Direct visual inspection of the cervix with acetic acid for the detection of premalignant lesions

Shammat, IM1*; Eissa, WA2; Mohammed, ZI2; Alnayal, MD3; Elsadig M,4
1 Faculty of Medical Laboratory Sciences, Omdurman Islamic University.
2 Royal Care International Hospital.
3 Department of Obst & Gyn, School of Medicine, Ahfad University for Women.
4 Department of Obst & Gyn, Faculty of Medicine & Health Sciences, University of Al-Neelien.

Abstract

Background This cross-sectional study aimed to compare the performance of visual inspection with acetic acid (VIA) against routine Pap smear for early detection of cervical intraepithelial neoplasia in Omdurman Province settings. Methods The study was conducted in Ahfad Family Health Centre (AFHC). A total of 65 women aged 18-60 years had a Pap smear followed by visual inspection of the cervix using 4 - 5% acetic acid. The 19 women who had positive VIA or abnormal Pap smear tests were referred for colposcopy together with randomly selected women as ones of the test-negative women. Results Of the all 29 women who were referred for colposcopy, 14 (48.3%) had abnormal result. Half of the 14 women with abnormal result (7) were detected by VIA, of which 4 cases were missed by cytology. Almost the other half 6 (40.9%) of these 14 positive cases were detected by the conventional cytology test as well, 3 cases of these were missed by VIA. Four women out of the 10 who were tested negative by both tests returned back after colposcopy as positive. Conclusion The study indicated that VIA had similar or better performance than cytology in detecting cervical neoplasia. Therefore, VIA worth further evaluation as an appropriate alternative to Pap smear in resource-challenged settings like ours in Sudan.

Keywords: cervical cancer, VIA, conventional cytology, colposcopy

*Corresponding author: Email imanshammat@hotmail.com.
Introduction
National screening programs with efficient cytologic screening have accomplished a significant reduction in cervical cancer in developed countries. Carcinoma of the cervix continues to be the most common cause of death among middle-aged women in many developing countries. In Sudan, cervical cancer is the leading gynecological cancer accounting for 40% cases of female cancers (1,2). Lack of cervical-cancer screening program contributes largely to this high occurrence. As a result, there are few opportunities to diagnose precancerous disease, and most patients present with invasive cancer.

Although limited by low specificity, visual inspection with acetic acid (VIA), has demonstrated high sensitivity for detecting cervical intraepithelial neoplasia (CIN) and cervical cancer (3). VIA involves naked eye examination of the uterine cervix, after swabbing it with 3-5% acetic acid (vinegar) and using artificial bright illumination. Findings of characteristic well-defined acetowhite lesions are considered positive (4). The objective of VIA is to detect acetowhite lesions leading to the early diagnosis of high-grade cervical intraepithelial neoplasia and early preclinical, asymptomatic invasive cancer. A major advantage with VIA is that it is a real-time screening test, as the outcome is known immediately after the administration of the test, so that further investigations and treatment can be planned and carried out during the same visit for women who test positive (5).

Materials and methods
Study area
This study was carried out in Ahfad Family Health Centre (AFHC). The AFHC according to its location promotes the health of the local community especially in Ombada locality and part of displacement area by providing quality comprehensive services (curative, preventive and promotive) to a defined population in the context of primary health care (PHC) strategies.

Study subject
All women attending the outpatient unit of obstetrics and gynecology clinic of Ahfad Family Health Centre for routine gynecological problems were invited to take part in this study. The study objectives were explained to all women, and verbal consent was obtained from those who were interested to participate in the study. Women aged between 18 and 60 years, who were not pregnant and had no previous history of cervical cancer or hysterectomy, were eligible for enrolment. The study design is summarized in figure 1.
Sample processing and screening test
The screening tests were performed in the following order: After obtaining social and medical history, each participant was asked to move to the examination table to be examined by the gynecologist, then a disposable speculum was inserted into the vagina, and the cervix and vaginal walls were inspected. Pap specimens were then taken using Aryes spatula, smeared on a glass slide, and fixed immediately with alcohol. A 4-5% dilute solution of acetic acid was then applied to the cervix using a cotton swab. After 1-2 minutes, a naked-eye examination was performed by the same gynecologist under a flash light illumination. Categories of VIA findings were recorded on the study questionnaire, and immediately thereafter, any excess liquid was removed from the posterior vaginal fornix using a cotton swab. Finally, the fixed slides were stained and then screened by two independent cytologists who were unaware of VIA results.

