

Acetic Acid Test Performed by Vulvoscopy Had Revealed an "O Sign" in the Inner Vulvar Ring of Patients with Vulvodynia

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Abstract: This study examined the distribution and characteristics of acetowhite staining (AWS) in three vulvar rings in women with chronic vulvar discomfort. AWS was monitored as a secondary outcome measure in the study DATRIV (Diagnostic Accuracy of Three Rings Vulvoscopy). The study included 328 consecutive participants, divided into four groups based on their medical history, results from the vulvodynia questionnaire designed by the International Society for the Study of Vulvovaginal Disease, and clinical examination. Asymptomatic participants were classified into a normal vulva group (N = 82) and an impaired vulvar skin group (N = 82). Patients with chronic vulvar discomfort were divided into primary vulvar distress/vulvodynia (N = 82) and secondary discomfort caused by vulvar dermatosis (N = 82). The clinical data collected from the three rings vulvoscopy (TRIV) were analyzed using statistical software such as StatSoft (Dell, Austin, TX, USA), Statistica 12 (TIBCO®, Palo Alto, CA, USA), and SPSS 20 (IBM, Armonk, NY, USA). The study was conducted with the approval of the Institutional Review Board of Polyclinic Harni, and all participants provided written informed consent. The study's findings support the notion that AWS patterns can vary across different histological areas of the vulva and can indicate specific conditions. The higher incidence of AWS in the outer vulvar ring among patients with vulvar dermatosis (45.1%) suggests a widespread involvement in this area. In contrast, the study found that patients with vulvodynia (96.3%) and impaired vulvar skin (78.0%) exhibited a higher AWS frequency and a distinct pattern called the "O sign" in the inner vulvar ring. AWS in the middle vulvar ring was observed in all participants. However, there were noticeable differences between patients with vulvodynia and vulvar dermatosis, highlighting the potential diagnostic value of AWS distribution in this area. The faster onset of AWS in patients with vulvodynia (mean 51.6 seconds), a significantly higher frequency of sharp demarcation of AWS (49.4%), nontransparent AWS (16%), the "O sign" (81.7%) and provoked erythema (11.1%) supports the role of a potential inflammatory component in this condition in response to AWS. This specific pattern suggests that AWS combined with the TRIV approach can enhance the clinical recognizability of vulvodynia. The similarity in the distribution and characteristics of AWS in patients with vulvodynia and impaired vulvar skin suggests a potential connection between the two conditions and the progression or evolution of vulvodynia in the presence of impaired vulvar skin over time.

Keywords: Acetic Acid Test, Acetowhite Staining, O Sign, Three Rings Vulvoscopy, Chronic Vulvar Discomfort, Vulvodynia, Vulvar Dermatitis

1. Introduction

The acetic acid test (AAT) or acetowhite staining of the epithelium (AWS) is an inseparable component of colposcopy, which involves the application of a 3% to 5% solution of acetic acid to the cervix [1-3]. The hyperosmolar solution leads to dehydration of the epithelial cells and coagulation of the nuclear proteins and cytokeratin, resulting in the shrinking of cells and compaction of nuclei [4]. As a result, cellular proteins swell, and the epithelium loses transparency and becomes opaque, making abnormal areas where cell nuclei are enlarged and numerous more visible [5, 6]. The acetic acid solution causes the abnormal cells to turn white or "acetowhite" while healthy cells remain unchanged.

Differentiating between normal and abnormal AAT requires consideration of various criteria such as dynamics/onset (fast vs. slow formation and disappearance), density (thick and opaque vs. thin and translucent), and boundary (well and sharp vs. weak and not clear) of AWS in comparison to the surrounding normal epithelium. However, it's important to note that AWS is not specific to intraepithelial neoplasia and can be observed in other conditions with an increase in nuclear protein – immature squamous metaplasia, congenital transformation zone, epithelial regeneration associated with inflammation, leukoplakia with hyperkeratosis, and condylomas.

Visual examination of the cervix after applying acetic acid (VIA) without magnification has been shown to be more effective in identifying abnormal epithelium than colposcopy without AAT. VIA has demonstrated a sensitivity ranging from 82.14% to 85.29% compared to cytological findings and colposcopy. The specificity was 50.00% and 68.75%, the positive predictive value (PPV) was 67.64% and 85.29, and the negative predictive value (NPV) was 68.75% and 68.75%, respectively [7, 8]. It has been considered an acceptable screening method for cervical cancer and a practical and cost-effective approach for detecting high-grade cervical dysplasia [9].

However, it's important to note that AAT does not have sufficient sensitivity to detect cervical human papillomavirus (HPV) infection. The specificity of AAT in detecting HPV infection is approximately 90%, while the sensitivity is low at around 22%. Combining AAT with cytology does not significantly improve its diagnostic value in detecting HPV infection [10]. Overall, the acetic acid test is a valuable tool in colposcopy, aiding in identifying abnormal cervical epithelium. It is essential to cervical cancer screening and can provide helpful information for further diagnostic evaluations and management decisions.

The acetic acid test of the vulva presents particular challenges [11]. When 3% to 5% acetic acid is applied during vulvar colposcopy, dysplastic vulvar lesions that are not visible become apparent as acetowhite epithelium, thereby accentuating suspicious vulvar lesions. The cellular changes responsible for AWS are temporary, lasting

between 15 seconds to two minutes, and depend on the number of cells, nucleus size, and amount of cellular cytoplasm [12, 13]. It is important to note that this reaction is not specific, and the liberal use of acetic acid may lead to AWS unrelated to vulvar neoplasia [14]. Vulvar findings can be present in up to 50% of average women, and nonspecific positive AAT in the vulva is common and can be confusing [15].

Despite the widespread use of acetowhite changes on the vulva as an indication for vulvar biopsy, AAT of the vulva has high sensitivity (97%) but low specificity (40%) as a predictor of high-grade vulvar intraepithelial neoplasia. The high sensitivity of AAT on the vulva means that it effectively detects high-grade vulvar lesions when they are present. However, the low specificity indicates that it may produce false-positive results, leading to a higher rate of unnecessary biopsies or interventions [16]. The absence of acetowhite lesions on the vulva after performing AAT can provide reassurance that high-grade vulvar lesions are likely to be absent. That confirms AAT's high negative predictive value (98%) for ruling out high-grade vulvar lesions. However, it's important to note that a negative AAT result does not entirely rule out the presence of other types of vulvar abnormalities or lesions. Overall, while AAT can help identify the extent and location of lesions on the vulva and has a high sensitivity for detecting high-grade vulvar intraepithelial neoplasia, it should be used in conjunction with other diagnostic methods and clinical judgment to ensure accurate assessment and appropriate management of vulvar abnormalities [16].

