Effectiveness of Visual Inspection with Acetic Acid and Cervical Cytology in Early Detection of Precursors of Cervical Cancer in Nepal

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ABSTRACT

Background

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Cervical cancer ranks as the first, most frequent cancer amongst Nepalese women. This can be prevented through primary prevention and early detection using screening techniques. Though cervical cytology is a standard screening tool in most of the developed countries, it fails to be the screening method of choice in low and middle income countries (LMICs) with limited resources due to financial and technical constraints.

Objective

This study aims at early detection of pre-cancerous lesion in cervix with cervical cytology and visual inspection with acetic acid.

Method

This is a hospital based, descriptive cross-sectional study. It included 104 women attending Obstetrics and Gynecology out-patient department (OPD) in Dhulikhel Hospital. Visual inspection of cervix with acetic acid (VIA) and liquid based cytology (LBC) was performed followed by cervical biopsy for positive VIA, or having abnormal cytology, or having unhealthy appearance of cervix even after treatment.

Result

The incidence of positive VIA and cytology screening was 38.5% and 7.7% respectively. The negative predictive value of VIA and cytology were similar whereas VIA was found to be more sensitive than cytology.

Conclusion

Cytology screening is used as an investigation of choice for screening of cervical cancer. In Nepal, VIA can be a good alternative for early detection of cervical cancer as it is found to be more sensitive than cytology.

KEY WORDS

Cervical cancer, Cervical cytology, Premalignant lesion, Screening, Visual inspection with acetic acid

INTRODUCTION

Cervical cancer has a major impact on the lives of women worldwide and is the second most common cancer among women constituting 15-51% of all cancers.¹ It is the fourth most common cause of cancer death in women accounting for 7.5%. Almost nine out of ten (87.0%), cervical cancer deaths occurs in the LMICs.² Cervical cancer has a long premalignant phase and if it is detected and treated in such phase, advanced disease can be prevented. Unfortunately, more than 80.0% of cases are diagnosed at an advanced clinical stage and survival is less than 40.0%. In 2012, 528,000 new cases of cervical cancer were diagnosed worldwide, of which about 85.0% occurred in LMICs. In the same year, 266,000 women died of cervical cancer worldwide and 231,000 women died in LMICs.³

Nepal has a population of 10.2 million women aged between 15 years and older who are at the risk of developing cervical cancer. Current estimates according to Human Papilloma Virus (HPV) information center 2018, indicate that every year 2,942 women were diagnosed with cervical cancer and 1,928 die from this disease. Cervical cancer ranks as the first most frequent cancer among women in Nepal and most frequent cancer among women between 15 and 44 years of age. Screening with cervical cancer has greatly helped to reduce the incidence of, and death from, cervical cancer in developed countries through the detection and treatment of cervical precancerous lesions so that they do not progress to invasive cancer, and possibly death. Hence, the main aim of our study is early detection of precancerous lesion with cervical cytology and visual inspection with acetic acid.4

METHODS

This study was conducted in the Obstetrics and Gynecology out-patient department (OPD) of Dhulikhel Hospital from March 2019 till September 2020. This is a hospital based, descriptive and cross-sectional study. The inclusion criteria were with women who were sexually active, had suspicious symptoms such as leucorrhoea, post-coital bleeding, intermenstrual or postmenopausal bleeding, had suspicious cervix such as hypertrophied, unhealthy cervix or cervix which bleeds on touch on examination. The women who were pregnant, having heavy bleeding per vaginum at the time of examinations, diagnosed case of cervical cancer or who denied consents were excluded from the study. The sample size required was 79 and calculated by using sample based formula ($(Z^2 P Q)/e^2$) with prevalence of 28.9%. However, 104 were included to increase the power of this study. The ethical approval was received from IRC-KUSMS and data was collected after written informed consent.

The cervix was examined and defined as healthy or unhealthy on the basis of naked eye observation. A healthy cervix was defined as the one that looked pink, round, smooth with a central external os. The cervix was considered unhealthy if it had abnormalities present, either single or in various combinations such as hypertrophy, irregular, congested with increased vascularity and polyp or looked clinically suspicious for malignancy.⁵ The cytobrush was used for collecting cervical samples for liquid based cytology. Then the cytobrush was placed into the fixative and was send to laboratory. The report of the cytological test was obtained and the reporting of the cytological test was done using Bethesda III System (2001).

For VIA, 5% acetic acid was used and the solution was applied to the cervix with cotton swab and after one minute visual inspection of cervix was done for the development of aceto-white area near the squamo-columnar junction or close to the external os or presence of aceto-white growth.

All participants with abnormal cytology or VIA positive were counseled for cervical biopsy. Then, cervical biopsy was taken from the particular aceto-white lesion. In case of VIA negative with abnormal cytology biopsy was taken from all the four quadrants. The samples were sent for the histopathology. All the data were recorded in the proforma and then entered in an excel spread sheet as a master chart and was analyzed using statistical package for the social science software (IBM SPSS Statistics 26).

