



# Preinvasive lesion creation prevalence of other HPV types other than HPV type 16-18

## HPV tip 16-18 dışındaki diğer HPV tiplerinin preinvaziv lezyon oluşturma prevalansı

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### Abstract

**Objective:** This study aims to investigate the prevalence of preinvasive lesions in cytology cases of high-risk human papillomavirus (HR-HPV) types other than types 16-18.

**Materials and Methods:** A retrospective file scan of 342 patients with normal cytology was performed between January 2016 and April 2019 in our hospital's obstetrics and gynecology outpatient clinic. In the first group, with the exception of HR-HPV type positivity, normal cytology and preinvasive lesions were present as a result of biopsy. In the second group, women were HPV type 16-18 positive, had normal cervical cytology, and were found to have preinvasive lesions as a result of biopsy. At the end of the study, we calculated the percentages of HPV types seen in preinvasive lesions.

**Results:** Three hundred and forty-two patients with normal cytology were included in our study. The average age of women was  $41.09 \pm 10.61$ . In 58 (16.9%) patients with 342 HR-HPV type positivity, preinvasive lesions were detected as a result of biopsy. High-grade squamous intraepithelial lesion-low-grade squamous intraepithelial lesion was reported in 54 (15.7%) cases, squamous cell carcinoma in 3 (0.92%) cases, and mixed surface epithelial carcinoma (endometrioid adenocarcinoma 95%, clear cell carcinoma 5%) in 1 (0.3%) case. The age variable was not significant in biopsy subgroups ( $p > 0.05$ ).

**Conclusion:** Among the biopsy results with preinvasive lesions, approximately half were positive for HPV type 16 or 18, and these cases were identified accordingly. Colposcopy and biopsy should be recommended in suspicious lesions, even if cytology is normal, since other HR-HPV types may also have certain rates of preinvasive and invasive lesions.

**Keywords:** Cervical cancer, human papilloma virus, colposcopy

### Öz

**Amaç:** Bu çalışmanın amacı, 16-18 tipleri dışındaki diğer yüksek riskli insan papilloma virüsü (HR-HPV) tiplerinin pozitif olduğu sitoloji olgularında preinvaziv lezyonların prevalansını araştırmaktır.

**Gereç ve Yöntemler:** Ocak 2016-Nisan 2019 tarihleri arasında hastanemiz kadın hastalıkları ve doğum polikliniğinde normal sitolojiye sahip 342 hastanın dosyaları geriye dönük olarak incelendi. Birinci grupta; 16-18 dışındaki HR-HPV tipleri pozitif, sitolojisi normal, biyopsi sonucu preinvaziv lezyon saptanın hastalar yer aldı. İkinci grupta ise; HPV tip 16-18 pozitif, servikal sitolojisi normal, biyopsi sonucu preinvaziv lezyon saptanın hastalar yer aldı. Çalışma sonunda preinvaziv lezyonlarda görülen HPV tiplerinin yüzdesi hesaplandı.

**Bulgular:** Normal sitolojiye sahip 342 hasta çalışmaya dahil edildi. Kadınların ortalama yaşı  $41,09 \pm 10,61$  idi. HR-HPV pozitifliği olan 342 hastanın 58'inde (%16,9) biyopsi sonucunda preinvaziv lezyon saptandı. Olguların 54'ünde (%15,7) yüksek dereceli skuamöz intraepitelial lezyon-düşük dereceli skuamöz

**PRECIS:** This study highlights that high-risk HPV types other than 16 and 18 may also play a significant role in the development of preinvasive cervical lesions, emphasizing that focusing solely on these two types may be insufficient for early detection.

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intraepitelyal lezyon, 3'tünde (%0,92) skuamöz hücreli karsinom, 1'inde (%0,3) ise miiks yüzey epitelial karsinom (endometrioid adenokarsinom %95, berrak hücreli karsinom %5) rapor edildi. Yaş değişkeni biyopsi alt grupları arasında anlamlı bulunmadı ( $p>0,05$ ).

**Sonuç:** Biyopsi sonucunda preinvaziv lezyon saptanan olguların oranı HPV tip 16-18 pozitif olanlarda daha yüksek bulunmuş olup, preinvaziv lezyon saptananların yaklaşık yarısında bu tipler belirlenmiştir. Sitoloji normal olsa bile, diğer HR-HPV tiplerinin de belirli oranlarda preinvaziv ve invaziv lezyonlara yol açabildiği göz önüne alınarak, şüpheli olgularda kolposkopi ve biyopsi önerilmelidir.

