

# Acetic Acid and Lugol Tests in Colposcopic Assessment and Surgical Management of Cervical Intraepithelial Lesions

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*Colposcopy is used predominantly to evaluate women with abnormal Babes - Papanicolaou test results, to locate abnormal appearing epithelium and to direct biopsies as well as the surgical management, in areas in which cervical intraepithelial neoplasia is suspected. The purpose of our study is to analyze the value of acetic acid and Lugol tests in the colposcopic assessment and surgical management of cervical intraepithelial neoplasia, and to evaluate the patient outcome after uterine cervix surgery, by postoperative colposcopic determination of the disease-free resection margins.*

*Keywords: uterine cervix, lesions, assessment, surgery, follow-up*

Although the performance of exfoliative cytology has improved the ability to indirectly identify cervical neoplasia, it certainly has limitations, but the enhancements to the conventional Papanicolaou (Pap) smear, such thin-layer preparations and computerized Pap smear reading, resulted in improved outcomes of this process [1].

Colposcopy is used predominantly to evaluate women with abnormal Pap test results [2].

The main role of colposcopy is to locate abnormal appearing epithelium and to direct biopsies [3] as well as the surgical management, in areas in which cervical intraepithelial neoplasia (CIN) is suspected [3, 4].

Once the cervix is visualized clearly through the colposcope, attention is directed to identifying the squamocolumnar junction (SCJ) and the surrounding transformation zone [5].

It has been also traditionally stated that if the colposcopic examination is performed according to acceptable protocols and is guided by a colposcopic assessment method that grades colposcopic findings according to severity, an accurate histologic diagnosis can be obtained [3, 6, 7].

Application of acetic acid (3-5%) followed by the application of diluted Lugol's Iodine solution on the uterine cervix, are both essential steps of colposcopic examination.

Lugol's iodine, also known as aqueous iodine and strong iodine solution, is a solution of potassium iodide with iodine in water [8].

The most commonly used 15% solution consists of 5% (wt/v) iodine (I<sub>2</sub>) and 10% (wt/v) potassium iodide (KI) mixed in distilled water and has a total iodine content of 142.5 mg/mL (126.5 mg/g). The 15% solution thus has a total iodine content of 7.13 mg per drop of 0.05 mL, while the 2% solution has 0.95 mg total iodine content per drop. Potassium iodide renders the elementary iodine soluble in

water through the formation of the triiodide (I<sub>3</sub>) ion [8-10].

The purpose of our paper is to analyze the value of acetic acid and Lugol tests in the colposcopic assessment and surgical management of CIN, and to evaluate the patient outcome after uterine cervix surgery, by postoperative colposcopic determination of the disease-free resection margins in CIN.

## Experimental part

In this multicenter study (see affiliation of the authors) we included 74 women aged between 19-54 years, taking into account the following inclusion criteria: Pap test cytologic abnormality (table 1), apparently unexplained lower genital tract bleeding, associated vulvar or vaginal Human papillomavirus (HPV) associated lesion, erosion, ulceration or cervical tumor at the classic speculum gynecological examination, patient concern over partner with lower genital tract lesion or condyloma and postsurgical follow-up examination.

Colposcopic evaluation has been performed in all selected cases, using a videocolposcopic equipment with the facility to capture and store images in order to set up the database of the study (Alltion® AC-2000 series colposcope, with LED coaxial illuminator and brightness adjustment; light intensity > 25.000 LUX; magnification - 3.75x, 7.5x; 15x; 300mm focal length; field of view - 79mm, 39mm, 19mm; depth field - 4.5mm, 1.13mm, 0.76mm; user-selectable built-in green filter and integrated video camera).

Colposcopy has been performed only by trained and certified doctors to perform this maneuver, according to the legislation.

The colposcopic examination considered the following objectives in the examination protocol: to visualize the

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cervix, identify the SCJ and transformation zone, identify and assess the size, shape, contour, location and extent of the lesions, correlate the Pap test and colposcopic findings, and to develop the therapeutic management plan.

In order to have a correct cervical exposure at colposcopic examination, the widest speculum, which did not cause any discomfort of the patient has been used, to maximize visualization.

