ORIGINAL ARTICLE
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Efficacy of Acetic Acid and Lugol's Iodine Assisted Colposcopic Imaging in Cases with Anormal Pap Smear Test Results

Anormal Pap Smear Testi Sonucu Olan Olgularda Asetik Asit ve Lugol İyot ile Kolposkopik Görüntülemenin Etkinliği

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ABSTRACT

Aim: This study was conducted to investigate the diagnostic efficacy of colposcopic imaging with Acetic Acid and Lugol's Iodine in cases with abnormal pap smear test results.

Material and Method: Hundred and five patients who presented to the Maltepe University Medical Faculty Hospital Gynecology Outpatient Clinic between 2017 and 2020 with abnormal pap smear results and whose biopsy was taken using colposcopic imaging were evaluated retrospectively. The study included patients with Pap smear results indicating atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL), or high grade squamous intraepithelial lesion (HSIL).

Result: 19% (n=20) of the patients with abnormal pap smear test results were diagnosed with HGSIL, 43.8% (n=46) were diagnosed with LGSIL, and 37.1% (n=39) were diagnosed with ASCUS. As a result of the biopsy performed on these patients, chronic cervicitis was diagnosed in 48.6%, CIN 1 in 28.6%, and CIN 2-3 in 22.9%. Initially, Acetic Acid was applied during colposcopy. In detecting premalignant cervical lesions, aceto-white areas were determined to have a sensitivity of 74.1%, a positive predictive value of 65.6%, a specificity of 58.8%, and a negative predictive value of 68.2%. Upon application of Lugol's Iodine, the sensitivity of areas with no uptake in detecting premalignant cervical lesions was 83.3%, while the positive predictive value was 61.6%, the specificity was 45.1%, and the negative predictive value was 71.9%.

Conclusion: We determined that colposcopic imaging with Acetic Acid is more accurate than Lugol's Iodine for detecting cervical premalignant lesions in patients with abnormal pap smear test results.

Keywords: Acetic acid, cervical cancer, colposcopy, hpv, lugol's iodine

ÖZ

Amaç: Bu çalışma anormal pap smear test sonucu olan olgularda asetik asit ve lugol iyot ile kolposkopik görüntülemenin tanısal etkinliğini araştırmak amaçlı yapılmıştır.

Gereç ve Yöntem: Maltepe Üniversitesi Tıp Fakültesi Hastanesi Jinekoloji Polikliniği'ne 2017-2020 yılları arasında başvuran hastalara yapılan pap smear testinde anormal sonuç saptanan ve kolposkopik görüntüleme yapılarak biyopsi alınan 105 hasta retrospektif olarak değerlendirildi. Pap smear test sonuçları; önemi belirlenemeyen atipik skuamoz hücreler (ASCUS), düşük dereceli skuamöz intraepitelyal lezyon (LSIL) ve yüksek dereceli skuamöz intraepitelyal lezyon (HSIL) olarak saptanan hastalar çalışmaya alındı.

Bulgular: Anormal pap smear test sonucu olan hastaların %19'u (n=20) HGSIL, %43.8'i (n=46) LGSIL ve %37.1'i (n=39) ASCUS tanısı aldı. Bu hastalara yapılan biyopsi sonucunda hastaların %48.6'sında kronik servisit, %28.6'sında CIN 1, %22.9'unda CIN 2-3 patolojik tanıları tespit edildi. Kolposkopik görüntüleme esnasında öncelikle asetik asit uygulaması yapıldı. Aseto-beyaz alanların premalign servikal lezyonları saptamadaki duyarlılığı %74.1, pozitif prediktif değeri %65.6, özgüllüğü %58.8, negatif prediktif değeri %68.2 olarak tespit edildi. Ardından yapılan lugol iyot uygulaması sonrası tutulum izlenmeyen alanların premalign servikal lezyonları saptamadaki duyarlılığı %83.3, pozitif prediktif değeri %61.6, özgüllüğü %45.1, negatif prediktif değeri %71.9 olarak bulundu.

Sonuç: Anormal pap smear testi sonucu olan hastalarda servikal premalign lezyonlarını saptamada asetik asit kullanarak yapılan kolposkopik görüntülemenin lugol iyota göre daha yüksek tanısal doğruluğa sahip olduğunu saptadık.