**Anahtar Kelimeler:** servikal kanser, insan papilloma virüsü, kolposkopi

## Introduction

Zur Hausen, who found a 99.9% relationship between human papillomavirus (HPV) and cervical cancer, received the 2008 Nobel Prize in Physiology or Medicine. HPV can cause genital and laryngeal warts as well as preinvasive and invasive lesions<sup>(1)</sup>. In the 2014 guideline by the American Society for Colposcopy and Cervical Pathology, if the smear is normal and women test positive for HPV type 16 and/or 18, they undergo colposcopy. If the smear is normal and women aged 30 years or older, are positive for other HPV types, different follow-up is recommended. Cotest (smear + HPV) is recommended after 1 year. Today, as a result of research, HPV types that are defined as high-risk (HR)-HPV have been identified due to their association with cancer. HPV types with high oncogenic risk; 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. The HPV types most frequently detected in invasive cervical carcinoma in Türkiye are 16, 18, 45, 31, and 33.

In this study, we evaluated biopsies with preinvasive lesions and HPV types 16 and 18 positivity in the follow-up of patients who were found to be positive for HR-HPV types other than types 16 and 18 at the Gynecology and Obstetrics outpatient clinic of Kayseri Erciyes University Faculty of Medicine between 2016 and 2019. By comparing it, we aimed to discuss the necessity of colposcopy and biopsy.

## Materials and Methods

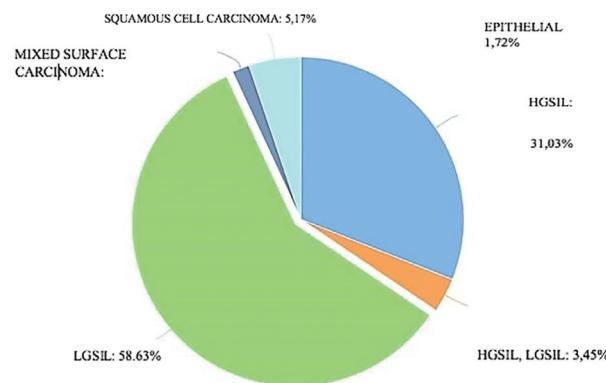
Our study was planned as a retrospective cohort study in the Gynecology and Obstetrics Clinic of Erciyes University Hospital. After obtaining ethical approval from the Clinical Research Ethics Committee of Erciyes University Hospital (approval number: 2019/430; date: 12.06.2019), 342 patients who applied to the gynecology outpatient clinic between January 2016 and April 2019 were examined. Cervical cytology was reported as normal in all of these patients. The common feature of these 342 patients was positivity for HR-HPV types other than HPV types 16 and 18. The control group consisted of patients with normal cervical cytology and positive results for HPV types 16-18 who were included in the study. All patients with normal cytology who tested positive for high-risk HPV types other than 16-18 between January 2016 and April 2019 at the Erciyes University Gynecology and Obstetrics Clinic were included. Patients who had abnormal cervical cytology, were positive for HPV types 6 or 11, or were under follow-up for premalignant or malignant cervical diseases were excluded from the study.

## Statistical Analysis

Patient data, pathological diagnoses, ages, and HPV types were recorded in a computer database and analyzed using the IBM Statistical Package for the Social Sciences, version 20 (SPSS Inc., Chicago, IL, USA). Mean  $\pm$  standard deviation values were reported for continuous variables and frequency (n) and percentage (%) for categorical variables. The normality of data distribution was assessed using histograms, Q-Q plots, and the Shapiro-Wilk test. Variance homogeneity was evaluated with Levene's test. For comparisons between groups, the independent samples t-test was applied to quantitative variables, and one-way analysis of variance was used for comparisons among more than two groups. The Pearson  $\chi^2$  test was used for the comparison of categorical data. A p-value of  $<0.05$  was considered statistically significant. The results of precancerous lesions were compared by calculating frequencies and percentages (n, %).