This study utilized descriptive analysis for mean and standard deviations for continuous variables and frequency (percentages) for categorical variables. The categorical variables (VIA positivity, cytology positivity and unhealthy cervix) were compared between groups with positive histopathology using Chi-square test. For all analysis, a two tailed p-value of < 0.05 was considered statistically significant. Then the sensitivity, specificity, positive predictive values and negative predictive values were calculated for different categorical variables based on positive histopathology.

RESULTS

The age range of women participated were from 23 to 53 years. The mean age of women who underwent VIA and cytological screening in this study was 37 years. The mean age of marriage of the participants was 19.3 years. The socio-demographic characteristics were presented in Table 1 below.

Out of 104 participants, 39 (37.5%) women had vulval itching, 80 (76.9%) had per vaginal (PV) discharge, 78 (75%) had lower abdominal pain, 72 (69.2%) had back pain, 6 (5.8%) had post-coital bleeding, 16 (15.4%) had dysparunia and 22 (21.2%) had inter-menstrual bleeding.

Among the participants, 53 (51%) had unhealthy cervix on per-speculum examination. When VIA were performed on them, 40 (38.5%) were identified having positive findings. The cytological examination was performed as a part of examination to the study participants and analysis found 81 (77.9%) participants had satisfactory results whereas 15
 Table 1. Distribution of women according to socio-demographic characteristics

Characteristics	Frequency (N=104)	Percentage (%)		
Age group (Years)				
≤ 24	5	4.8		
25-29	14	13.5		
30-34	18	17.3		
35-39	26	25		
40-44	22	21.2		
45-49	12	11.5		
≥ 50	7	6.7		
Age of marriage/sexual debut (Years)				
≤ 19	61	58.7		
20-25	39	37.5		
26-29	3	2.9		
30-34	0	0		
≥ 35	1	1		
Occupation				
Homemakers	87	83.7		
Service	6	5.8		
Vendor	5	4.8		
Teacher	2	1.9		
Student	4	3.8		
Smoking				
Yes	8	7.7		
No	96	92.3		
Parity				
0	3	2.9		
1-3	90	86.5		
≥ 4	11	10.6		
Contraceptive use				
Implant	15	14.4		
Pills	11	10.6		
IUCD	2	1.9		
Depo-Provera	12	11.5		
Barrier	8	7.7		
None	56	53.8		
Menstrual cycle				
Regular	84	80.8		
Irregular	20	19.2		
Cervical cancer screening by cytology				
Not screened	32	30.8		
Screened	70	67.3		
Treatment for other purpose	2	1.9		

(14.4%) had inflammatory smears. The abnormal cytology finding was identified among 8 (7.7%) participants, 2 (1.9%) had ASCUS, 3 (2.9%) each had LSIL and HSIL.

Though vaginal discharge was the most common complain, VIA positivity was noted to be more in participants with

post coital bleeding 5 (62.5%). Cytology screen positivity noticed more in patient with inter menstrual bleeding 3 (15%) followed by vaginal discharge 6 (7.2%) and lower abdominal pain 5 (6.6%). Screening results in relation to symptoms are presented in Table 2.

Table 2. Screening results in relation to symptoms

		VIA positive			Epithelial cell abnormality in cytology			
Symptoms	No. of cases	(%)	N	%	p- value	Ν	%	p- value
Vulval itching	43	41.3	21	48.8	.068	2 ª	4.6	.387
PV discharge	83	79.8	32	38.5	.969	6 ^b	7.2	.711
Lower ab- dominal pain	76	73.1	28	36.8	.576	5°	6.6	.958

2°: 2LSIL; 6b:1ASCUS, 3LSIL, 2HSIL; 5° 1ASCUS, 2LSIL, 2HSIL; 4d: 1ASCUS, 1LSIL, 2HSIL; 3°: 1ASCUS, 1LSIL 1HSIL

The relation between screening positive i.e. VIA positive and epithelial cell abnormality in cytology and gross cervical appearance is shown in Table 3.

Table 3. Relation between gross cervical appearance and screening positivity

		VIA positive		Epithelial cell abnorm cytology			ality in
Gross cervi- cal appearance	N = 104	N (%)	Chi square p value	ASCUS	LSIL	HSIL	Chi square p value
Healthy	51	14 (27.5)	.010	2 (3.9)	1 (1.9)	1 (1.9)	.456
Unhealthy	53	26 (49)		0 (0)	2 (3.8)	2 (3.8)	

The results of comparison of VIA results and cytology with histopathology were done and shown separately in Table 4 and 5. For VIA, a positive predictive value (PPV) of 12.5% and negative predictive value (NPV) of 89.5% was received. Similarly, the sensitivity of the test is 71.4% and specificity is 32.7%. For cytology screening, the positive predictive value is 50% and negative predictive value is 94.1%. Furthermore, the sensitivity is 57.1% and specificity is 92.3%.