Anahtar Kelimeler: Asetik asit, lugol iyot, kolposkopi, serviks kanseri

INTRODUCTION

Cervical cancer, the fourth most prevalent cancer in women, is responsible for 8% of all cancer-related fatalities (1,2). In the development of invasive cervical cancer, a protracted phase of preinvasive disease is observed, characterized by the progression of precursor lesions from cellular atypia to varying degrees of cervical intraepithelial neoplasia (CIN).

Epidemiological studies have identified many risk factors for the development of CIN and cervical cancer. These include human papillomavirus (HPV), early sexual activity, multiparity, multiple sexual partners, long-term use of combined oral contraceptives, smoking, and low socioeconomic status. HPV types are highly associated with CIN and invasive cancer (3). CIN is the abnormal growth of cells on the surface of the cervix that may have the potential to cause malignancy. CIN, which refers to potential precancerous transformation of cervical cells, most commonly occurs at the squamocolumnar junction of the cervix. It is rated from 1 to 3.

According to the American Society of Colposcopy and Cervical Pathology (ASCCP) guidelines, the standard screening method for cervical cancer is the pap smear test. Screening programs for women should begin at the age of 21 and be repeated every three years. In women over the age of 30, screening strategies differ. According to the ASCCP guidelines, both pap smear test and HPV-DNA test (co-test) are recommended every 5 years after the age of 30 (4). With HPV-DNA testing, it is possible to detect high-risk HPV genotypes that can cause cervical cancer. The addition of HPV testing to screening programs after the age of 30 is due to the fact that, after this age, the likelihood of HPV infection clearance decreases and the persistence of HPV increases (5, 6).

The incidence of cervical cancer has decreased by more than 50 percent in the last 30 years as a result of the implementation of screening programs. This rate decreased from 14.8 per 100,000 women in 1975 to 6.7 per 100,000 women in 2011. The disease's mortality rate decreased from 5,5 per 100,000 women in 1975 to 2,3 per 100,000 women in 2011 (7). Studies indicate that 50% of women diagnosed with cervical cancer have never undergone a pap smear test, while 10% have not undergone a screening test within 5 years prior to diagnosis (8).

Pap smear test results are reported as normal, atypical squamous cells of uncertain significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), or high-grade squamous intraepithelial lesion (HSIL). While the regression rate of CIN 1 cases is 57%, this rate is around 32% in CIN 2-3 cases. The potential of CIN cases to progress to cervical cancer is the most important risk factor to be considered. In the absence of early detection, patients with low- and high-grade squamous intraepithelial lesions have an increased risk of progression to cervical

squamous cell carcinoma or invasive carcinoma. CIN 2 cases have a 5% progression rate to cervical cancer, while CIN 3 cases have a 13% progression rate (9). Colposcopic imaging is a procedure performed on women with abnormal pap smear test results to determine the biopsy site and improve diagnostic accuracy (10). Colposcopic imaging is required for patients over the age of 24, who have ASCUS and are infected with oncogenic type HPV virus, as well as women with HGSIL or ASCUS-H, regardless of age (4).

The application of Acetic Acid and Lugol's Iodine to the cervix during colposcopic imaging is crucial for detecting cervical lesions. Studies have shown that colposcopic imaging methods with Acetic Acid and Lugol's Iodine are more sensitive than pap smear tests (11). These applications, which provide easy results and do not require any laboratory support, are frequently used today. Acetic Acid and Lugol's Iodine are used in colposcopic imaging because of the color changes they create in abnormal tissues. While normal tissues are unaffected by the application of Acetic Acid, areas with an increased nuclear/cytoplasmic ratio, such as CIN, become white. Another solution utilized is Lugol's Iodine, which reacts with glycogen in normal squamous epithelium. This solution, which causes browning in normal tissue, cannot stain high-grade CIN lesions brown because they contain low amounts of glycogen and produce negative uptake regions (12). In this study, we aimed to demonstrate the diagnostic effectiveness of Acetic Acid and Lugol's Iodine application in colposcopic imaging.

MATERIAL AND METHOD

The study included 105 patients who underwent a pap smear test at the Gynecology Outpatient Clinics of Maltepe University Medical Faculty Hospital between 2017 and 2020 and whose abnormal cytology results necessitated a colposcopy biopsy. LSIL, HSIL and ASCUS were considered as a result of abnormal cytology.