Colposcopic assessment started at low magnification (3.75x), followed by the next degree of magnification (7.5x), for detailed examination of the entire cervix and lesion, and for fine details of a certain lesion area we used the last degree of magnification (15x).

The first step in the assessment of cervical lesions has been the application of the normal saline solution, focusing on leukoplakia and abnormal blood vessels (fig. 1,2).



Fig.1. Low grade cervical lesion demonstrated at 8-12 o' clock after the application of normal saline. Note the indistinct borders. Magnification 15x.



Fig.2. Low grade cervical lesion demonstrated at 8-12 o' clock after the application of normal saline and green filter. Blood vessels appear dark, easy to discern from the green background. Magnification 15x

Normal saline (0.9% Sodium Chloride Solution) is a sterile, nonpyrogenic solution containing 9 g/L Sodium Chloride with an osmolarity of 308 mOsmol/L and also 154 mEq/ L Sodium and Chloride.

Further on, acetic acid (3-5%) has been appropriately applied to the cervix and left in contact with it, until the reaction has been maximally expressed (1-2 min), in order to help discriminate normal from abnormal cervical epithelium, resulting in acetowhiteness of the tissue (fig. 3).



Fig.3. Low grade cervical lesion. Colposcopy after the application of 3-5% acetic acid demonstrating map-like geographic borders and pale acetowhite color. Magnification 7.5x

The next step in the colposcopic examination was represented by the application of diluted Lugol's iodine solution (iodine solution diluted half-strength or quarter-strength) (fig. 4), checking that the patient is not allergic to iodine.



Fig.4. Low grade cervical lesion following application of Lugol's iodine solution, demonstrating an angular and geographic lesion. Magnification 7.5x

After completing colposcopy and recording patients in the database, 48 patients received the recommendation for ablative surgery.

The techniques used for ablation surgery were loop electrosurgical excision procedure (LEEP) and electrosurgical conization. LEEP consisted of a single excision pass to a depth of 5-8 mm, with the electrosurgical unit set on the cutting mode at 30-50 Watts, and the loop positioned 4-6 mm outside the lateral perimeter of the lesion.

Electrosurgical conization has been achieved using special sized and shaped electrodes, by single or multiple excision pass.

Both procedures were performed under colposcopic control, after the Lugol's solution has been applied in order to delimit the margins of the cervical lesion (fig. 5-7).



Fig.5. Large high grade cervical lesion. Colposcopy after the application of 3-5% acetic acid demonstrating dense opaque acetowhite epithelium. Magnification 7.5x.

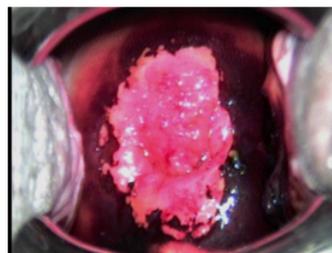


Fig.6. High grade cervical lesion following application of Lugol, mostly rejecting iodine solution. Magnification 7.5x.

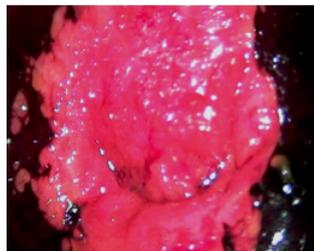


Fig.7. High grade cervical lesion following application of Lugol's iodine solution, demonstrating smooth and irregular margins. Magnification 15x

In cases where cervical surgery was performed, cytologic and colposcopic follow-up has been expected at 6 months and 1 year, according to the study protocol (fig. 8-10), (table 1).



Fig.8. Colposcopic reassessment at 12 months after electrosurgical conization, following the application of normal saline, demonstrating pink, stratified squamous epithelium of the cervix and clear mucus. Magnification 15x.



Fig.9. Colposcopic reassessment at 12 months after electrosurgical conization, following application of normal saline and green filter demonstrating the surgically cured cervix.