Recent research has emphasized the study of vulvar conditions considering the complex anatomy, histology, and embryology of the vulva, which is organized into three rings [17]. The vulvoscopy index and the N-S-P scheme, which assesses the three rings vulvoscopy (TRIV) outcomes, have shown a sensitivity of 100%, specificity of 96.1%, and positive and negative predictive values of 0.88 and 1.00, respectively, in diagnosing vulvar dermatoses [18, 19]. TRIV has also demonstrated usefulness in studying primary and secondary chronic vulvar discomfort by distinguishing specific lesions from nonspecific ones concerning three vulvar rings. Nonspecific changes in the outer vulvar ring were more common in patients with vulvar dermatosis (70.7%). In comparison, a higher frequency of nonspecific findings in the inner vulvar ring was observed in patients with vulvodynia (98.8%) and impaired vulvar skin (96.3%) [20]. The inner vulvar ring in patients with vulvodynia showed a distinct profile with more frequent nonspecific findings, suggesting that vulvodynia is a clinically recognizable disease with a complex background. This complexity involves dysfunction of muscles, fascia, blood vessels, and nerve fibers, mediated by inflammatory cytokines. The study also highlights the potential association of vulvodynia with another chronic pain syndrome known as Complex Regional Pain Syndrome (CRPS) type I [20].

This study investigates AWS distribution and

characteristics in different histological areas of the vulvar rings in women with chronic vulvar distress. The objective is to obtain reference data for diagnostic purposes and better understand women with chronic vulvar discomfort and abnormal AAT in the vulva. By examining the AWS distribution and characteristics such as onset, dynamics, density, demarcation, density, and provoked erythema in different histological areas of the vulvar rings, the study aims to provide insights into the diagnostic process for women with chronic vulvar discomfort and abnormal AAT. Understanding these patterns and features can help healthcare professionals better comprehend and manage the condition, leading to improved diagnostic accuracy and appropriate treatment strategies.

It is important to note that this study is specific to the context of vulvar discomfort and vulvodynia, and its findings contribute to the existing knowledge in this field. Clinical evaluation and diagnosis of vulvar conditions should involve a comprehensive assessment, considering multiple factors such as symptoms, medical history, physical examination, and additional diagnostic tests, to ensure an accurate diagnosis and tailored management approach for individual patients.

2. Methods

2.1. Study Design

The distribution and characteristics of AWS concerning three vulvar rings were monitored as a secondary outcome measure in a prospective experimental study using diagnostic interventions called DATRIV study (Diagnostic Accuracy of Three Rings Vulvoscopy), which included a total of 328 consecutive participants [18, 19]. The study distinguished four groups of patients based on their history, results from the International Society for Study of Vulvovaginal Disease (ISSVD) Vulvodynia Pattern Questionnaire, and clinical examination [21].

According to a previous observational study, based on clinical examination, inspection, and cotton swab test, asymptomatic participants were divided into a normal vulva group (N = 82), showing no changes on the vulva, and an impaired vulvar skin group (N = 82), diagnosed with nonspecific changes on the vulva [17]. The definition of a normal vulva was taken from previous vulvoscopy classifications [22-24].

Patients with chronic vulvar discomfort were categorized into primary, idiopathic vulvar pain/vulvodynia (N=82) and those with secondary discomfort caused by vulvar dermatosis (N=82). According to the existing classification, vulvodynia was diagnosed according to Friedrich's criteria [25-27]. Therefore, the vulvar lesions found in patients with vulvodynia were irrelevant to the diagnosis.

An asymptomatic patient was randomly assigned to each symptomatic patient. Exclusion criteria were vulvar infection, benign tumors, pre-/malignancy, incomplete medical records, and protocol violation. This study was conducted at the

Polyclinic Harni in Zagreb, Croatia, between December 1, 2011, and December 31, 2016.

Diagnosing vulvar dermatosis involves identifying specific dermatological lesions with secondary morphological presentations [24, 28]. These specific lesions can aid in differentiating vulvar dermatosis from other conditions. Some examples of specific lesions associated with vulvar dermatosis include:

- 1) Red, flat, and diffuse lesions on the vulvar skin appear as areas of eczematous inflammation with thickened and excoriated skin. The red color and a flat, diffuse appearance characterize them.
- 2) Irregularly and extensively diffuse white plaques and patches on the skin and mucosa: These lesions present as hypopigmented or white areas on the vulvar skin. They can occur in irregular patterns and may extend to involve both the skin and mucosal surfaces.
- 3) White reticular pattern to extensive erosion, especially in the vestibule, refers to a pattern of white lines or reticulated patterns on the vulvar skin. In some cases, these areas may progress to extensive erosion, particularly in the vestibule area.
- 4) Erythematous papules with silver, scaly plaques, agglutination, and fusion: These lesions manifest as small, red papules on the vulvar skin. They may be accompanied by silver or scaly plaques and can exhibit agglutination (sticking together) and fusion of adjacent lesions.
- 5) Resorption of the labia minora and clitoral hood, loss of vulvar architecture, and sclerotic changes: In advanced cases, vulvar dermatosis may cause resorption or shrinking of the labia minora and clitoral hood. That can lead to a loss of typical vulvar architecture and the development of sclerotic (hardened) changes in the affected area [22, 23].

In addition to specific lesions, the presence and distribution of nonspecific lesions in the vulva are crucial for diagnosing and classifying vulvar dermatosis. Nonspecific lesions refer to various morphological changes and findings that are not specific to a particular condition but may indicate the presence of inflammation, infection, or viral pathology [23, 29]. These nonspecific lesions were evaluated according to their distribution across the three vulvar rings. The nonspecific lesions that were assessed in the study included:

- 1) Nonspecific erythema refers to redness in any part of the vulva that does not exhibit the characteristic morphological features of specific lesions. Nonspecific erythema can indicate inflammation or irritation in the vulvar area.
- 2) Punctuation and papillae are minor, raised bumps or projections on the vulvar skin. The punctuation and papillae may suggest inflammation or a response to an underlying pathology.
- 3) Pallor refers to areas of white discoloration or paleness on the vulvar skin or mucosa. Whiteness can be a nonspecific finding associated with various vulvar

conditions.

- 4) Smoothness refers to the absence of normal texture or irregularities on the vulvar skin. Smoothness may indicate a loss of typical architecture or changes in the surface of the vulvar tissues.
- 5) Fissures are small cracks or splits in the vulvar skin or mucosa. They can indicate dryness, irritation, or inflammation in the vulvar area.

These nonspecific lesions, although not specific to a particular condition, provide valuable information about the overall appearance and health of the vulva. Evaluating their presence and distribution across the three vulvar rings can aid in diagnosing, classifying, and managing vulvar dermatosis.

All four patient groups underwent TRIV, AAT on the vulva using a 5% acetic acid solution, and a vulvar biopsy with histopathology. TRIV was used to map the lesions precisely concerning three vulvar rings. When performing AAT on the vulva, the AWS was observed uniformly across the inner ring structures in some cases, creating a circular pattern that resembles the letter "O" (*Figure 1*).

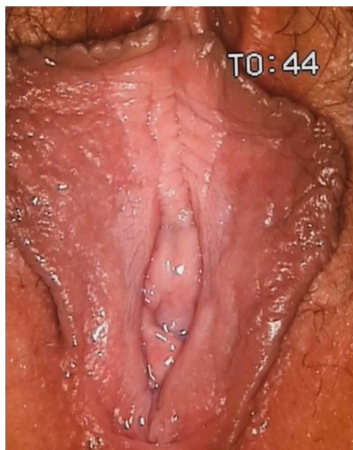


Figure 1. The term "O sign" denotes the acetowhitening of the epithelium in all structures of the inner vulvar ring.