From the patient files, the age, gradivy, parity, and cervical pathology results of the women were retrospectively analyzed and recorded. In addition, the results of these patients' colposcopic imaging, observation with Acetic Acid and Lugol's Iodine were recorded.

Colposcopic imaging in our clinic includes the use of Acetic Acid and Lugol's Iodine. Positive Acetic Acid uptake was determined by the presence of well-defined, dense, acetowhite lesions near the squacolumnar junction or cervical os one minute after Acetic Acid administration. Following the application of Lugol's Iodine solution, the presence of mustard or saffron yellow areas in the transformation zone was accepted as the evaluation criterion for the presence of Lugol's Iodine negative area (13). The sensitivity, specificity,

positive and negative predictive values of Acetic Acid and Lugol's Iodine applications used during colposcopic imaging were investigated according to biopsy results. The study was approved by the Ethics Committee of the University of Maltepe.

Statistical Method

In order to summarize the study's findings, descriptive statistics for continuous (numerical) variables were tabulated as median, minimum, and maximum, depending on the distribution. Numbers and percentages were used to summarize categorical variables.

Kappa test was used for negative compatibility of Acetic Acid and Lugol's Iodine with pathology. The specificity, sensitivity, negative and positive predictive values were computed based on the pathology and states of Acetic Acid and Lugol's Iodine.

Statistical analysis was carried out using the "Jamovi project (2022), Jamovi (Version 2.2.5.0) and JASP (Version 0.16.1) programs and the level of significance was taken into account as 0.05 (p-value).

RESULTS

The mean age of the 105 patients in the study was 39.1±9.4 years (range: 22-64), and 36.1% were childless. Twenty (19%) of the study's patients were diagnosed with HSIL, forty-six (43,8%) with LGSIL, and thirty-nine (37,1%) with ASCUS. While aceto-white uptake was observed in 61 patients (58.1%) when Acetic Acid was applied, no staining was observed in 73 patients (69.5%) when Lugol's Iodine was applied. According to the results of the colposcopic biopsy, 48.6% were reported to have chronic cervicitis, 28.6% CIN 1, and 22.9% CIN 2-3. (**Table 1**). In terms of age, gravida, and parity distribution, there was no statistically significant difference (p> 0.05) between patients diagnosed with chronic cervicitis, CIN 1, and CIN 2-3.

When the biopsy results were analyzed according to the Pap smear test results, 30% (n=9) of the cases with ASCUS were diagnosed as CIN 1 and 25% (n= 6) of them were diagnosed as CIN 2-3. While 53.3% (n=16) of LGSIL cases were diagnosed as CIN 1 and 41.7% (n=10) CIN 2-3, 16.7% (n=5) of HGSIL cases were diagnosed as CIN 1 and 33.3% (n=8) was diagnosed with CIN 2-3 (**Table 2**).

According to pathology results, the distribution of patients with Acetic Acid uğtake was similar (34.4%, 31.1%, and 34.4% for chronic cervicitis, CIN 1 and CIN 2-3, respectively). The rate of chronic cervicitis, CIN 1 and CIN 2-3 in patients without Lugol's Iodine uptake was calculated as 38.4%, 32.9% and 28.8% (**Table 3**). Statistically significant levels of concordance were observed between the pathology results and the presence of Acetic Acid uptake and the absence of Lugol's Iodine uptake (**Table 4**).

Table 1. Demographic and clinical characteristics

	Mean±SD / n (%)	Median (Min. - Max.)
Age	39.1±9.4	38.0 (22.0- 64.0)
Gravida (%)		
Nulligravida	38 (36.2)	
Primigravida and Multigravida	67 (63.8)	
Parity (%)		
Nulliparous	41 (39.0)	
Primiparous and Multiparous	64 (61.0)	
Pap smear test result (%)		
ASCUS	39 (37.1)	
LGSIL	46 (43.8)	
HGSIL	20 (19.0)	
Acetic Acid uptake (%)		
Yes	61 (58.1)	
None	44 (41.9)	
Lugol's Iodine uptake (%)		
Yes	32 (30.5)	
None	73 (69.5)	
Pathology (%)		
Chronic Cervicitis	51 (48.6)	
CIN 1	30 (28.6)	
CIN 2-3	24 (22.9)	

Table 2. Distribution of pathology results according to Pap smear test results.

