

# Comparison of biopsy results of HPV 16/18 and non-16/18 HPV positive patients with a normal PAP test: A tertiary center experience

Kazibe Koyuncu<sup>1</sup>, Mustafa Kurt<sup>2</sup>, Önder Sakin<sup>1</sup>, Emine Eda Akalın<sup>1</sup>, Ramazan Denizli<sup>1</sup>, Abdulmecit Öktem<sup>3</sup>, Yasemin Alan<sup>4</sup>, Mustafa Gökkaya<sup>1</sup>

<sup>1</sup>University of Health Sciences, Kartal Training and Research Hospital, Obstetrics and Gynecology Clinic, Istanbul, Turkey

<sup>2</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Hitit University, Corum, Turkey

<sup>3</sup>Ministry of Health, Cizre State Hospital, Obstetrics and Gynecology Clinic, Şırnak, Turkey

<sup>4</sup>Metropolitan Municipality Esrefpaşa Hospital, Obstetrics and Gynecology Clinic, Izmir, Turkey

## ORCID ID of the author(s)

KK: 0000-0001-9070-3962  
MK: 0000-0002-9017-9643  
ÖS: 0000-0001-6036-9975  
EEA: 0000-0002-3593-5063  
RD: 0000-0003-1128-7169  
AÖ: 0000-0003-4956-1813  
YA: 0000-0003-2680-814X  
MG: 0000-00032-0477-157X

## Corresponding Author Yasemin Alan

Metropolitan Municipality Esrefpaşa Hospital,  
Obstetrics and Gynecology Clinic, Izmir, Turkey  
E-mail: gozdealan@hotmail.com

## Ethics Committee Approval

This study was approved by the Medical Specialty Training Board of Dr. Lutfi Kirdar Training and Research Hospital (Approval ID: 2019/514/148/26). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

## Conflict of Interest

No conflict of interest was declared by the authors.

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## Abstract

**Background/Aim:** Optimal management for HPV positive and cytology negative patients remains a controversial issue. Immediate colposcopy is suggested for HPV 16/18 positive patients, whereas patients with non 16/18 HPV oncogenic virus positive are recommended to co-test after a year. In this study, we aim to compare the immediate colposcopic biopsy results between HPV 16/18 and non-16/18 HPV positive patients with cytology negative patients.

**Methods:** In this prospective cross-sectional study, we included 1028 HPV positive and cytology negative patients who were screened for cervical cancer between January 2017 and 2019. Liquid based preparations were used for cytology samples (ThinPrep Pap Test). Cervical specimens were analyzed with Hybrid Capture for HPV types. Patients underwent colposcopic examination, biopsy procedure and endocervical curettage.

**Results:** A total of 424 (41.2%) patients were HPV 16/18 positive, while 604 (58.8%) were non-16/18 oncologic HPV positive. Colposcopic biopsy results of the patients revealed that of the HPV 16/18 positive patients, 246 (23.9) had no dysplasia, 101 (9.8) had LGSIL and 77 (7.5%) had HGSIL. Among the non 16/18 positive patients, 422 (41.1%) had no dysplasia, 144 (14%) had LGSIL and 38 (3.7) had HGSIL. All patients were referred for endocervical curettage, which resulted as follows: Among HPV 16/18 patients, 384 (37.4%) had no dysplasia, 21 (2%) had LGSIL and 19 (1.8%) had HGSIL. Five hundred seventy-one non 16/18 positive patients had no dysplasia, 26 (2.5%) had LGSIL and 7 (0.7) had HGSIL. The comparison of colposcopic biopsy results of HPV 16/18 and non-16/18 HPV positive patients were different in terms of no dysplasia and HGSIL ( $P=0.001$  and  $P=0.001$ , respectively), while LGSIL results were similar. The endocervical curettage biopsy results of the patients revealed a significant difference in HGSIL results ( $P=0.03$ ). The two groups were similar with respect to reports of no dysplasia and LGSIL.