## Results

An abnormal cervical pathology was detected by biopsy in 58 (16.9%) of 342 patients who were HR-HPV-positive and had normal cytology. The ages of the patients included in the study ranged from 18 to 67 years, with a mean of  $41.09 \pm 10.61$  years. Among these 342 HR-HPV-positive patients, 54 (15.7%) had preinvasive lesions [high-grade squamous intraepithelial lesion (HGSIL) or low-grade squamous intraepithelial lesion (LGSIL)] according to biopsy results, 3 (0.92%) had squamous cell carcinoma (SCC), and 1 (0.3%) had mixed surface epithelial carcinoma (endometrioid adenocarcinoma 95%, clear cell carcinoma 5%) (Figure 1).

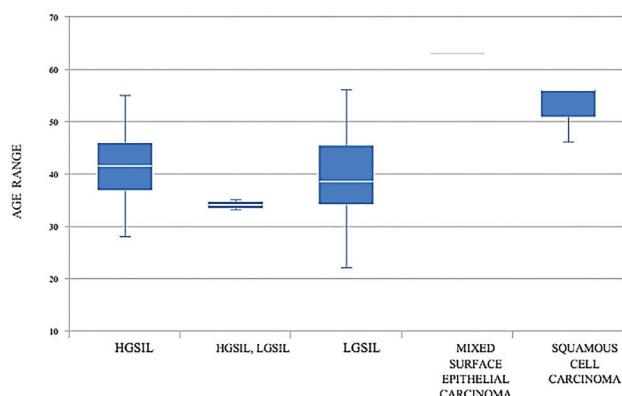


**Figure 1.** Distribution of abnormal bioscopy results in HR-HPV type positivities

HR-HPV: High-risk human papillomavirus, LGSIL: Low-grade squamous intraepithelial lesion, HGSIL: High-grade squamous intraepithelial lesion

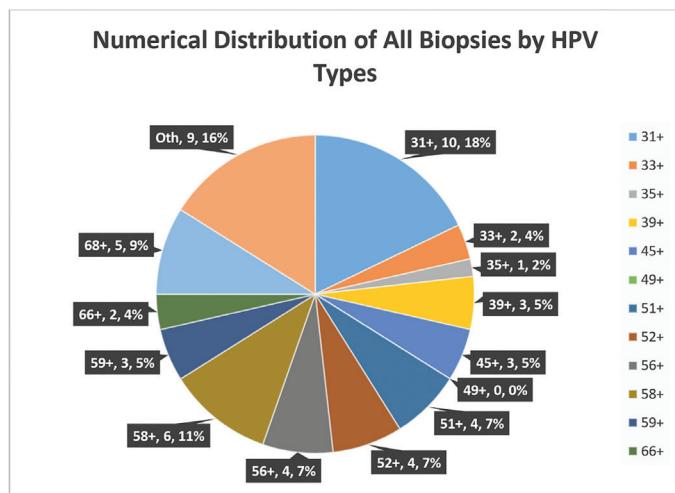
The mean age of patients with HGSIL as a result of biopsy was  $40.9 \pm 6.9$ , the mean age of patients with LGSIL was  $34 \pm 1.4$ , the mean age of patients with mixed surface epithelial carcinoma was  $63 \pm 2.3$ , and the mean age of patients with SCC was  $52.6 \pm 5.7$  (Figure 2).

The most common HPV type in patients with HGSIL was type 31 (27.7%) (Figure 3). The rates of other types were as follows: HPV 39 (11.1%); HPV 45, 51, 52, 56, 58, 59, and 66 (5.5%). Multiple HPV infections were detected as HPV 31+39 (5.5%), HPV 35+66 (5.5%), HPV 39+51 (5.5%), and HPV 51+58 (5.5%). In patients with LGSIL, the most common HPV types were HPV 58 (14.7%) and HPV 68 (14.7%), followed by HPV 31, 51, and 52 (8.8%), HPV 33, 45, 56, and 59 (5.8%), and HPV 35, 39, and 66 (2.9%). Among patients with SCC, HPV 31 (33.3% each), HPV 56 (33.3% each), and HPV 45 or 49 (33.3% each) were identified. In two cases positive for both HGSIL and LGSIL, HPV 31 (50%) and HPV 35 (50%) were detected.