	Pap smear test result (%)		
Pathology Result	**ASCUS (n=39)**	**LGSIL (n=46)**	**HGSIL (n=20)**
Chronic Cervicitis (n=51)	24 (47.1)	20 (39.2)	7 (13.7)
CIN 1 (n=30)	9 (30.0)	16 (53.3)	5 (16.7)
CIN 2-3 (n=24)	6 (25.0)	10 (41.7)	8 (33.3)

Table 3. Distribution of pathology results according to Acetic Acid and Lugol's Iodine application results.

	Acetic Acid uptake (+) (n=61)	Lugol's Iodine uptake (-) n=73
Chronic Cervicitis	21 (34.4)	28 (38.4)
CIN 1	19 (31.1)	24 (32.9)
CIN 2-3	21 (34.4)	21 (28.8)

Table 4. Diagnostic accuracy of Acetic Acid and lugol's iodine in the diagnosis of premalignant cervical lesions

	Sensitivity	Specificity	PPD	NPD	Kappa	p value
Acetic Acid Uptake (+)	74.1%	58.8%	65.6%	68.2%	0.117	0.001
Lugol's Iodine Uptake (-)	83.3%	45.1%	61.6%	71.9%	0.303	0.002

PPD. Positive predictive value, NPD. Negative predictive value

Table 4 summarizes the diagnostic accuracy of Acetic Acid and Lugol's Iodine in the differential diagnosis of cervical premalignant lesions (CIN 1, 2, and 3). The calculated sensitivity and specificity of Acetic Acid in predicting CIN lesions were 74.1% and 58.8%, respectively. The sensitivity of Lugol's Iodine in predicting CIN lesions was 83.3%, while the specificity was 45.5%.

DISCUSSION

Colposcopic imaging is based on the observation that malignant and premalignant epithelium exhibit distinct visual characteristics regarding contour, color, and vascularity. During colposcopic imaging, the transformation zone must be identified. In colposcopic imaging, a broad-band light spectrum is used for visual scanning. In imaging, solutions of Acetic Acid and Lugol's Iodine are used to enhance the detection of differences between normal and abnormal tissues. The most significant advantage of utilizing Acetic Acid and Lugol's Iodine is that they do not require any special procedures (11,13).

The application of Acetic Acid accelerates and reverses the coagulation of cellular proteins. It helps to coagulate and clear mucus secretions from the cervix by causing swelling of columnar and abnormal squamous epithelial areas and dehydration of cells in epithelial tissue. Normal squamous epithelium appears pink, while columnar epithelium appears red due to light reflected from the stroma. Acetic Acid coagulates the cellular proteins in the epithelium and causes the stroma to become discolored. Thus, a color change to aceto-white occurs. Consequently, the action of Acetic Acid is dependent on the quantity of cellular proteins present in the epithelium. Increased nuclear activity and DNA concentration are associated with the most prominent white color change while normal tissues are unaffected by the application of Acetic Acid. Areas with an increased nuclear/cytoplasmic ratio, such as CIN, become white (3).

All patients' cervixes were treated with 3% Acetic Acid in order to highlight suspicious and abnormal areas during colposcopic imaging. Acetic Acid application resulted in aceto-white areas in 41.2% of chronic cervicitis cases, 63.3% of CIN 1 cases, and 87.5% of CIN 2-3 cases (**Figure 1**). The group with CIN 2-3 had a significantly higher rate of Acetic Acid uptake than the group with chronic cervicitis and CIN 1. Similar to our study, another study carried out by Denny et al. determined that Acetic Acid inspection with sensitivity for CIN 2-3 lesions was 74% (14). In a study conducted by Bhattachan et al. in 2019, the sensitivity of observation with Acetic Acid during colposcopy imaging was found to be 80%, and the specificity was 88.5%, for the detection of cervical lesions. (15). Similar to other studies, the negative predictive value of Acetic Acid application was determined to be 68.2% (16, 17).