**Conclusion:** Direct referral of the patients, who are expected to be lost to follow-up, could be convenient for non-16/18 HPV positive patients with negative cytology to reduce progression of cervical cancer and the psychological burden of HPV positivity.

**Keywords:** HPV 16, HPV 18, Colposcopy, PAP test

## Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection [1] which is proven to cause cancers in anogenital tract (cervical, vaginal, vulvar, anal), along with the head and neck regions [2, 3]. Cervical cancer screening is adopted worldwide. Pap cytology or HPV genotyping are the most common methods. In our national screening program in Turkey, only HPV genotyping is conducted. If the result is positive, cytology is used for triage. HPV test alone is found to be more sensitive than cytology alone [4]. New triage methods are also under investigation, such as p16/Ki-67 dual-stained cytology or molecular triage markers [5, 6].

The debate for best option of cervical cancer screening continues. The most prevalent co-test result is "Cytology negative, non-16/18 high-risk HPV positive" [7]. According to The American Society for Colposcopy and Cervical Pathology (ASCCP), colposcopy should be suggested to the patients with abnormal cytology results regardless of the type of a positive HPV result [8]. Patients with positive tests for HPV 16/18 should also be referred for colposcopy even they are negative for intraepithelial lesions and malignant cytology (NILM). Patients positive for HPV types other than 16/18 are recommended to have co-tests repeated after one year because of the possibility of spontaneous regression [8, 9]. However, there are studies suggesting that non 16/18 HPV positive patients should undergo immediate colposcopy to avoid the risk of overlooking cervical intraepithelial neoplasia [9]. Direct referral for colposcopy is not suggested in ASCCP guidelines, however, larger studies should be conducted regarding follow-up results and patients lost to follow up in this approach.

In this study, we aimed to evaluate the colposcopic biopsy and endocervical curettage results of NILM and HPV-positive patients who presented to our clinic to determine the optimal management.

## Materials and methods

In this retrospective cross-sectional study, we included patients with NILM cytology results and HPV positive patients aged between 30-65 years, who were admitted to a tertiary center between January 2017-2019. Exclusion criteria included patients with a history of cervical intraepithelial neoplasia, abnormal Pap test results and pregnancy. Patients' ages, education level, monthly income, marital status, obstetric history, and employment status were noted. Ethics approval was obtained from the local ethics committee, and all patients signed informed consent forms (2019/514/148/26).

Liquid based preparations were used for cytology samples (ThinPrep Pap Test). Cervical specimens were analyzed with Hybrid Capture for HPV types 16, 18 and twelve other high-risk HPV types including type 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. Colposcopic biopsy and endocervical canal curettage were performed according to pathological findings in colposcopy and history of the patients. Colposcopy was performed with colloquial device (colposcope 1D-21100, Leisegang GmbH, 2014-03, Germany), which can augment up between 4.5- 30 using a green filter. First, the cervix was cleaned with saline, acetic acid was applied, and acetowhite areas and

vascular pathologies were determined. Then, the cervix was stained with Lugol's solution, and iodine-free areas were spotted. Random biopsies were taken from four quadrants if there were no specific lesions. Endocervical curettage was performed if there were suspicious lesions or inadequate transformation zone observation. Pap test results were reported in accordance with the Bethesda system. Cervical biopsy results were interpreted according to The Lower Anogenital Squamous Terminology system, as LGSIL and HGSIL. Biopsy results were rendered pursuant to World Health Organization. Follow-ups after the procedures were planned in accordance with the 2012 ASCCP [4].

## Statistical analysis

The data was analyzed using Statistical Package for the Social Sciences software version 24. The normality of data distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. Levene's test was used for homogeneity of variances. Nominal variables were given as number of cases and percentages, descriptive variables were shown as mean (standard deviation). Chi-square test was used to compare the categorical data and ratio comparisons. Post-hoc analysis was performed for binary comparisons of groups. Adjusted p values were calculated after Bonferroni correction. Statistical significance was  $P < 0.05$ .