Fig.10. Colposcopic reassessment at 12 months after electrosurgical conization, following application of Lugol's iodine solution demonstrating a rich mahogany brown color and disease-free cervix. Magnification 15x

**Table 1**  
PATIENTS DISTRIBUTION ACCORDING TO THE INDICATION OF COLPOSCOPY AND POST-SURGICAL FOLLOW-UP

Colposcopy indication		N (%)
Pap test cytologic abnormality	ASC-US	11 (14.86)
	HR-HPV	33 (44.59)
	ASC-H	1 (1.35)
	LSIL	15 (20.27)
	HSIL	38 (51.35)
	AGC	3 (4.05)
Apparently unexplained lower genital tract bleeding		42 (56.75)
Associated vulvar or vaginal HPV associated lesion		7 (9.45)
Erosion, ulceration or cervical tumor at the classic speculum gynecological examination		19 (25.67)
Patient concern over partner with lower genital tract lesion or condyloma		5 (6.75)
Postsurgical follow-up examination	6 months	46 (95.83)
	1 year	31 (64.58)
N - number of cases; ASC-US - Atypical squamous cells of undetermined significance; HR-HPV - High risk HPV; ASC-H - Atypical squamous cells - cannot exclude HSIL; LSIL - Low-grade squamous intraepithelial lesion; HSIL - High-grade squamous intraepithelial lesion; AGC - Atypical glandular cells		

The research meets the conditions of the ethical guidelines and legal requirements and was approved by each Ethical Committee of the Universities of Medicine and Pharmacy (see authors' affiliations). Informed consent was obtained from every patient included in the study.

### Results and discussions

Of the 74 patients included in the study, 48 (64.86%) patients received indications for surgical treatment and 26 (35.13%) received conservative medical treatment. LEEP has been practiced in 32 (66.66%) cases, while electrosurgical conization has been performed in 16 (33.33%).

Residual disease has been found in 2 (4.16%) cases after surgery, both HR-HPV positive. Concordance between cytology, colposcopy and histology in selected cases was found in 71 (95.94%) cases. In all 3 cases where we found discordance between colposcopy and histology there was HR-HPV infection.

In terms of colposcopic examination, small abnormal vessels are best visualized after the application of normal saline and prior to acetic acid application [2].

A green filter assessment of the cervix (fig. 2), which absorbs red light, causing blood vessels to appear black, enhancing the angio-architecture and making them easier to discern against the green background, is often used during the saline examination of the cervix [2, 3].

Acetic acid interacts with both normal and neoplastic epithelia, causing them to swell and change color.

Acetic acid also causes tissue edema that exerts a mild, transient, vasoconstrictive effect on fine caliber blood vessels [2, 11].

Following acetic acid application, the columnar epithelium blanches slightly white and this effect is of short duration [1-3, 12].

Once the acetic acid is applied, both normal and abnormal tissues composed of cells with an increased nuclear-to-cytoplasmic ratio appear transiently white,

while normal squamous epithelium retains its usual pink color [2, 11-13].

Lugol's solution stains normal mature nonkeratinized squamous epithelium a dark, mahogany color, indicating that glycogen is present in the cells [3]. This color enhancement method is based on the colorimetric determination of glycogen, using iodine solution [14].

Thus, the absence of staining denotes a nonglycogenated state or keratinized surface [3].

Original squamous epithelium of post-pubertal women and areas of mature metaplasia are heavily glycogenated and will appear mahogany brown following the application of Lugol's solution, while neoplastic epithelium, normal columnar epithelium and leukoplakia contain little or no glycogen and therefore do not stain [2, 3, 13-17].

Also, squamous metaplasia may exhibit variegated staining, whereas columnar epithelium stains a mustard yellow color [3, 17].

Even if discomfort or isolated allergic reactions have been reported [18], the lugol staining test is easy to use, reproducible and safe [19].

Considering that iodine application prevents assessment of the underlying vasculature and other colposcopic signs, the assessment with 3-5% acetic acid must be done before applying iodine [2, 17, 20].

### Conclusions

Acetic acid and Lugol tests are essential stages of the colposcopic examination. Lugol test is a helpful way of assessing disease free margins in surgery for cervical intraepithelial lesions. Both the Lugol staining and acetic acid tests are easy to use, reproducible and safe. Significant concordance between cytology, colposcopy and histology in our study support that Lugol staining and acetic acid tests are reliable solutions in colposcopic assessment and surgical management of CIN. Uterine cervix surgery under colposcopic control, respecting modern protocols, is effective in the treatment of CIN and HPV induced lesions.

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