Pap staining method
The wet fixed smears were stained using Pap stain. The smears were fixed in 95% alcohol for at least 15 minutes, and then hydrated through descending grades of alcohol: 90% and 70, and then soaked in distilled water for two minutes. Cell nuclei were stained with regressive Harris haematoxylin for 3 minutes, and then differentiated in 0.5% aqueous hydrochloric acid for 10 seconds and immediately rinsed in distilled water to stop discolorations, then bluing in running tap water for 10 minutes and dehydrated in ascending alcohol concentrations from 70% through two changes of 95% alcohol 2 minutes for each change.

For cytoplasmic staining, smears were then treated with OG6 for 2 minutes, rinsed in 95% alcohol and treated in EA50 for three minutes. Finally, smears were dehydrated in 95% alcohol through absolute alcohol, cleared in xylene and mounted in distyrene plasticizer & xylene (D.P.X), to preserve permanent staining preparations, then examined under the light microscope. All smears showed fair staining quality with dark blue to black nuclei, pale greenish to bluish cytoplasm.

Colposcopic examination
Colposcopy was done in Fath-Elrahman Albashir hospital by a specialized gynecolposcopist. The examination was performed after the cervix was bathed with 5% acetic acid for at least one minute.

Data management and analysis
Cytology was processed manually according to the conventional routine manner. Cytology smears were reported according to Bethesda system. VIA was performed immediately after the collection of cervical swaps. The findings of VIA were classified according to WHO.
Result from both tests were compared and evaluated. Women with any positive test were referred for further investigation with colposcopy. If no lesion was found during colposcopy, women were asked to return back for annual routine follow-up. Women assessed as abnormal on colposcopy were referred for appropriate treatment and followed up. All data were analyzed using statistical software SPSS version 12. Sensitivity, specificity, positive and negative predictive values were calculated for each test. Data were presented in tables and figures.

**Results**

*Socio-demographic characteristics and risk factors reported by the participants*

A total of 65 women aged 18-60 years who fulfilled the eligibility criteria and
provided informed consent to participate in the study and were interviewed using a structured questionnaire. Four questionnaires were incomplete and were excluded from the analysis. The structured questionnaire included questions on sociodemographic characteristics and relevant risk factors for cervical neoplasia including smoking, oral contraceptive use, number of full pregnancy, and others.

**Performance of VIA and Pap smear tests**  
The overall result of the 65 women who were screened by VIA and Pap smear was summarized in Table 1. The result demonstrated that 12 (18.5%) had acetowhite lesions on naked-eye inspection, and 10 (15.4%) had ASCUS or worse lesions on Pap smears. Of these, 3 (4.6%) were reported positive on both VIA and cytology, 9 (13.8%) were positive on VIA only, and 7 (10.8%) were positive on cytology only. The performance of VIA and Pap smear was not found to be significantly different in our study (P-value = 0.267).

**Correlation between performances of VIA, Pap smear with colposcopy**  
All women who had positive VIA or abnormal Pap smear tests (19) were referred for colposcopy together with 10 women who were randomly selected as ones of the test-negative women to receive the reference-standard test, (Table 2). Of the all 29 women who were referred for colposcopy, 15 (51.7%) had a normal result, and 14 (48.3%) had abnormal result. The performance of VIA and Pap test were correlated with colposcopy and presented in Table 2. Half of the 14 women with abnormal results were detected by VIA, of which 4 cases were missed by cytology. Almost the other half were detected by the conventional cytology test as well, 3 cases of these were missed by VIA. However, 4 (28.6%) out of the 10 cases who were tested negative by both VIA and cytology tests returned back as positive.