Lugol's Iodine solution was applied following the application of Acetic Acid. Iodine is glycophilic and iodine uptake occurs in epithelial cells containing glycogen after administration. Columnar epithelium lacks glycogen, while squamous metaplastic

epithelium contains abundant glycogen. It is found in very small amounts in CIN and invading cancer cells. As a result, after iodine application, squamous epithelium containing normal glycogen stains brown or black. Columnar epithelium is not stained because it does not retain iodine. Areas of immature squamous metaplastic epithelium may remain only partially iodine-stained. Iodine does not stain inflammatory areas of the squamous epithelium, so they appear colorless against the surrounding black or brown background. CIN and invasive cancer areas do not contain glycogen, so they do not retain iodine and appear as thick mustard-yellow or saffron-colored areas (3).
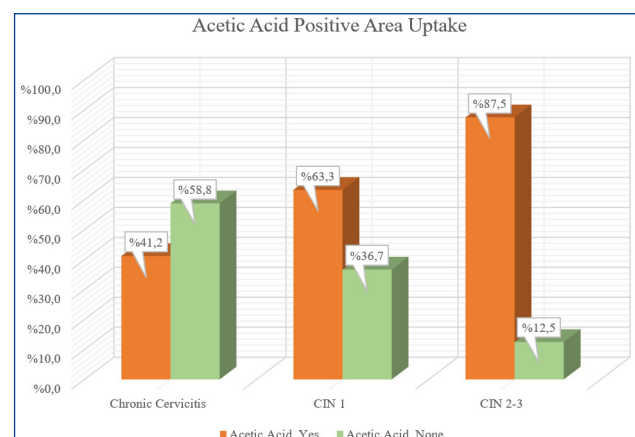

Figure 1. Acetic Acid Positive Area Uptake

The incidence of non-uptake areas after Lugol's iodine application was 54.9% in chronic cervicitis, 80% in CIN 1, and 87.5% in CIN 2-3 (**Figure 2**). Although the presence of Lugol's Iodine negative area in CIN 1 and CIN 2-3 cases was not found to be statistically significant, it was observed to be significantly increased compared to chronic cervicitis cases. While our study found the sensitivity of Lugol's Iodine application to be 84.5%, another study found it to be 92.2%, which is consistent with our findings (18).
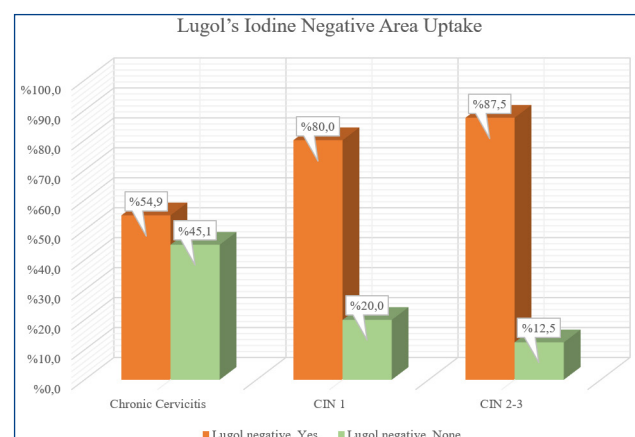

Figure 2. Lugol's Iodine Negative Area Uptake

In a meta-analysis of data from nineteen studies, the sensitivity and specificity for the diagnosis of CIN2+ with Lugol's Iodine were 88.1% (95% CI: 81.5-94.7%) and 85.9% (95% CI: 81.7-90.0%), respectively (19). Meanwhile, the use of different parameters as Acetic Acid uptake criteria can be attributed to the disparities in results in some studies (16).

In our study, 46 patients whose pap smear test results revealed LGSIL had colposcopic imaging and biopsy results reported CIN 2-3 in 21.7% of the cases. Therefore, based on the findings of the current study, we can conclude that colposcopic imaging is more effective than a pap smear test at identifying cervical lesions that are malignant and premalignant.

Studies have shown that an increased number of biopsies increases the diagnostic accuracy of cervical malignancies. During colposcopic imaging, the probability of high-grade lesions in random biopsies taken from nonsuspicious areas ranges from 13% to 37% (20). In our study, we preferred to obtain biopsies from aceto-white regions, Lugol's Iodine-negative regions, metaplastic regions, and suspicious regions. Four quadrant biopsies were taken from the squamocolumnar junction from all patients, even though colposcopic imaging did not reveal abnormal areas. Our study's most significant limitations are its small sample size and retrospective nature.

CONCLUSION

Acetic Acid and Lugol's Iodine applications in colposcopic imaging are effective applications that increase the success rate in the diagnosis of cervical neoplasia. In this study, we demonstrated that the use of Acetic Acid during colposcopic imaging for the detection of premalignant cervical lesions is more accurate than the use of Lugol's Iodine.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Maltepe University Medical Ethics Committee Reference Number: 2020/900/70.

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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