17.50% positive HPV-DNA test rate results. These 80 patients had 6.25% HPV 16, 1.25% HPV16 and HR (other high risk HPV types), 1.25% 18 and 8.75% HR types respectively. Patients that had abnormal Pap smear were younger than the general Pap smear group. This supported that screening programs were beneficious even in early ages. Cervical cancer is highly related to HPV infection and can be screened with easy and minimally invasive methods. Whatever the method is, screening of cervical cancer decreases the morbidity and mortality rates when applied population wide. Furthermore, it is a fact that vaccination and education programs about venereal diseases are long-term but feasible and cost-effective investments for the future to prevent cervical cancer. At this stage this this study could contribute to the vaccination and screening programs.

Cite as: Dilsiz G. Evaluation of Pap-Smear Tests in Accordance with the Results of HPV-DNA and Colposcopical Examinations Performed in Bülent Ecevit University Hospital in the Year of 2020. Acta Med Eur. 2024;6(2):20-23. doi: 10.5281/zenodo.10848854

INTRODUCTION

Cervical cancer shares the third place after breast and colorectal malignancies in women throughout the World and the second place among gynecological cancers. In Turkey the exact number is not known due to shortness of screening frequency but it is estimated that cervical cancer shares the ninth place among female cancer cases (1). In the USA cases with dysplasia are being constantly followed up. Information concerning cervical diseases have been available for centuries but the concept of cervical cancer has been present for the last 150 years. After the development of cytologic screening methods by Papanikolaou and their routine utilization dropped the frequency rate of cervical cancer cases to the eleventh place and the mortality rate to the thirteenth place among cancer cases in the USA whereas it shared the second place before (2).

Cervical cancer screening can be regarded as one of the most cost-effective method among other cancer screening procedures in meaning of frequency and mortality reduction. With accurate orientation and treatment , cervical cancer is easier to handle and this reduces the social burden (3).

Human Papilloma Virus (HPV). infection is frequently encountered in the society and leads the path to the development of cervical cancer. 99,7% of Cases with cervical cancer share HPV infection as causative factor (4). Furthermore, the development of cervical cancer due to HPV infection by its impact on cervical epithelial cells and host immunity has been largely understood. Due to this fact it is a

must to perform cytological screenings on pre-neoplastic lesions and HPV-DNA testing and typing must be performed on patients that are infected with high risk HPV species. Even if patients infected with high HPV types turn out to be cytologically normal, a colposcopical evaluation must be performed (5).

Pap smear testing has a high specificity but a low sensitivity. Since developed by Papanikolaou in 1950, this test decreased incidence and mortality rates of cervical cancer by 75% (3).

The most controversial cytological result is atypical squamous cells of undetermined significance (ASC-US). Some authorities suggest direct colposcopy, some request a cytological re-examination 6 months later and a reflex HPV-DNA testing prior to colposcopical examination for the evaluation of high risk patients. When combined with cytological examination, high oncogenical risky HPV-DNA testing sensitivity increases significantly (6).

In this study we aimed to compare cytological examinations with HPV-DNA typing analysis and colposcopic biopsy specimens in our clinic.

METHODS

Time and localization of the study: This study was performed on 1079 out of1332 women that aged between 30-65 years and underwent a Pap smear test in the gynecology outpatient clinic Zonguldak Bülent Ecevit University Hospital between 1 January 2020 and 31 December 2020. Clinical files of enrolled patients were scanned with the permission of the ethical board via the utilization of the hospital database retrospectively. Data concerning smear, HPV-DNA and colposcopical results were recorded for analysis.

Research Universe and Sampling

Research universe consists of 1332 patients that underwent a smear test in the gynecology outpatient clinic of the Zonguldak Bülent Ecevit University hospital between the dates of 1 January-31 December 2020. No samples were chosen and all patients were enrolled.

1079 patients were enrolled in the study that met the criteria. All patients that met enrollment criteria underwent analyses and 100% of the study universe was reached cumulatively.

Definition of the Study and Variables

This study is of an analytical epidemiological type. Outpatient data was analyzed with minimum loss and turns out to be of representative qualification.