**Figure 2.** Numerical distribution of HR-HPV type positions abnormal biopsy results according to age range

HR-HPV: High-risk human papillomavirus LGSIL: Low-grade squamous intraepithelial lesion, HGSIL: High-grade squamous intraepithelial lesion



**Figure 3.** The ratio of all other high risk human papillomavirus types in preinvasive lesions

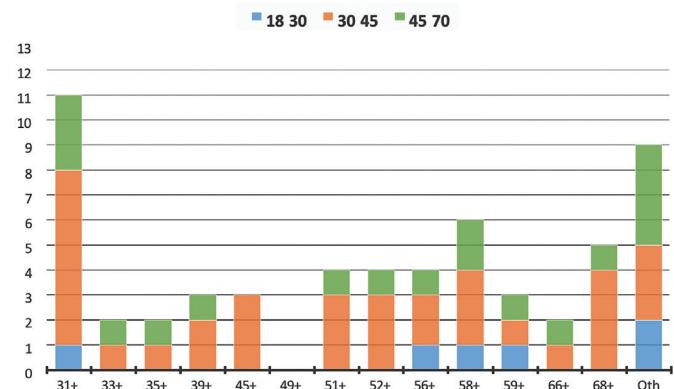
When all biopsy results were evaluated according to HPV type distribution, the most common types were HPV 31 (18%), HPV 58 (11%), HPV 68 (5.9%), HPV 51, 52, and 56 (4.7%), HPV 39, 45, and 59 (3.5%), HPV 33 and 66 (2.4%), HPV 35 (1.2%), and positivity for multiple other HPV types (9.16%).

HPV types 31 (17%), 56 (17%), 58 (17%), and 59 (17%) were the most common in the 18-30 age group. In the 30-45 age group, the most frequent types were HPV 31 (21%), HPV 68 (12%), HPV 45, 51, 52, and 58 (9%), HPV 39 and 56 (6%), and HPV 33, 35, 59, and 66 (3%). Among patients older than 45 years, HPV 31 (17%) and HPV 58 (11%) were the most common, followed by HPV 33, 35, 39, 51, 52, 56, 59, 66, and 68 (6%). Multiple HPV type infections were observed in 33% of patients aged 18-30, 9% of those aged 30-45, and 22% of those over 45 years (Figure 4).

The average age of patients with HPV type 16-18 positivity was found to be  $41.49 \pm 9.26$ . The average age of HR-HPV type positivity was  $41.09 \pm 10.61$  (Table 1). The age variable was not statistically significant in HPV subgroups ( $p > 0.05$ ). HPV type is 16-18 positive, the average age of patients with HGSIL after biopsy is  $42 \pm 7.85$ , the average age of patients with LGSIL is  $41.53 \pm 8.9$ , the average age of patients with SCC is  $46.12 \pm 7.12$ , the age of patients with adenocarcinoma the mean was found to be  $46 \pm 12.04$  (Table 2). Age variable was not significant in biopsy subgroups ( $p > 0.05$ ).

When the biopsies of 209 patients who were HPV type 16-18 positive were examined, 4 cases (1.91%) were diagnosed with adenocarcinoma, 40 cases (19.14%) with HGSIL, 30 cases

**Numerical Distribution of HPV Types by Age Range**



**Figure 4.** Numerical distribution of other high risk human papillomavirus types by age range

**Table 1.** Average age of HPV type 16-18 and HR-HPV types

HPV type	n	Mean
HPV type 16-18	209	$41.49 \pm 9.26$
Other HPV types	342	$41.09 \pm 10.61$

Data are expressed as  $\pm$  standard deviation. P-value was calculated using two independent-samples t-test, HPV: Human papillomavirus

(9.57%) with LGSIL, and 5 cases (2.39%) with SCC (Table 3). A statistically significant difference was found between the HPV type variable and the biopsy variable ( $p>0.05$ ). It was observed that the numbers of adenocarcinoma, HGSIL, and SCC were higher in the HPV type 16-18 group than in other HPV types. It was observed that the number of LGSIL in the HPV type 16/18 group was lower than the other HPV types.