## Results

In this study, we included 1320 patients with NILM cytology and positive HPV results who were admitted to our hospital. Among them, 292 patients were excluded due to history of cervical intraepithelial neoplasia and abnormal cytology. Two patients positive for HPV 16/18 had cervical cancer in their cervical biopsy results; they were also excluded from the study to preserve the normality of distribution. The study was completed with 1028 patients and the biopsy results were evaluated.

The mean age of the patients was 44.28 (8.82) (26-66) years. In the study population, we found that 424 patients (41.2%) were HPV 16/18 positive, and 604 (58.8%) were non-16/18 oncologic HPV positive (Figure 1). All patients underwent cervical biopsy and endocervical curettage. Pathology results of the patients are summarized in Table 1.

The patients were divided into two groups and cervical biopsy results were compared. Group 1 consisted of HPV 16/18 positive patients, and Group 2 included patients positive for non-16/18 oncogenic types. Results of the patients were classified as normal, LGSIL and HGSIL. We found that no dysplasia results were significantly higher in non-16/18 HPV oncogenic type group (n=422 (69.8%)), compared to the HPV 16/18 positive group (n=246, (58.0%)) ( $P=0.001$ ), while HGSIL results were significantly lower (n=38, 6.2% vs. n=77, 18.1%,  $p=0.001$ ). LGSIL results were similar (Table 2).

Figure 1: Flow-chart of the study

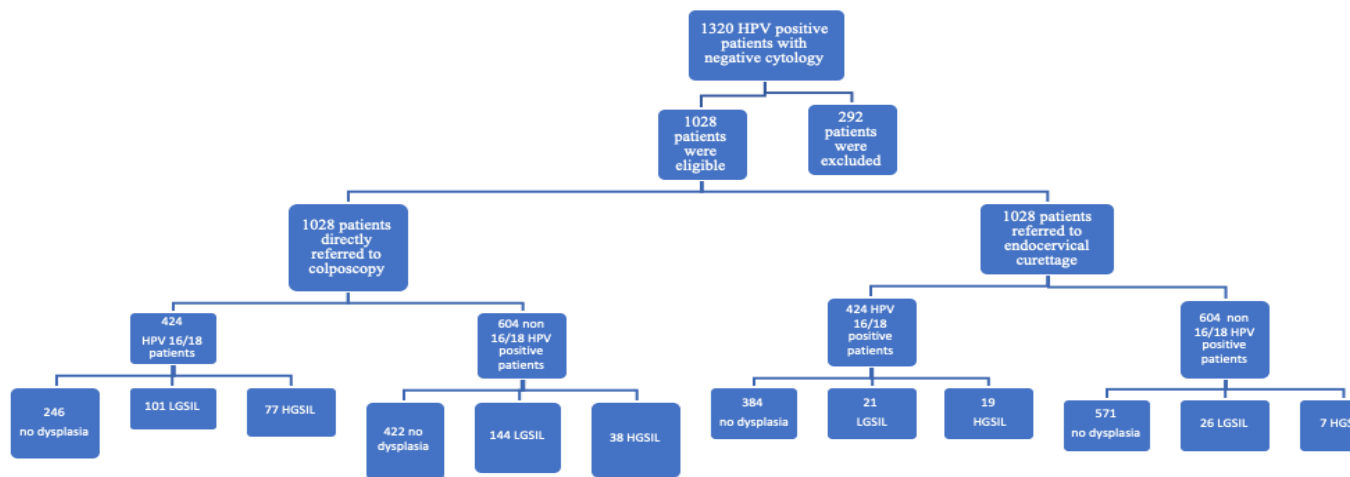


Table 1: HPV, cervical cancer and ECC biopsy results of the patients

HPV screening result	Eligible female patients n=1028	
	n	%
HPV 16/18 +	424	41.2
Non 16/18 +	604	58.8
Cervical biopsy results		
Normal	668	65
LGSIL	245	23.8
HGSIL	115	11.2
Endocervical curettage		
Normal	955	92.9
LGSIL	47	4.6
HGSIL	26	2.5