Some sociodemographic data and physical features (age, sex). cervical cytological results, HPV-DNA typing tests and colposcopical biopsy results have been recorded. Cervical cancer screening results and their relation to HPV-DNA infection status and results of colposcopical biopsy and their relation with cancerous and pre-cancerous lesion producing percentages have been analyzed.

HPV-DNA Test, Pap Smear and Biopsy

Cervical smear samples taken with a plastic brush were sent for pathological analyses via a conventional slide. Grading was made according to the Besthesda 2001 system. Specimens obtained via a cervical brush were sent to the microbiology lab stored in Abbott Cerci-Colect Specimen Collection kits.

DNA isolation was carried out by Roche Cobas 4800 System Liquid Cytology Preparation Kit and Sample Preparation Kit on liquid based cervical cytology specimens

HPV-DNA genotyping was performed by Roche Cobas 4800 systems Cobas 4800 HPV amplification/detection kit using real time PCR method. This genotyping test detects 600 copies/mL or more HPV-DNA genotypes. This test detects HPV-DNA genotype 16,18 and HPV-DNA HR (other high risk HPV types). and genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. HPV-DNA low risk genotypes (e.g. 6, 11, 42, 43, 44). that don't have a proven relationship with cervical cancer and its precursors are out of the scope of this test. Biopsies performed via colposcopy were obtained using validified methods as punch biopsy, Loop Electrosurgical Excision Procedure (LEEP), conization by the gynecology clinic and were sent for pathological examination.

Study Enrollment and Exclusion Criterion

Pap smear tests of overall 1079 patients were examined and compared out of 1332 patients aged between 30-65 years that applied to the Gynecology out-patient clinic of Bülent Ecevit University hospital during 2020.

Data Collection and Data Collection Tools

A data form has been utilized that was prepared according to current literature. Variables constituting the data forms were extracted from the patients' files with obtained necessary permissions and analyzed using SPSS 17.0 program. While evaluating the data descriptive statistical methods have been used (e.g. Number, percentage, mean, standard deviation).

Table 1. Numerical distribution and mean age of smear results.

	N	%	Mean age
Negative	986	91.29	46
ASC-US	76	7.04	41
AGUS	3	0.28	63
ASC-H	1	0.09	65
LSIL	9	0.83	42
HSIL	3	0.28	43
Carcinoma	2	0.19	41
Total patient number	1079	100	45.94

ASC-US: Atypical squamous cells of unidentified importance, AGUS: Glandular cells of unidentified importance, ASC-H: Atypical squamous cells without exclusion of high-grade squamous intraepithelial lesions, LSIL: Low grade cervical intraepithelial lesion, HSIL: High grade cervical intraepithelial lesion.

Table 2. Distribution of HPV types among patients with ASC-US, AGUS and ASC-H.

	N	%
Negative	**66**	**82.50**
Positive	**14**	**17.50**
16	**5**	**6.25**
16 and HR	**1**	**1.25**
18 and HR	**1**	**1.25**
HR	**7**	**8.75**

ASC-US: Atypical squamous cells of unidentified importance, AGUS: Glandular cells of unidentified importance, ASC-H: Atypical squamous cells without exclusion of high-grade squamous intraepithelial lesions, HR: Other high risk HPV types.

Study Preliminary Trials

As this study is of retrospective design no preliminary trials were held. Technical information on hospital computing system, patient data access and the scope of this study were evaluated prior to the study.

RESULTS

Of 1079 enrolled patients 7.04% turned out to be ASC-US, 0.28% atypical glandular cells of undetermined significance (AGUS), 0.09% Atypical squamous cells without exclusion of high-grade squamous intraepithelial lesions (ASC-H), 0.83% low-grade squamous intraepithelial lesion (LSIL), 0.28% high-grade squamous intraepithelial lesion (HSIL) and 0.19% carcinoma respectively. The rest 91.29% were negative on Pap smear testing. (Table 1).

When the age of patients was taken into account the results were as follows; the ASC-H group 41, AGUS 63, ASC-H 65, LSIL 42, HSIL 43, carcinoma 41 and the mean age was 45,95. (Table 1).

Totally 80 patients with smear results ASC-US,AGUS and ASC-H, 82,50% were negative and 17,50% were positive on HPV-DNA typing test. When the typing results of 80 patients were taken into account 6,25% were HR, 1,25% were 16 and HR (other high risk HPV types), 1,25% were 18 and HR and 8,75% were HR respectively. (Table.2).