The term "O sign" had been used to describe this specific extended pattern of the acetowhitening of the epithelium in all structures of the inner vulvar ring, forming a circular or "O" shape.

Additionally, the study looks after provoked erythema following the AAT, a phenomenon where the skin becomes visibly red and looks inflamed after exposure to specific triggers or stimuli (*Figure 2*).

The AAT reaction time was measured, and qualitative analysis of AWS was performed using video colposcope EDAN C6. Vulvar biopsies were conducted as part of routine clinical care. The clinical data and findings from TRIV, AAT, and vulvar biopsy were collected, organized, and analyzed in the study. Asymptomatic participants were recruited from women undergoing elective labiaplasty, and vulvar biopsy was performed on vulvar specimens approved for further investigation.

2.2. Data Analysis

Data collected from TRIV were analyzed using software packages, including StatSoft (Dell, Austin, TX, USA), Statistica 12 (TIBCO®, Palo Alto, CA, USA), and SPSS 20 (IBM, Armonk, NY, USA). Basic statistics were performed, including the calculation of mean (arithmetic mean, quartiles, and mode) and measures of dispersion (variance and standard deviation).



Figure 2. Provoked erythema with punctuation in the urethral sulcus and Hart's line following the acetic acid test.

In addition to descriptive statistics, various statistical tests were employed to analyze the data in the study. Parametric tests, such as t-tests, were used when the distribution of random variables was known, and nonparametric tests, such as the Mann-Whitney U test, were used when the distribution was unknown. Chi-square and Fisher's exact tests were used to analyze categorical data on a nominal or ordinal scale. These tests allowed for the comparison of distributions and the identification of significant differences between groups.

2.3. Ethical Approval

In the study, ethical considerations were taken into account to ensure the rights and well-being of the participants. Before their participation, all individuals were provided with comprehensive information about the study, including its purpose, procedures, potential risks and benefits, and their right to refuse participation. Written informed consent was obtained from the patients specifically for the vulvoscopy and vulvar biopsy procedures. Notably, no form of incentive or compensation was provided to the participants.

The research's study protocol and ethical aspects were reviewed and approved by the Polyclinic Harni Institutional Review Board, with the approval number 20111201001 issued on December 1, 2011. The study was also registered on ClinicalTrials.gov with the identifier NCT02732145, indicating its presence in the clinical trial registry.

3. Results

3.1. The Distribution of AWS According to the Three Vulvar Rings

Table 1. The prevalence of acetowhite staining (AWS) or positive acetic acid test (AAT) in patients with and without vulvar discomfort related to the vulvar rings (number and percentage).

Vulvar ring	Normal vulva (N=82)	Impaired vulvar skin (N=82)	Vulvodynia (N=82)	Vulvar dermatosis (N=82)
Positive AAT (AWS)	37 (45.1%)	74 (90.2%)**	81 (98.8%)**	57 (69.5%)
Outer vulvar ring (O)	0 (0%)	4 (4.9%)	12 (14.6%)	37 (45.1%)**
Middle vulvar ring (M)	23 (28.0%)	61 (74.4%)	75 (91.5%)**	48 (58.5%)
Inner vulvar ring (I)	27 (32.9%)	64 (78.0%)	79 (96.3%)**	32 (39.0%)
t-test proportion/p-value				
Middle vs. outer vulvar ring	p = 0.0000**	p = 0.0000**	p = 0.0000**	NS
Inner vs outer vulvar ring	p = 0.0000**	p = 0.0000**	p = 0.0000**	NS
Middle vs. inner vulvar ring	NS	NS	NS	p=0.0124*

*=p<0.05; **=p<0.001

The prevalence of AWS or positive AAT in patients with and without vulvar discomfort related to the vulvar rings has been presented in number and percentage in *Table 1* and *Figure 3*.

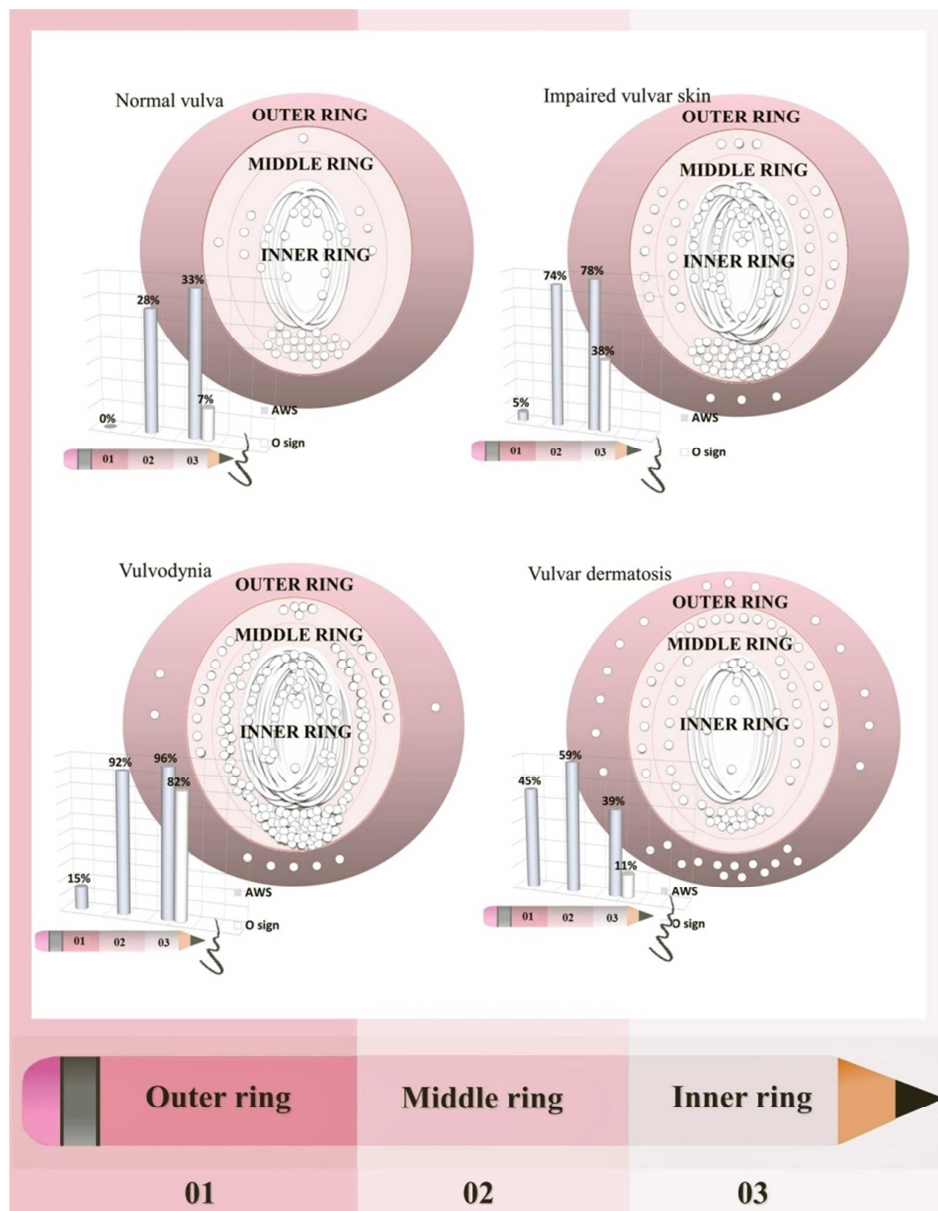


Figure 3. Distribution of acetowhite staining (AWS) and O sign in patients with and without vulvar discomfort related to the vulvar rings.