**Distribution of cervical neoplasia grading according to colposcopy outcomes**  
Table 3 summarizes the distribution of cervical neoplasia as reported by colposcopy. The result showed that among the 14 women with confirmed positive result, over half 9 (64.3%) had CIN I, 4 (28.6%) had CIN II-III and only 1 (7.1%) had invasive cancer. At the first follow-up visit, all women, even those who tested negative with VIA, were showed satisfaction with their decision to return for cryotherapy for suspect cervical cancer. However, all women with high-grade lesions CIN II-III and invasive cancer detected in this study were referred for treatment, and all women with low-grade lesions CIN I were reported for follow-up after 6 months.
Accuracy and efficacy of the screening tests in detecting cancer lesions

The sensitivity, specificity, positive and negative predictive values for both tests VIA and Pap smear are given in Table 4. VIA had a higher sensitivity (50%) than cytology (42.9%) in detecting lesions, but cytology had a higher specificity (73.3%) than VIA (66.7%). The positive predictive values were found to be 58.3% and (60%), respectively for VIA and Cytology, and the corresponding negative predictive value were 58.8% and 57.9%, respectively.

Table 1: Results of screening tests

<table>
<thead>
<tr>
<th>VIA</th>
<th>Pap smear</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>4.6</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>10.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10</strong></td>
<td><strong>15.4</strong></td>
</tr>
</tbody>
</table>

*P-value = 0.267

Table 2: Referral for gold–standard colposcopy

<table>
<thead>
<tr>
<th>Reason for colposcopy</th>
<th>Attended colposcopy</th>
<th>Colposcopy positive</th>
<th>Colposcopy negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>%</td>
<td>#</td>
</tr>
<tr>
<td>Normal Pap and negative VIA</td>
<td>10</td>
<td>34.5</td>
<td>4</td>
</tr>
<tr>
<td>Abnormal Pap and negative VIA</td>
<td>7</td>
<td>24.1</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of cervical neoplasia

<table>
<thead>
<tr>
<th>Clinical grading</th>
<th>No. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN I</td>
<td>9</td>
<td>64.3</td>
</tr>
<tr>
<td>CIN II- III</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>14</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
**Table 4:** Sensitivity, specificity and predictive values of VIA and Pap smear

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIA</td>
<td>50</td>
<td>66.7</td>
<td>58.3</td>
<td>58.8</td>
</tr>
<tr>
<td>Cytology</td>
<td>42.9</td>
<td>73.3</td>
<td>60</td>
<td>57.9</td>
</tr>
</tbody>
</table>

*PPV:* positive predictive value  
*NPV:* negative predictive value

**Discussion**

This cross-sectional study has been carried out in Omdurman province to compare the performance of VIA against routine Pap smear for early detection of cervical intraepithelial neoplasia.

Even though cytology screening may be feasible in middle-income countries, there are technical, human resource and financial constraints in implementing such programs in low-income countries. In view of this, alternative methods based on visual examination of the cervix have been investigated for the control of cervical cancer in low-resource settings (7).

Among the visual inspection approaches, VIA has been more widely investigated for its performance characteristics (accuracy) in detecting cervical neoplasia, in various settings, by different providers. Visual inspection of the uterine cervix after the application of 3-5% freshly prepared acetic acid appears to be the most promising low-technology alternative to cytology (4), which led several international agencies such as WHO to study its efficacy in reducing incidence of and mortality from cervical cancer.

Several reports suggest that VIA can lead to the satisfactory detection of cervical lesions which are missed by cervical cytology (8, 9) and we wished to evaluate this technique provided by a gynecologist in our primary health care setting. Some previous reports have observed that VIA can reach similar or better results than the Pap smear (8, 10) in the detection of CIN. In our study, the results was in agreement with these studies and showed that VIA can effectively identify more cases of cervical intraepithelial neoplasia than did the routine cytology test. This study demonstrated a significant sensitivity of visual inspection of the cervix with acetic acid (VIA) in detecting premalignant lesions of the cervix comparable with that of Pap smear cytology. Some studies tested VIA in high-risk patients in sexually transmitted disease clinics (11). Patients in
the family health centre were chosen for this study as they represent a broad spectrum of supposedly normal and sexually active population and, therefore, the family health center was a good screening point. The sensitivity of VIA, even with the small number of subjects studied was found to be 50%. This showed that VIA may be equally effective or better in detecting lesions otherwise missed by a Pap smear.