When the LSIL group was taken into account, 55.6% were HPV negative, 22.2% 16 HR and 22.2% were HPV-HR. (Table.3).

When the HSIL group was analyzed , 33,3% were HPV 18 and HR and 33,3% HPV HR and33,3% were HPV negative (Table.4).

Table 3. Distribution of HPV types among patients with smear results of LSIL.

LSIL	N	%
HPV Negative	5	55.6
16 HR	2	22.2
HPV HR	2	22.2

HPV: Human Papilloma Virus, LSIL: Low grade cervical intraepithelial lesion, HR: Other high risk HPV types.

Table 4. Distribution of HPV types among patients with smear results of HSIL.

HSIL	N	%
HPV 18+HR	1	33.3
HPV HR	1	33.3
HPV NEGATİVE	1	33.3

HPV: Human Papilloma Virus, HSIL: High grade cervical intraepithelial lesion, HR: Other high risk HPV types.

Eight patients out of 9 with a cytological result of LSIL underwent a colposcopical evaluation. Biopsy results were as follows; 4 Cervical intraepithelial neoplasia (CIN) -1, CIN-2 and 4 were negative. 3 out of 5 patients with a biopsy result were found to be HPV-DNA negative.

DISCUSSION

Cervical cancer is generally the outcome of asymptomatic precancerous lesions. It shares the first rank among gynecological malignancies and is seen in earlier ages. With proper screening, treatment and follow-up most of the mortal events can be prevented as the development of cervical cancer takes a long time span. Identification of precancerous lesions via screening programs and proper treatment decreases incidence and mortality rates dramatically. Pap smear test was developed approximately 70 years ago and demonstrates a good sample to this issue. In our country a cervical cancer screening program has been initiated in 2014 and includes the age span of 30-65 years.

When applied properly the Pap smear test decreases cervical cancer development drastically. Also, it increased the diagnosis of CIN and besides the early diagnosis. This is a cost effective screening test and has many requested properties. But unfortunately has a high false positive rate in the diagnosis of precancerous lesions. In a study 62% of false positive results were due to clinical sampling mistakes, 22% due pathological evaluation and 16% due to cytotechnological screening errors (7). False negative results also reach a rate of 50%. (8). This limits the reliability of the test and can be overcome by repeated smears. The route from normal cervix to cervical cancer takes 10-15 years and this provides the performance of repeated scans.

This is a retrospectively designed study which was held on Pap smears performed in our gynecology outpatient clinic where tests were scanned and compared with HPV-DNA and cervical biopsy results. Pap smear tests of overall 1079 patients were examined and compared out of 1332 patients aged between 30-65 years that applied to the Gynecology out-patient clinic of Bülent Ecevit University hospital during 2020. 986 out of 1079 Pap-smear tests were found to be negative, 76 ASC-US, 3 AG-US, 1 ASC-H, 9 LISL, 3 HSIL and 2 carcinomas respectively. In a different study held in Tokat in 2012 carried out by Çimşir et al frequencies of smear tests were evaluated and ASC-US frequencies were found to be less than 1% (9). In a similarly designed study that was held in Istanbul in 2012 by Arslan et al ASC-US frequency was found to be 3% among women aged 18-60 (10). By means of diagnostical standards ASC-US frequency is expected to be over 5% (11). In our study ASC-US

frequency was found to be high and this can be attributed to the subjectivity of pathological evaluation and the high rate of HPV infection in our region. Especially LSIL,HSIL and carcinoma frequencies were close to our study but ASC-US numbers were different and this can be explained by a different patient profile and the utilization of conventional Pap smear tests in our clinic. When the results of HPV tests are taken into account it can be seen that HPV types with high risk are more than 16 and18%. This leads to the result that 9 repentant vaccinations will be more effective than 2 or 4. This vaccination schedule was approved in our country but due to sales and distribution problems this has not been initiated yet. In a study performed by Benli et.al. held in Kayseri in2018, high risk HPV types were evaluated separately and were found to be less than HPV 16 (12). In our center distinguishment of HPV types is not performed due to this risk evaluation cannot be done properly. To keep light on future vaccination programs similar and more comprehensive studies must be carried out.