According to the table, the overall prevalence of AWS in patients with impaired vulvar skin (90.2%) and vulvodynia (98.8%) indicates that most patients with these conditions exhibited positive AWS during the examination.

In patients with vulvar dermatosis, positive AAT was significantly more common in the outer vulvar ring (45.1%) than in other groups. Additionally, AWS was more frequently observed in the middle vulvar ring compared to the inner vulvar ring in these patients, suggesting a specific pattern of AWS distribution in patients with vulvar dermatosis.

AWS was significantly more common in patients with vulvodynia in the middle vulvar ring (91.5%) and the inner vulvar ring (96.3%). Moreover, positive AAT was significantly more common in the middle and inner vulvar ring compared to the outer ring in patients with impaired vulvar skin and those with vulvodynia. These findings suggest a distinct distribution pattern of AWS and positive AAT in patients with vulvodynia, with a higher prevalence in

the middle and inner vulvar rings.

The distribution of AWS in the outer vulvar ring in patients with and without vulvar discomfort has been described in *Table 2*.

The prevalence of positive AAT in the outer vulvar ring was statistically significantly higher (45.1%) in patients with vulvar dermatosis than in other groups. *Table 2* indicates a higher frequency of AWS in the mons pubis (9.8%), labia majora (24.4%), and perineum (37.8%) in these patients.

A similar pattern of AWS was observed in the outer vulvar ring in all patients with chronic vulvar discomfort, including vulvodynia and vulvar dermatosis. Among these patients, increased AWS was found more frequently in the labia majora and perineum compared to the mons pubis.

Furthermore, participants with impaired vulvar skin exhibited a higher frequency of positive AAT in the perineum. Positive AAT in the perineum was significantly more frequent than positive AAT in the mons pubis and labia majora in participants with impaired vulvar skin.

Table 2. The distribution of acetowhite staining (AWS) or positive acetic acid test (AAT) in patients with and without vulvar discomfort in the outer vulvar ring (number and percentage).

Outer vulvar ring	Normal vulva (82)	Impaired vulvar skin (82)	Vulvodynia (82)	Vulvar dermatosis (82)
Positive AAT (AWS)	0 (0%)	4 (4,9%)	12 (14,6%)	37 (45,1%)**
Mons pubis	0 (0%)	0 (0%)	0 (0%)	8 (9,8%)**
Labia majora	0 (0%)	0 (0%)	4 (4,9%)	20 (24,4%)**
Perineum	0 (0%)	4 (4,9%)	10 (12,2%)	31 (37,8%)**
t-test proportion/p-value				
Labia majora vs. mons pubis	-	NS	p = 0,0429*	p = 0,0128*
Perineum vs. mons pubis	-	p = 0,0429*	p = 0,0011**	p = 0,0000**
Perineum vs. labia majora	-	p = 0,0429*	NS	NS

*=p<0.05; **=p<0.001

According to *Table 3*, patients with vulvodynia had a significantly higher prevalence of AWS in the middle vulvar ring (91.5%) compared to other groups. In patients with vulvodynia, positive AAT was significantly more common in the posterior commissure (87.8%) and labia minora (52.4%).

On the contrary, patients with vulvar dermatosis had a

statistically significant higher prevalence of AWS in the anterior commissure (18.3%).

Additionally, *Table 3* shows that the posterior commissure had a significantly higher frequency of positive AAT compared to other structures of the middle vulvar ring in all four groups of patients.

Table 3. The frequency of acetowhite staining (AWS) or positive acetic acid test (AAT) in patients with and without vulvar discomfort in the middle vulvar ring (number and percentage).

Middle vulvar ring	Normal vulva (82)	Impaired vulvar skin (82)	Vulvodynia (82)	Vulvar dermatosis (82)
Positive AAT (AWS)	23 (28,0%)	61 (74,4%)	75 (91,5%)**	48 (58,5%)
Anterior commissure	1 (0%)	2 (2,4%)	4 (4,9%)	15 (18,3%)**
Interlabial sulci	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Labia minora	6 (7,3%)	20 (24,4%)	43 (52,4%)**	27 (32,9%)
Posterior commissure	23 (28,0%)	53 (64,6%)	72 (87,8%)**	40 (48,8%)
t-test proportion/p-value				
Anterior commissure vs. interlabial sulci	NS	NS	p = 0,0429*	p = 0,0001**
Labia minora vs. anterior commissure	NS	p = 0,0000**	p = 0,0000**	p = 0,0318*
Labia minora vs. interlabial sulci	p = 0,0126*	p = 0,0000**	p = 0,0000**	p = 0,0000**
Posterior vs. anterior commissure	p = 0,0000**	p = 0,0000**	p = 0,0000**	p = 0,0000**
Posterior commissure vs. interlabial sulci	p = 0,0000**	p = 0,0000**	p = 0,0000**	p = 0,0000**
Posterior commissure vs. labia minora	p = 0,0005**	p = 0,0000**	p = 0,0000**	p = 0,0389*

*=p<0.05; **=p<0.001

Table 4. The prevalence of acetowhite staining (AWS) or positive acetic acid test (AAT) and the O sign in patients with and without vulvar discomfort in the inner vulvar ring (number and percentage).

Inner vulvar ring	Normal vulva (82)	Impaired vulvar skin (82)	Vulvodynia (82)	Vulvar dermatosis (82)
Positive AAT (AWS)	27 (32.9%)	64 (78.0%)	79 (96.3%)**	32 (39.0%)
Clitoris	8 (9.8%)	43 (52.4%)	76 (92.7%)**	34 (41.5%)
Hart's line	21 (25.6%)	68 (82.9%)	81 (98.8%)**	55 (67.1%)
Urethral sulcus	16 (19.5%)	69 (84.1%)	81 (98.8%)**	55 (67.1%)
Urethral meatus	7 (8.5%)	45 (54.9%)	72 (87.8%)**	43 (52.4%)
Hymenal remnants	6 (7.3%)	37 (45.1%)	69 (84.1%)**	30 (48.8%)
Bartholin's gland opening	8 (9.8%)	49 (59.8%)	77 (93.9%)**	33 (40.2%)
Vestibule	22 (26.8%)	59 (72.0%)	80 (97.6%)**	61 (74.4%)
O sign	6 (7.3%)	31 (37.8%)	67 (81.7%)**	9 (11.0%)
t-test proportion/p-value				
Urethral sulcus vs. clitoris	NS	p = 0.0287*	NS	NS
Urethral sulcus vs. urethral meatus	p = 0.0220*	NS	NS	NS
Urethral sulcus vs. Hart's line	p = 0.0220*	p = 0.0125*	NS	NS
Hart's line vs. clitoris	NS	p = 0.0191*	NS	NS
Hart's line vs. urethral meatus	NS	NS	p = 0.0148*	NS
Hart's line vs. hymenal remnants	NS	p = 0.0079**	p = 0.0085**	NS
Vestibule vs. clitoris	p = 0.0078**	p = 0.0287*	NS	NS
Vestibule vs. urethral meatus	p = 0.0016**	NS	NS	NS
Vestibule vs. Hart's line	p = 0.0016**	p = 0.0125*	NS	NS
Vestibule vs. Bartholin's gland opening	p = 0.0078**	NS	NS	p = 0.0360*

*= $p < 0.05$; **= $p < 0.001$

Table 4. provides insights into the prevalence and distribution of AWS in various structures of the inner vulvar ring among different patient groups.