In a study conducted in South Africa (8), the authors concluded that because VIA detected more than 60% of the high grade CIN, it merited consideration as an alternative to cytology in low resources settings. On the other hand, many other studies (10, 12) provided additional evidence on the performance of VIA as an alternative to cytology as a primary screening test indicated that VIA and Pap smear had similar performance in detecting high grade CIN. The performance of VIA and Pap smear in detection of high grade CIN in our study was found to be similar and consistent with these studies.

In studies where VIA was compared to cytology in the same setting, VIA performed similar sensitivity to (sometimes better than) cytology in terms of detecting high-grade lesions or cancer, but was less specific (13, 14). Another study in China showed that the sensitivity of VIA (70.9%) was lower than its specificity (74.3%) (15). However, in a workshop in Baltimore, a review of preliminary or final results from several studies investigating the performance of VIA in detecting cervical neoplasia in low-resource settings in Asia (India and Indonesia) and sub-Saharan Africa (Kenya, Zimbabwe, and South Africa) suggested that VIA performs similar or better compared to the Pap smear and/or other screening tests being investigated in those settings (16). Our results are consistent with those studies that have shown that VIA is more sensitive (50%) than cytology (42.9%), but usually less specific (66.7%) than cytology (73.3%) in terms of its efficacy. However, in general, the range of sensitivity and specificity of VIA in our study was lower than that observed from the above cross-sectional studies. Considering our study conditions, our results are likely to represent the lower end of the range for sensitivity and specificity of VIA. Higher test qualities for both tests are likely to be observed under better conditions and with more standardized VIA training than was available in our study. Additional improvements in VIA specificity could result from repeat VIA testing in women likely to return to the same site for healthcare.

In terms of accuracy, several studies (4, 10, 17) have observed that VIA had a lower positive predictive value (PPV) than
cytology, but their negative predictive value (NPV) was usually higher than cytology. In our study, the PPV of VIA (58.3%) and Pap smear (60%) reported was consistent with all these studies as well as the NPV of the VIA which had a higher NPV than Pap smear in detecting cervical lesions. The 58.8% NPV for VIA reported in the present study indicated that in resource-poor settings, where a Pap smear is not available, VIA can be used for mass screening of premalignant lesions, with some degree of confidence. However, the range of PPV of VIA and Pap smear reported in our study are higher than some those reported by other studies \(12, 14\). It is worth mentioning that these values are referring for all grades of CIN in order to ensure as many true-positive lesions as possible, whereas many other studies have looked mainly at high grade CIN only. However, one study \(18\) recommended that it would be better to consider all grades of lesions as threshold for doing colposcopy as we did in our study.

A study conducted in rural Thailand reported that a single-visit approach with VIA and cryotherapy seems to be safe, acceptable, and feasible in rural Thailand, and is a potentially efficient method of cervical-cancer prevention in such settings. \(19\). At this level of our study, the high response rate for follow-up and treatment as well as partner’s support was considered as an indicator of the high level of acceptance of the VIA method.

Finally, our study indicates that VIA and cytology has almost similar performance in detecting cervical lesions. In terms of technical effectiveness, the results in our study and of the other reported studies \(20, 21\) indicate that VIA is a simple, objective test. The results of this procedure (positive or negative) available immediately, allowing of further investigation to be carried out for the identification of cervical lesions. It has been shown that follow-up colposcopy and treatment of pre-invasive lesions can be performed immediately (during the same visit), which not only avoids recalls but also increases compliance to diagnostic investigation and treatment. The feasibility of offering colposcopy and large-loop excision of the transformation zone under local anesthesia during same visit, following a positive screening test, has been well demonstrated in South Africa \(8\). The test is not expensive, and it is possible to train providers (both medical and paramedical) to detect acetowhite lesions with the naked eye \(22, 23\). Despite the above advantages, the major concern is low specificity, which means that many subjects must be recalled for colposcopy. It is likely that standardized training of the providers, development of quality control procedures, and uniform definitions of
VIA test outcomes may contribute to some improvement of the specificity of visual inspection based screening approach without substantially lowering sensitivity.

Acknowledgements
We are grateful for Administration of Fath-Elrahaman Albashir hospital. We also acknowledge Miss Asma Alamir for assisting in the study. Authors declare no conflict of interest.

References