Table 2: Comparison of colposcopic biopsy results of HPV 16/18 and non 16/18 patients

Colposcopic biopsy results	HPV 16/18 positive n (%)	non 16/18 HPV positive n (%)	P-value	P-value <sup>1</sup>
No dysplasia	246 (58.0)	422 (69.8)	<0.001	0.001
LGSIL	101 (23.8)	144 (23.8)		0.600
HGSIL	77 (18.1)	38 (6.2)		0.001

<sup>1</sup> P-value of post-hoc analysis after Chi-square test

The endocervical curettage (ECC) results of the patients were also compared. Results of the patients were classified as normal, LGSIL, HGSIL. According to the post-hoc analysis, patients with no dysplasia were insignificantly higher in the non-16/18 HPV positive group ( $P=0.34$ ), and no significant differences were found in terms of LGSIL results ( $P=5.29$ ). However, HGSIL results differed, with 7 (1.1 %) detected among non 16/18 HPV-positive patients and 19 (4.4 %) detected among those with non-16/18 HPV positivity ( $P=0.03$ ). When the three categories of cytology results (no dysplasia, LGSIL and HGSIL) were compared between the two groups, ECC results significantly differed ( $P<0.001$ ) (Table 3).

Table 3: Comparison of endocervical curettage biopsy results of HPV 16/18 and non 16/18 patients

ECC biopsy results	HPV 16/18 positive n (%)	non 16/18 HPV positive n (%)	P-value	P-value <sup>1</sup>
No dysplasia	384 (90.5)	571 (94.5)	<0.001	0.340
LGSIL	21 (4.9)	26 (4.3)		0.529
HGSIL	19 (4.4)	7 (1.1)		0.030

<sup>1</sup> P-value of post-hoc analysis after Chi-square test

## Discussion

Cervical cancer is a public health problem worldwide, with an estimated 528,000 new cases and 266,000 deaths per year [10, 11]. In Turkey, cervical cancer is the 10<sup>th</sup> most observed cancer [12]. Mortality and morbidity due to cervical cancer mostly affects low and middle-income countries [10]. This may be due to low socio-cultural status or inappropriate screening programs. Optimal management strategies for cervical cancer screening are still controversial. The aim is to detect cervical cancer in the early phase with a low-cost and less invasive method.

Addressing the Need for Advanced HPV Diagnostics (ATHENA), a large population-based study, confirms that HPV screening is more sensitive than cytology alone for detecting  $\geq$ CIN 3 lesions. Besides, a negative HPV test is more reliable for long-time protection than cytology [13]. Therefore, it was expected to exclude cytology and move on with HPV in the first place for cervical cancer screening [14]. However, cytology and HPV test are still being interpreted together in guidelines. The algorithms of positive HPV and abnormal cytology is well-established in ASCCP guidelines [15]. Approach to HPV-positive patients who are negative for intraepithelial lesion and malignancy cytology (NILM) remain a controversy. The results of the Population Based Screening Study Amsterdam (POBASCAM) study revealed that only HPV screening is safe, also stating that the lack of further evaluation of the normal cytology results could constitute bias between groups [16]. In our study, we evaluated the colposcopic and endocervical curettage results of the patients.

The most common result obtained in screening programs is non-16/18 oncologic positivity with normal cytology [17]. National screening programs aim to lower the risk below 2% for  $\geq$ CIN 3 lesions [18, 19]. In our study, we found a HGSIL incidence of 3.7%, which is higher than expected. This could be related to consequences of the discrete health-care policies of the countries [20]. In the Netherlands, according to Vrije Universiteit Medical Centre-Saltro laboratory population-based cervical screening (VUSA- screen) study, patients are followed with cytology in 0<sup>th</sup>, 6<sup>th</sup> and 18<sup>th</sup> months for a corresponding negative predictive value [21]. However, this procedure is patient-dependent and up to 28-33% of the patients were lost to follow-up in the studies [19]. We also have a lot of patients who did not attend their follow-up regularly in our clinic, which decreases the

benefits of screening, as these patients could not be treated in the early stages. If optimal follow-up is not expected, more invasive methods would be justified to prevent any delays in treatment.