Patients with vulvodynia had a significantly higher overall prevalence of AWS in the inner vulvar ring (96.3%) compared to other groups. AWS in all individual structures of the inner vulvar ring was significantly more frequent in patients with vulvodynia than in other participants. Patients with vulvodynia exhibited a substantially higher prevalence of the "O sign" (81.7%) compared to other groups. The "O sign" refers to the formation of AWS in all structures of the inner vulvar ring, creating a shape resembling the letter "O." In patients with vulvodynia, AWS of Hart's line had a significantly higher frequency than the urethral meatus and hymenal remnants.

In the normal vulva group, AWS was significantly more absent when comparing different structures, such as the urethral sulcus and urethral meatus, urethral sulcus and Hart's

line, vestibule with the clitoris, urethral meatus, Hart's line, and Bartholin's gland opening.

Participants with impaired vulvar skin had a significantly higher frequency of AWS in the urethral sulcus compared to the clitoris and Hart's line. AWS significantly differed between Hart's line and clitoris, vestibule and hymenal remnants, and between the vestibule and clitoris in patients with impaired vulvar skin.

AWS was significantly more frequently observed in the vestibule than in Bartholin's gland opening in patients with vulvar dermatosis.

3.2. Dynamics / Onset of AWS in Patients with Positive AAT

Table 5 provides information on the velocity of AWS onset in participants with positive AAT. The measurements were conducted for 180 seconds in each participant.

Table 5. The acetowhite staining (AWS) onset velocity in participants with positive acetic acid test (AAT).

Participants	Normal vulva (82)	Impaired vulvar skin (82)	Vulvodynia (82)	Vulvar dermatosis (82)
Positive AAT (AWS)	37 (45.1%)	74 (90.2%)**	81 (98.8%)**	57 (69.5%)
Mean (sec)	69.2	54.9	51.6*	65.9
SD (sec)	24.3	21.5	21.2**	34.7
Median (sec)	64.5	51.5	49**	58
Range (sec)	30-120	21-131	16-120**	18-184
Mann-Whitney U test				
Vulvodynia vs. vulvar dermatosis	p = 0.0231**			
Vulvodynia vs. normal vulva	p = 0.0000**			
Impaired vulvar skin vs. normal vulva	p = 0.0006**			

*= $p < 0.05$; **= $p < 0.001$

In the normal vulva group, 45.1% of the participants had a positive AAT. The average onset time for AWS in this group was 69.2 seconds (mean), and the median onset time was 64.5 seconds.

Participants with impaired vulvar skin had a higher prevalence of positive AAT (90.2%) than the normal vulva group. The average onset time for AWS in this group was significantly faster, with a mean of 54.9 seconds, and the

median onset time was 51.5 seconds.

Among patients with vulvodynia, the prevalence of positive AAT was 98.8%. The average onset time for AWS in patients with vulvodynia was significantly faster, with a mean of 51.6 seconds, and the median onset time was 49

seconds.

The vulvar dermatosis group had the slowest AWS onset compared to the other groups. This group's average AWS onset time was 65.9 seconds (mean), and the median was 58 seconds.

Table 6. Quality parameter of acetowhite staining (AWS) in patients with positive acetic acid test (AAT).

Participants	Normal vulva (82)	Impaired vulvar skin (82)	Vulvodynia (82)	Vulvar dermatosis (82)
Positive AAT (AWS)	37 (45.1%)	74 (90.2%)**	81 (98.8%)**	57 (69.5%)
Demarcation				
Well and sharp	6 (16.2%)	23 (31.1%)	40 (49.4%)*	21 (36.8%)
Weak and not clear	31 (83.8%)	51 (68.9%)	41 (50.6%)	36 (63.2%)
Density				
Semitransparent AWS	37 (100%)	71 (95.9%)	68 (84.0%)	53 (93.0%)
Nontransparent AWS	0 (0%)	3 (4.1%)	13 (16.0%)*	4 (7.0%)
Provoked erythema	0 (0%)	2 (2.7%)	9 (11.1%)*	0 (0%)

*=p<0.05; **=p<0.001

3.3. Qualitative Characteristics of AWS

Based on the data from *Table 6*, the following observations can be made regarding demarcation, density and occurrence of provoked erythema after the AAT:

Demarcation of AWS: Over half of the patients in each group had a weak and unclear demarcation of AWS. However, patients with vulvodynia had a significantly higher frequency (49.4%) of well-defined and sharp boundaries of AWS.

The density of AWS: Semitransparent and nontransparent AWS patterns varied among the patient groups. The majority of patients across all groups demonstrated semitransparent AWS. The highest percentage of semitransparent AWS was observed in the normal vulva group (100%), followed by the impaired vulvar skin group (95.9%), the vulvar dermatosis group (93.0%), and the vulvodynia group (84.0%). Nontransparent AWS, indicating a more robust grade of AAT, was significantly more common in patients with vulvodynia, with an occurrence of 11.1%.

Provoked erythema: Provoked erythema which refers to redness-like inflammation following AWS, was observed in participants with impaired vulvar skin (2.7%) and patients with vulvodynia (11.1%). The occurrence of provoked erythema was significantly more common in these two groups.

4. Discussion

Vulvoscopy or colposcopy of the vulva and the colposcopic examination of the cervix are two distinct procedures that require separate considerations. While they share similarities in the use of magnification and the application of acetic acid, there are notable differences in vascular patterns, lesion appearance, and the response to acetic acid application between the vulva and cervix. The vulva has a unique vascular anatomy and a different distribution of blood vessels compared to the cervix. Therefore, the vascular patterns observed during vulvoscopy or colposcopy of the vulva may differ from those seen during

the examination of the cervix. Lesion appearance can also vary between the vulva and cervix. Different types of lesions, such as specific dermatological lesions or other vulvar conditions, may present distinct morphological characteristics on the vulva. These variations in lesion appearance necessitate particular expertise in evaluating and interpreting vulvar lesions [12, 15, 31].