## Discussion

Screening programs for cervical cancer are becoming increasingly common, as the slow natural progression of the disease allows for early recognition of dysplastic lesions and prevention of their progression to invasive cancer<sup>(2)</sup>. Cervical cancer typically begins with mild dysplasia and progresses toward invasive carcinoma. Cervical dysplasia generally occurs in women in their 20s, carcinoma in situ in their 30s, and invasive disease after the age of 40<sup>(3)</sup>. With the introduction of liquid-based cytology, the accuracy of screening tests has improved, leading to better detection and treatment outcomes. However, despite the increased sensitivity and specificity of these tests, no single screening method has yet proven to be completely reliable. In one study, the sensitivity of the Pap test alone for detecting CIN 2-3 or cancer was reported to be between 33% and 94%, with a specificity of 87-98%. When HPV DNA testing was added, sensitivity increased to 87-100%, while specificity ranged from 69% to 95%<sup>(4)</sup>. In a meta-analysis published by Arbyn et al.<sup>(5)</sup>, it was demonstrated that adding cytology to HPV DNA testing provides no additional diagnostic benefit. Accordingly, cytological examination does not hold significant value when colposcopy is performed in all HR patients identified by HPV genotyping. However, this approach leads to an increased number of colposcopies, biopsies, and pathological evaluations, which in turn raises the cost per patient for cervical cancer screening.

**Table 2.** Age average of patients according to HPV type 16-18 positive, smear negative preinvasive lesion types

Biopsy (n)	HGSIL	LGSIL	SCC	Adenocarcinoma
Age	42±7.85	41.53±8.9	46.12±7.12	46±12.04

Data are expressed as ± standard deviation. P-value was calculated using one-way analysis of variance, HPV: Human papillomavirus, LGSIL: Low-grade squamous intraepithelial lesion, HGSIL: High-grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma

According to the ATHENA study conducted by Wright et al.<sup>(6)</sup>, which included 42,209 participants over a three-year period in the United States, HPV DNA testing showed higher sensitivity than cytology or hybrid screening strategies for detecting CIN3+ lesions, particularly in women aged 25 years or older (28.3%) compared to those aged 30 years or older (24.3%). Studies have also shown that the risk of invasive cancer among HR-HPV carriers is significantly higher than in non-carriers. Similar results were observed in long-term follow-up studies of women enrolled in Kaiser Permanente cohorts in Portland, Oregon, and Northern California<sup>(7,8)</sup>. After five years of surveillance, the cumulative probability of CIN3 positivity was 0.17% [95% confidence interval (CI): 0.11-0.28] in HPV-negative women and 0.16% (95% CI: 0.06-0.39) in those negative for both cytology and HPV. Based on these findings and cost-effectiveness modeling analyses, both Australia and the Netherlands have adopted HPV testing as the primary screening method in their national cervical cancer prevention programs<sup>(9,10)</sup>.

In the POBASCAM study conducted by Rijkaart et al.<sup>(11)</sup> in the Netherlands between January 1999 and September 2002, 22,420 women underwent cervical cancer screening. Among 19,999 women screened within the study group, 724 were classified as cytology-negative but HPV DNA-positive. Among these women, 31 (4.28%) had CIN2 and 29 (4.0%) had CIN3 lesions. In our study, colposcopic evaluation and biopsy of 342 patients with normal cytology revealed preinvasive lesions (HGSIL or LGSIL) in 54 (16.6%) cases, SCC in 3 (0.92%) cases, and mixed surface epithelial carcinoma (endometrioid adenocarcinoma 95%, clear cell carcinoma 5%) in 1 (0.3%) case. The rates of LSIL and HSIL in our study were higher than those reported in the POBASCAM trial.

In a study of 7,747 patients with cervical intraepithelial neoplasia in China, Wenbo Long et al.<sup>(12)</sup>, reported that HPV type 16 was the most common carcinogenic subtype, followed by HPV 58 (15.2%) and HPV 33 (5.09%). In our study, HPV 58 was observed in 11% and HPV 33 in 4% of preinvasive lesions. Similarly, in a study by Chiang et al.<sup>(13)</sup> conducted in Taiwan involving 1,086 patients positive for HPV genotypes, HPV types 16 and 18 were detected in 21.3% of CIN2-3 cases among women over 50 years of age, while HPV 52, 58, and 33 were positive in 55.5% of cases.