Castle et al. [13] performed a sub-analysis of the patients of ATHENA study and concluded that adding HPV typing to cytology enabled a more sensitive and efficient screening method for cervical cancer. Combined liquid-based cytology and HPV testing are shown to increase the sensitivity by 4% compared to HPV alone for  $\geq$ CIN 3 lesions. However, the number of positive patients screened also increased by 35.2%. Adding HPV genotyping to the triage was found to increase colposcopy sensitivity, and addition of positive predictive value (PPV) proved more successful than ASC-US, but less successful when alone. When HPV 16,18 genotyping is adopted for NILM cytology, the sensitivity and PPV for  $\geq$ CIN 3 are 53.8 % and 10.2%, respectively. HPV genotyping reduces interobserver reproducibility and workforce. There are other cytology and molecular triage strategies. For example, p16/Ki 67 dual- stained cytology has a similar sensitivity for  $\geq$ CIN 3, but higher specificity [22-24]. Wright Jr. published a sub-study nested into ATHENA trial and concluded that p16/Ki-67 dual stained cytology was more sensitive and efficient for colposcopy than routine cytology or HPV 16/18 genotyping [13]. Furthermore, the increase of adenocarcinoma necessitates better triage methods for HPV-positive/ NILM cytology patients [7]. It is found that 50% of HPV-positive/NILM women could develop cervical adenocarcinoma, which cytology is less effective in identifying [25].

The potential harms of direct referral to colposcopy are increase in patients' anxiety and the risk of the procedure [26]. We found that HPV results of the patients decreased their quality of life, irrespective of their cytology result, in a study conducted in our center. As direct referral to colposcopy is the most sensitive method for detecting high grade lesions with a sensitivity of 89.9% for  $\geq$ CIN 3 lesions, we used this method on our patients [27] and found that we would have missed 3.7% percent of the patients for follow-up with HGSIL had we not utilized cytology for triage. It is also shown that screening with HPV alone had the highest relative sensitivity and lowest relative specificity for  $\geq$ CIN 2 lesions (1.68 and 0.71, respectively) [28].

HPV with cytology and HPV genotyping triage had the highest relative specificity (1.04) and lowest relative sensitivity (0.92 and 0.85, respectively) [29]. Cytology and colposcopy should be used as complementing methods to reduce more invasive procedures such as conization or LEEP [30].

Non 16/18 HPV types have a prominent place in HPV screening programs because they are the most reported result of co-testing [7]. In the assessment of colposcopic biopsy results of 300 patients who tested positive for oncogenic non 16/18 HPV types, no significant associations were found between the age groups and in the number of HPV types detected [31]. On the other hand, we found a statistically significant difference of colposcopy results between patients positive for HPV 16/18 and non-16/18 oncogenic type. We also determined that HPV 16/18 and oncogenic types significantly differed in terms of endocervical curettage biopsy results. Clinicians should consider that the possibility of cervical intraepithelial neoplasia is 23.8% for LGSIL and 6.2% for HGSIL in colposcopic biopsy and 4.3 %

for LGSIL and 1.1% for HGSIL in endocervical curettage [31,32]. To reduce mortality and morbidity related to cervical cancer, more precise algorithms should be constituted [33].

The strengths of our study include the high number of patients and the fact that it was carried out in a tertiary center. However, there are also some limitations: We did not assess the risk factors thoroughly, such as smoking and multiple sexual partners, and long-term follow-up results were not evaluated.

### Conclusion

Direct referral of the patients could increase the number of the colposcopies performed. However, it is known that the half of the patients left to follow up would also require colposcopy eventually. Patients who are presumed to be lost to follow-up could be also directly referred for colposcopic biopsy. This approach would increase the benefit of the cervical cancer screening by reducing the number of patients lost to follow-up, HPV-related psychological burden, and advanced cervical cancer treatment costs.

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