Furthermore, the response of tissues to acetic acid application may differ between the vulva and cervix. The AAT is commonly used in both vulvoscopy and colposcopy to enhance the visibility of abnormal cells or lesions. However, the interpretation of the AAT response in the vulva may differ from that in the cervix due to tissue composition and reaction variations. Considering these dissimilarities, it is essential to recognize that vulvoscopy or colposcopy of the vulva should be treated as a complementary tool rather than a direct equivalent to examining the cervix. By understanding the unique characteristics and responses of the vulva, healthcare professionals can enhance their ability to study and diagnose vulvar conditions effectively [15].

Studies have shown that AWS of the vulva can be observed in healthy individuals without any vulvar complaints. This finding emphasizes the lack of specificity of AWS as a diagnostic marker. It highlights the importance of considering additional clinical factors when interpreting vulvoscopy or colposcopy findings in the vulvar region. Acetowhite changes can occur due to various factors, including physiological variations, normal epithelial variations, or benign conditions. Therefore, AWS alone may not be sufficient to diagnose a pathological condition or disease definitively. It is crucial to consider the overall clinical picture, including patient symptoms, medical history, and additional diagnostic tests. Using AWS as a diagnostic tool may lead to misleading results and unnecessary interventions. Comprehensive evaluations considering the individual patient's characteristics and context are necessary to avoid overdiagnosis or misinterpreting findings [15, 29, 31].

Acetowhitening of the cervix and vulva has been found to have low sensitivity as a predictor of human papillomavirus

(HPV) infection compared to more specific diagnostic methods such as polymerase chain reaction (PCR). While acetowhite changes may indicate cellular changes associated with HPV infection, it is not a definitive or specific indicator of HPV presence. Several factors can influence the appearance of acetowhitening, including the type and concentration of HPV and the host immune response. Therefore, relying solely on acetowhitening as a diagnostic tool for HPV infection may lead to false-negative results and missed cases of the disease. It is essential to consider multiple diagnostic methods and clinical factors when evaluating HPV infection and its associated risks to make informed decisions about patient management and follow-up [10].

When it comes to high-grade vulvar dysplasia, the use of acetic acid during vulvoscopy has shown high sensitivity (97%), which means that it effectively detects most cases of high-grade lesions. However, it has low specificity (40%), which may produce false-positive results, identifying lesions that are not genuinely high-grade dysplasia. The absence of an acetowhite lesion can provide some reassurance that a high-grade vulvar lesion is not present, as indicated by a high negative predictive value (98%). However, the PPV of AAT is low (37%), meaning that a positive finding of an acetowhite lesion does not definitively indicate the presence of high-grade dysplasia [16].

To obtain a definitive diagnosis, histopathological examination of tissue samples through biopsy is often necessary. A comprehensive approach that combines AAT with other diagnostic methods, such as colposcopy and histopathological analysis, is recommended to improve accuracy in diagnosing high-grade vulvar dysplasia [10-12, 15, 17].

The systematic assessment of AWS in the vulva, considering its presence, localization, and recurrence patterns across the three vulvar rings, was undertaken to overcome the limitations of previous studies that lacked a comprehensive examination of AWS. This approach allows for a more thorough understanding of the distribution and characteristics of AWS in different areas of the vulva. By examining the three vulvar rings separately, researchers can identify specific patterns and variations in the occurrence of AWS within each ring, aiding in the differentiation and diagnosis of a vulvar condition.

The study found that the overall prevalence of AWS was significantly higher in patients with impaired vulvar skin (90.2%) and vulvodynia (98.2%) compared to healthy individuals without vulvar discomfort and vulvar dermatosis. That indicates that AWS is more commonly observed in these patient groups, suggesting a potential association between AWS and vulvar pathology.

Furthermore, the differential distribution of AWS and positive AAT across the three vulvar rings in patients with vulvar dermatosis and vulvodynia provides crucial diagnostic information. The study highlights that patients with chronic vulvar discomfort, including vulvodynia, exhibit a higher frequency of AWS in the labia majora and perineum

compared to the mons pubis. That suggests that the outer vulvar ring, particularly the labia majora and perineum, is more affected by AWS in these patients. In addition, participants with impaired vulvar skin demonstrated a higher frequency of positive AAT in the perineum, indicating that the perineum is mainly affected by AWS in patients with impaired vulvar skin.

The overall prevalence of AWS in the middle vulvar ring was significantly higher (91.5%) in patients with vulvodynia compared to other groups. Patients with vulvodynia exhibited a significantly higher frequency of positive AAT in the posterior commissure (87.8%) and labia minora (52.4%).

In contrast, patients with vulvar dermatosis had a statistically significant higher frequency of AWS in the anterior commissure (18.3%).

The distribution of AWS in the posterior commissure compared to other structures of the middle vulvar ring was significantly more common in all four groups of patients. That suggests that the posterior commissure may be more affected by positive AAT across various vulvar conditions.

The study revealed that patients with vulvodynia had a significantly higher overall prevalence of AWS in the inner vulvar ring (96.3%) than other groups, indicating a strong association between AWS and vulvodynia in this region. AWS in all individual structures of the inner vulvar ring, such as the urethral sulcus, Hart's line, vestibule, clitoris and hymenal remnants, were significantly more frequent in patients with vulvodynia than in other participants.

In the normal vulva group, AWS was significantly less present when comparing different structures within the inner vulvar ring. That means that AWS was less commonly observed in combinations such as the urethral sulcus and urethral meatus, urethral sulcus and Hart's line, and vestibule with the clitoris, urethral meatus, Hart's line, and Bartholin's gland opening. Participants with impaired vulvar skin had a significantly higher frequency of AWS in the urethral sulcus compared to the clitoris and Hart's line. Significant differences in AWS were also observed between Hart's line and clitoris, vestibule and hymenal remnants, and between the vestibule and clitoris. These findings highlight the specific distribution patterns and differences in the occurrence of AWS within the inner vulvar ring in patients with vulvodynia, impaired vulvar skin, and normal vulva. They provide valuable insights into the involvement of different structures in these conditions and may have diagnostic and management implications.

The presence of the "O sign" observed during AAT by vulvoscopy in the inner vulvar ring of patients with vulvodynia indicates a uniform appearance of AWS across the structures of the inner ring, creating a circular pattern resembling the letter "O." This sign can be an applicable descriptive term to communicate and document findings during vulvoscopy, aiding in the diagnosis and management of vulvar abnormalities.

However, it's important to note that the O sign is not pathognomonic for any vulvar pathology. While it may suggest abnormal changes in the vulvar tissue, such as

inflammation, dysplasia, or other vulvar conditions, further evaluation is needed to determine the underlying cause. Histological examination through biopsy remains the gold standard for definitive diagnosis. Therefore, it is essential to interpret the presence of the "O sign" in the context of the patient's clinical presentation, medical history, and other diagnostic information. Considering additional factors and conducting a comprehensive evaluation will help arrive at an accurate diagnosis and guide appropriate treatment planning for vulvar abnormalities.

The analysis of AWS's dynamics and onset time in different patient groups demonstrates that patients with vulvodynia and impaired vulvar skin experience a significantly faster onset of AWS compared to the normal vulva group and the group with vulvar dermatosis. On the other hand, the group with vulvar dermatosis exhibited a slower AWS onset compared to the other groups. This information highlights the differences in the timing of AWS appearance among these patient groups and can contribute to understanding the underlying pathophysiology of these conditions.