Mean age in cervical smears performed in our hospital was 46 and the mean age of smears with the results of ASC-US, LSIL,HSIL and carcinoma was 41-43. This was in accordance with current literature and demonstrated the fact that cervical cancers are accounted in earlier ages among other malignancies.

Samples with the diagnosis of ASC-US were 17,5% positive for HPV tests. HPV infections are strongly connected with cervical cancers and the low percentage of ASC-US diagnosis must be aggressively evaluated. Cytological results of ASC-US are highly false positive and transformation to normal is frequently seen. Patients with the diagnosis of ASC-US who underwent HPV-DNA tests should be re-evaluated via repeated smears and colposcopy. 5 out of 9 patients who were cytologically evaluated as LSIL had pathological lesions and this provides information on false positivity. Furthermore, HPV infection underlying cervical cancers reach a percentage of 99,7% and in those with HPV-DNA negative result can be CIN-1 positive and this brings in mind false negative results. Due to this issue, when costs are ignored, the combination of two test or utilization of reflex tests seem to be the best choice.

Ethics

Non-interventional study permission was obtained from ethical board of ZBEU Medical Faculty. Furthermore, access to study data permission was obtained from the hospital management (with the ethical board decree of 20.10.2021 dating and the number 2021-20). Study data was only used for scientific purposes and personal data concerning reenrolled patients was not shared in study reports nor with third persons.

Funding

This study was planned by researchers, patient data was accessed, analysed and reported.

Stationary expenses were paid by researchers. No extra budget was necessary.

Conflict of Interest

All authors declare that they do not have any potential conflict of interest that could inappropriately influence the present study.

REFERENCES

1. Bray F, Farlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. doi:10.3322/CAAC.21492
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin. 2021;71(1):7–33. doi:10.3322/CAAC.21654
3. Quinn M, Babb P, J Jones, Allen E. Effect of screening on incidence of and mortality from cancer of cervix in England: evaluation based on routinely collected statistics. BMJ. 1999;318(7188):904. doi:10.1136/BMJ.318.7188.904
4. Cancer do colo de útero - Ginecologia - 4. Accessed: October 9, 2021. https://www.passeidireto.com/arquivo/81612135/3-cancer-do-colo-de-utero/4
5. Wentzensen N, Massad LS, Mayeaux EJ, Khan M, Waxman A, Einstein M et al. Evidence-Based Consensus Recommendations for Colposcopy Practice for Cervical Cancer Prevention in the United States. J Low Genit Tract Dis. 2017;21(4):216–222. doi:10.1097/LGT.0000000000000322
6. Cheung LC, Egemen D, Chen X, vd. 2019 ASCCP Risk-Based Management Consensus Guidelines: Methods for Risk Estimation, Recommended Management, and Validation. J Low Genit Tract Dis. 2020;24(2):90–101. doi:10.1097/LGT.0000000000000528
7. Sanders GD, Taira A V. Cost Effectiveness of a Potential Vaccine for Human papillomavirus. Emerg Infect Dis. 2003;9(1):37. doi:10.3201/EID0901.020168
8. Coppleson LW, Brown B. Estimation of the screening error rate from the observed detection rates in repeated cervical cytology. Am J Obstet Gynecol. 1974;119(7):953–958. doi:10.1016/0002-9378(74).90013-1
9. Çimşir MT. 01.01.2008- 31.03.2011 tarihleri arasında kliniğimizde yapılan servikal pap smear sonuçlarının değerlendirilmesi. Thesis of Obstetrics and Gynecology. Published online 2012.
10. Arslan E. Normal dışı pap smear sonuçlarının sıklığı ve kolposkopi sonuçları. Thesis of Obstetrics and Gynecology. Published online 2012.
11. Berek J. Novak's gynecology. Williams & Wilkins; 1996.
12. Yildiz S, Özdemir ME, Gökçek MB, Ünsal NE, Aslan B, Benli AR. Kayseri ili 2018 yılı HPV tarama sonuçlarının değerlendirilmesi. Turkish J Public Heal. 2021;19(3):244–250. doi:10.20518/TJPH.801654