In another study by Boumba et al.<sup>(14)</sup>, HPV 16 (47.1%), HPV 33 (22.6%), HPV 18 (15%), HPV 31 (11.3%), and HPV 69 (3.7%) were the most common types detected. In invasive cervical cancer, HPV 33 (28.8%), HPV 18 (11.8%), HPV 31 (5%), and HPV 35 (1.7%) were reported. Overall, HPV 33 and HPV 31 were found to be the most common types in HGSIL and invasive cervical cancer, excluding HPV 16 and 18.

A 13-year study conducted by Andrea Piana et al.<sup>(15)</sup> in Italy reported HPV 16 (49%) as the most common type in patients diagnosed with invasive neoplasia, followed by HPV 51 (19.4%) and other HR types (excluding HPV 16 and 18) collectively

**Table 3.** Distribution of preinvasive lesions in HPV type 16-18 types

Preinvasive lesion	Number	Percentage
Adenocarcinoma	4	%1.914
HGSIL	40	%19.14
LGSIL	20	%9.569
SCC	5	%2.392

Data are expressed as% n. Pearson  $\chi^2$  analysis was used for HPV and biopsy variable, HPV: Human papillomavirus, LGSIL: Low-grade squamous intraepithelial lesion, HGSIL: High-grade squamous intraepithelial lesion

with rates exceeding 20%. Matsumoto et al.<sup>(16)</sup> demonstrated that progression from LSIL to HSIL in women infected with HPV types 16, 18, 31, 33, 35, 52, and 58 occurred 3.5 times faster than in those with other HR types.

When considered in this context, cytological examination alone does not provide additional benefit if colposcopy is performed in all HR patients identified by HPV genotyping. However, this approach increases the number of colposcopies, biopsies, and histopathological examinations, consequently raising screening costs. To prevent missed preinvasive lesions and achieve early diagnosis, patient compliance with screening, diagnosis, and treatment should be improved. Therefore, colposcopy should be recommended for all patients positive for HR-HPV, despite the increased cost. Detection of low- and high-oncogenic-risk HPV DNA in cervical biopsy specimens is crucial for screening, early diagnosis, treatment planning, and follow-up in cervical cancer prevention. Based on the results of this study conducted at the Department of Obstetrics and Gynecology, Erciyes University Hospital, between January 2016 and April 2019, we believe that colposcopic biopsy in patients with HR-HPV positivity can significantly reduce the frequency of missed cervical lesions.

Despite ongoing research aimed at identifying the optimal global screening method, existing evidence indicates that HPV DNA testing is more effective than Pap smears, and the likelihood of missing preinvasive lesions decreases when a combined testing strategy is used. This study analyzed biopsy outcomes of patients with HR-HPV types excluding HPV 16 and 18, who were treated at the Obstetrics and Gynecology Outpatient Clinic of Kayseri Erciyes University Faculty of Medicine Hospital between January 2016 and April 2019. Similarly, Güzin et al.<sup>(17)</sup> reported that routine colposcopy in women with HR-HPV types other than 16 and 18 significantly increased the detection rates of CIN2+ lesions, even among those with normal cytology, emphasizing the importance of colposcopic evaluation in such cases.

## Conclusion

Our objective was to demonstrate that various HR-HPV types may also necessitate colposcopic biopsy for the early detection of cervical cancer. Consequently, our research has shown that colposcopic evaluation and biopsy may be essential in cases where other HR-HPV types yield positive results despite normal cytology findings. It should be considered that in situations where other HR-HPV types are positive and cytology is normal but colposcopy is not performed according to established guidelines, cervical preinvasive lesions or even neoplasms may still be present.

## Ethics

**Ethics Committee Approval:** Obtained ethical approval from the Clinical Research Ethics Committee of Erciyes University Hospital (approval number: 2019/430; date: 12.06.2019).

**Informed Consent:** Retrospective study.

**Acknowledgment:** This study is part of the MD thesis of Tugce Baykara.

## Footnotes

## Authorship Contributions

Surgical and Medical Practices: T.B., M.D., B.Ö., E.K., Concept: T.B., İ.S.S., B.Ö., E.K., Design: T.B., İ.S.S., B.Ö., E.K., Data Collection or Processing: T.B., M.D., B.Ö., E.K., Analysis or Interpretation: T.B., M.D., E.K., Literature Search: T.B., M.D., E.K., Writing: T.B., İ.S.S., E.K.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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