The study findings demonstrate that the demarcation and characteristics of acetowhite staining (AWS) varied among the patient groups. In general, over half of the patients in each group had a weak or unclear demarcation of AWS. However, patients with vulvodynia had a significantly higher frequency of well-defined and sharp boundaries of AWS, with 49.4% exhibiting this characteristic. That suggests that the appearance and delineation of AWS may be more pronounced and distinct in patients with vulvodynia compared to other groups.

The pattern of semitransparent and nontransparent AWS provides additional insights into the nature of epithelial changes in different groups. The majority of patients in all groups exhibit semitransparent AWS, suggesting a commonality in the appearance of the staining. However, the presence of nontransparent AWS, which indicates a more substantial or different type of epithelial change, was significantly more common in patients with vulvodynia. This finding suggests that vulvodynia may involve distinct pathophysiological mechanisms or epithelial alterations compared to other conditions.

The observation of provoked erythema after AWS in patients with damaged vulvar skin (2.7%) and vulvodynia (11.1%) supports the role of an inflammatory component in these conditions in response to AWS, which may contribute to the overall symptomatology occurring in these groups of patients.

The similarity in the distribution and characteristics of AAT in patients with vulvodynia and impaired vulvar skin suggests a potential connection between the two conditions and the possible progression or evolution of vulvodynia in the presence of impaired vulvar skin. This observation aligns with the previously described similarities in the distribution of nonspecific vulvar lesions [21].

The findings highlight the importance of close monitoring and evaluating these visual markers, including nonspecific

vulvar findings and positive AAT, as potential indicators of symptom progression in healthy women with compromised vulvar skin. By recognizing these visible markers and their possible association with the development or worsening of vulvodynia, healthcare providers can intervene early and implement appropriate preventive measures. Regular monitoring and evaluation of vulvar health and proper vulvar care can potentially help prevent or minimize the progression of vulvodynia in individuals with compromised vulvar skin. That emphasizes the importance of comprehensive care and proactive management strategies to address vulvar conditions and promote overall vulvar health [32].

The analysis of AWS in relation to the three vulvar rings highlights the significance of considering the intricate anatomy, histology, and embryology of the vulva when investigating vulvar lesions. This comprehensive approach acknowledges that the vulva comprises distinct structures with unique characteristics, and each structure may exhibit different patterns of AWS.

By conducting a systematic and detailed examination of AWS and AAT, the study aims to enhance the accuracy and effectiveness of vulvar diagnostics. The specific patterns of AWS observed in different vulvar structures and rings offer valuable insights for diagnosing and managing various vulvar conditions, such as vulvar dermatosis and vulvodynia.

Understanding the distribution and characteristics of AWS concerning the vulvar anatomy can assist in identifying specific lesions, determining their potential implications, and guiding appropriate treatment strategies. This comprehensive examination approach contributes to a better understanding of vulvar complaints and associated lesions, ultimately leading to improved patient care and outcomes.

The variability observed in the appearance and characteristics of AWS among different patient groups, particularly in vulvodynia and impaired vulvar skin, underscores the importance of evaluating both specific dermatological lesions and nonspecific findings in the vulva. This comprehensive assessment aids in the understanding and diagnosis of vulvar conditions.

In light of these observations, the question of appropriate vulvar care for asymptomatic patients with nonspecific vulvar findings and positive AWS becomes relevant. Early recognition of nonspecific vulvar findings and positive AWS in asymptomatic individuals may provide an opportunity for intervention and preventive measures. Implementing proper vulvar care practices, such as maintaining good hygiene, using suitable skin care products, and minimizing potential irritants, may help prevent the development or progression of vulvodynia in these individuals. However, it's important to note that further research is needed to determine the effectiveness of these preventive measures and their impact on the development of vulvodynia.

Further research is needed to address factors that can enhance AAT's understanding and clinical applicability with TRIV in assessing and managing patients with chronic vulvar discomfort. Validating and expanding upon the presented results through uncontrolled conditions is necessary to

determine their real-world applicability. Additionally, evaluating intra- and interobserver variability is important to ensure reliable and consistent examination results.

Moreover, investigating the impact of education and training on the implementation and duration of vulvar examination is crucial. Adequate training of healthcare providers in performing vulvoscopy is necessary for accurate interpretation of findings and reliable diagnosis. Assessing the educational aspect can optimize the implementation of vulvoscopy in clinical practice. Further research is warranted to explore these aspects and develop evidence-based strategies for managing and preventing vulvodynia.

5. Conclusion

The evaluation of AAT results, the distribution and characteristics of AWS across three vulvar rings in patients with chronic vulvar discomfort revealed characteristic and recognizable patterns of appearance in individuals with vulvar dermatoses and vulvodynia. These findings suggest specific patterns indicative of these conditions and can aid their diagnosis and differentiation.

This study found that patients with vulvar dermatosis exhibited a higher incidence of AWS in the outer vulvar ring, suggesting widespread involvement. On the contrary, patients with vulvodynia and impaired vulvar skin showed a higher frequency of nonspecific findings and the "O sign" in the inner vulvar ring, indicating a distinct and characteristic pattern of AWS in this region. These findings can help differentiate between the two conditions and contribute to their accurate diagnosis.

AWS in the middle vulvar ring was observed in all participants. However, there were noticeable differences between patients with vulvodynia and vulvar dermatosis, highlighting the potential diagnostic value of AWS distribution in this area.

The faster onset of AWS in patients with vulvodynia (mean 51.6 and median 49 seconds), a significantly higher frequency of sharp demarcation of AWS (49.4%), nontransparent AWS (16%), the "O sign" (81.7%) and provoked erythema (11.1%) supports the role of a potential inflammatory component in this condition in response to AWS, which may contribute to the overall symptomatology occurring in these groups of patients. By considering the complex background of vulvodynia, including factors like dysfunction of muscles, fascia, blood vessels, and nerve fibers mediated by inflammatory cytokines, the study contributes to a better understanding of this condition.

The similarity in the distribution and characteristics of AAT in patients with vulvodynia and impaired vulvar skin suggests a potential connection between the two conditions and the progression or evolution of vulvodynia in the presence of impaired vulvar skin over time. That highlights the importance of monitoring and evaluating these visual markers as potential indicators of symptom progression in healthy women with inferior vulvar skin.

Overall, the study provides valuable information about the

distribution and characteristics of AWS in three vulvar rings in different patient groups. These findings contribute to our understanding of the distinct patterns associated with vulvodynia and impaired vulvar skin, supporting the implementation of these diagnostic tools in clinical practice and highlighting the potential for improved diagnosis and monitoring of patients with these conditions.

References

- [1] Hinselmann H. Die Essigsäureprobe ein Bestandteil der erweiterten Kolposkopie. *Deutsche medizinische Wochenschrift* 1938; 40-42.
- [2] Apgar BS, Brotzman GL, Spitzer M. Colposcopy principles and practice: an integrated textbook and atlas, 2nd Edition. Elsevier, Philadelphia, 2008.
- [3] Prendiville W, Sankakaranyan R. Colposcopy and treatment of cervical Precancer. *IARC Technical Publication* 2017; N°45, ISBN-13 978-92-832-2458-7 <https://publications.iarc.fr/555>.
- [4] Ferenczy A. Diagnostic procedure for lesions of the cervix. In: Pathology of the female genital tract. Edited by Ancel Blaustein, 2. Edition, Volume 1, Springer Science-Business Medical LLC, 1982; 178-83.
- [5] Holtorff J. Kolposkopische Kriterien der atypischen Umwandlungszone. *Geburtsh u Frauenheilk* 1960; 20: 931.
- [6] Kolstad P, Stafl A. Atlas der Kolposkopie. Nach der 3. Auflage übersetzt von Fritz Hübner. Ferdinand Enke Verlag Stuttgart, 1983.
- [7] University of Zimbabwe and JHPIEGO Cervical Cancer Project. Visual inspection with acetic acid for cervical-cancer screening: test qualities in a primary-care setting. *Lancet* 1999; 353: 869-73.
- [8] Belinson JL, Pretorius RG, Zhabg WH et al. Cervical cancer screening by simple visual inspection after acetic acid. *Obstet Gynecol* 2001; 98: 441-4.
- [9] Ardahan, Melek PhD, RN; Temel, Ayla Bayik PhD, RN. Visual Inspection With Acetic Acid in Cervical Cancer Screening. *Cancer Nursing* 2011; 34 (2): p 158-163; DOI: 10.1097/NCC.0b013e3181efe69f.
- [10] Jonsson M, Karlsson R, Evander M et al. Acetowhitening of the cervix and vulva as a predictor of subclinical human papillomavirus infection: sensitivity and specificity in a population-based study. *Obstet Gynecol* 1997; 90 (5): 744-7.
- [11] Kesić, V.; Vieira-Baptista, P.; Stockdale, C. K. Early Diagnostics of Vulvar Intraepithelial Neoplasia. *Cancers* 2022; 14, 1822. <https://doi.org/10.3390/cancers14071822>.
- [12] Apgar B. Differentiating normal and abnormal findings of the vulva. 1996; *American Academy of Family Physicians* 2023. Available at: <https://indexarticles.com/reference/american-family-physician/differentiating-normal-and-abnormal-findings-of-the-vulva>; Last accessed on 18. February 2023.
- [13] González, M. G.-Y.; Muñoz Ledesma, A. M.; Monge, M. N.; Fontán, J. S. Colposcopy of the Vulva and Perineum Colposcopy of the Vulva and Perineum. Open Access Peer-Reviewed Chapter September. 2017. Available online: <http://dx.doi.org/10.5772/intechopen.68768>; Last accessed on February 19, 2023.

- [14] Micheletti L, Preti M, La Monica F. La vulvosopia, no debe ser destinada como el examen colposcopico de la vulva. *Arch. Med. Actual Tracto Genit. Inf* 2011; 3: 29-34.
- [15] Reid R. Rational management of cervical and vulvar neoplasia. *Contemp Ob Gyn* 1993; 38: 92-112.
- [16] Santoso J, Likes W. Colposcopic acetowhitening of vulvar lesion: a validity study. *Arch Gynecol Obstet* 2015; 292: 387-390. DOI 10.1007/s00404-015-3666-5.
- [17] Harni V, Babic D, Barisic D. "Three Rings Vulvoscopy" – A New Approach to the Vulva. *Gynaecol Perinatol* 2015; 24: 37-45.
- [18] Harni V, Babic D, Hadzavdic S, Barisic D. Diagnostic Accuracy of the Vulvoscopy Index for Detection of Vulvar Dermatitis (DATRIV Study, Part 1). *Journal of Gynecology and Obstetrics* 2022; 10 (1): 39-47. DOI: 10.11648/j.jgo.20221001.16.
- [19] Harni V, Babic D, Hadzavdic S, Barisic D. Clinical Value of the N-S-P Scheme for Detection of Vulvar Dermatitis (DATRIV Study, Part 2). *Journal of Gynecology and Obstetrics* 2022; 10 (3): 159-166. DOI: 10.11648/j.jgo.20221003.11.
- [20] Harni V, Babic D, Hadzavdic S, Barisic D. Nonspecific Lesions in Patients with Chronic Vulvar Discomfort Revealed Vulvodynia as Chronic Reflex Pain Syndrome (CRPS) Type I. *Journal of Gynecology and Obstetrics* 2022; 10 (6): 243-252. doi: 10.11648/j.jgo.20221006.12.
- [21] ISSVD Vulvodynia Pattern Questionnaire. Available at: <https://netforum.avectra.com/temp/ClientImages/ISSVD/3ef9c6ea-aac7-4d2b-a37f-058ef9f11a67.pdf>; Last accessed on May 23, 2015.
- [22] Byrne MA, Walker MM, Leonard J, Pryce D, Taylor-Robinson D. Recognising covert disease in women with chronic vulval symptoms attending an STD clinic: value of detailed examination including colposcopy. *Genitourin Med* 1989; 65: 46-9.
- [23] Audisio T, Zarazaga J, Vainer O. A Classification of Vulvoscopic Findings for Clinical Diagnosis. *J Lower Genit Tract Dis* 1999; 3: 7-18.
- [24] Bornstein J, Sideri M, Tatti S, Walker P, Prendiwill W, Haefner HK. 2011 Terminology of the Vulva of the International Federation for Cervical Pathology and Colposcopy. *J Lower Genit Tract Dis* 2012; 16: 290-5.
- [25] Friedrich EG Jr. Vulvar vestibulitis syndrome. *J Reprod Med* 1987; 32: 110-4.
- [26] Haefner H, Collins M, Davis GD, Edwards L, Foster D, Hartmann E, et al. The Vulvodynia Guideline. *J Lower Genit Tract Dis* 2005; 9: 40-51.
- [27] Stockdale CK, Lawson HW. 2013 Vulvodynia Guideline Update. *J Lower Genit Tract Dis* 2014; 18: 93-100.
- [28] van Beurden M, van der Vange N, de Craen AJM, Tjong-Ahung SP, ten Kate FJW, ter Schegget J, et al. Normal findings in vulvar examination and vulvoscopy. *J Br Obstet Gynaecol* 1997; 104: 320-4.
- [29] Reid R, Greenberg MD, Daoud Y, Husain M, Selvaggi S, Wilkinson E. Colposcopic findings in women with vulvar pain syndromes. A preliminary report. *J Reprod Med* 1988; 33: 523-532.
- [30] Kesic V. Colposcopy of the vulva, perineum, and anal canal. Chapter in Bosze P, Luesley DM. *EACG Course Book on Colposcopy*. Budapest, Primed-X-Press; 2003.
- [31] Lifits-Podorozhansky, YM; Podorozhansky, Y; Hofstetter, S; Gavard, JA. Role of Vulvar Care Guidelines in the Initial Management of Vulvar Complaints. *J Low Genit Tract Dis* 2012; 16 (2): 88-91.