Table 4. Comparison of VIA results with histopathology

VIA	Histopathology positive	Histopathology negative	Total
Positive	5	35	40
Negative	2	17	19
Total	7	52	59

Sensitivity: 5/7x100=71.4%; Specificity: 17/52x100=32.7%; Positive predictive value: 5/40x100=12.5%; Negative predictive value: 17/19 x 100=89.5%

A total of 49 participants underwent biopsies and 7 (14.3%) reports noted to be positive. VIA positivity and histopathology positive was in 5 participants among whom 2 had squamous cell carcinoma and 1 had CIN 1, 1 CIN 2

Table 5. Comparison of cytology screening with histopathology

Cervical cytology	Histopathology Positive	Histopathology Negative	Total
Positive	4	4	8
Negative	3	48	51
Total	7	52	59

Sensitivity: 4/7x100= 57.1 %; Specificity: 48/52x100=92.3% Positive predictive value: 4/8x100%=50%; Negative predictive value: 48/51x100=94.1%

and 1 cervicitis with koilocytic change. Cytology screen positivity and histopathology positive was identified in 4 participants. Comparison between VIA positivity, epithelial abnormality in cytology, cervical appearance and histopathological findings are presented in Table 6.

Table 6. Comparison between VIA positivity, epithelial cell abnormality in cytology, gross cervical appearance and histopathological findings

		VIA positive	Epithelial cell abnor- mality in cytology	Gross cervical appear- ance
Combination of total screen positive	101	40	8	53
Histopathologi- cally confirmed positive	7	5(12.5%)	4(50%)	6 (11.3%)
CIN1	1(14.3%)	1(20%)	0(0%)	1(16.7%)
CIN2	1(14.3%)	1(20%)	1a (25%)	1(16.7%)
CIN3	1(14.3%)	0(0%)	1b (25)%	0(0%)
SCC	3(42.8%)	2(40%)	2c (50%)	3(50%)
Cervicitis with koilocytic change	1(14.3%)	1(20%)	0(0%)	1(16.7%)

1^a: HSIL; 1^b: HSIL; 2^c: 1HSIL, 1LSIL

DISCUSSION

The current study was carried out to determine the effectiveness of VIA and cervical cytology in early detection of precursors of cervical cancer. In the developed countries, due to organized cytology screening, there is substantial reduction in cervical cancer. However, in the LMICs regular cytological screening is not possible due to financial and technical barrier. VIA based cervical cancer screening can be of choice for early detection of precancerous lesion. The national cervical cancer screening guidelines of Nepal included VIA for screening of cervical cancer in women of age group 30-60 years.

We included 104 participants in the study aged between 20 to 60 years. The maximum number of screened population was of the age group 35-39 years and is similar to the study conducted by Dhaubhadel et al.¹ VIA and cervical cytology positivity rate was highest among the age group 35-39 years which was different from the study done by

Ruchi et al. where highest VIA positivity was noted in the age group of 40 years and above.⁶ Early age of marriage and sexual debut can be the reason for early occurrence of premalignant lesions. Majority of the women in our study underwent marriage around the age of 19 years. Similar findings was noted in the study conducted by Krishnegowda et al.⁷ Early marriage also coincides with illiteracy rate and low socioeconomic status amongst the studied population.

In this study, we found 7.7% participants as a smokers and is lower than those reported by Dhaubhadel et al. and Cronje et al. as 16%.^{1,8} In the study conducted at Thapathali maternity hospital, the prevalence of positive VIA and Pap smear was more among the smokers, 3.9% and 1.3% respectively, compared to 2.6% and 0.4% among non-smokers.¹ Similarly, in our study, we found 90 (86.5%) participants were multiparous followed by 11 (10.6%) grand multipara. This is similar to study conducted by Agarwal et al.⁹ The prevalence of positive VIA and cervical cytology was more among multiparous. It has been reported that metaplastic changes are also influenced by the trauma and repair experienced during delivery, and increased risk of cervical carcinoma has been identified in participants who are highly parous.¹⁰

Our study found women using no contraceptive 56 (53.8%) were the one having risk for cervical cancer as VIA positive identified in 25 (44.6%) and abnormal cytology in 5 (8.9%). This was followed by implants 15 (14.4%) and Depo-Provera 12 (11.5%). In contrast, injectable were the most common form of contraceptive in the study done by Cronje et al.8 A data was collected from 8 countries (Thailand, the Philippines, Morocco, Brazil, Peru, Paraguay, Colombia, and Spain) with two studies of carcinoma in situ (CIS) and 8 studies of invasive cervical cancer (ICC) in women who tested positive for HPV DNA. These studies reported that the women who had used oral contraceptives for 5 years or more and had 7 or more pregnancies had the greatest risk compared to nulliparous.¹¹ The choice of oral or injectable contraceptive therefore seems not having protection for this group of women population.

The most common clinical presentation amongst the screened participants was discharge per vagina 83 (79.8%), followed by lower abdominal pain 76 (73.1%) which was similar to the study conducted by Krishnegowda et al.⁷ This finding was different from the study conducted by Dhaubhadel et al. where pelvic pain was the most common complain.¹ Discharge per vagina is related to poor hygiene during menstruation and lack of regular health checkup.¹ Of all different symptoms (Table 2), vaginal discharge seems related with pathology. Bacterial vaginosis and vaginal discharge might have a role in the development of cervical intraepithelial neoplasia.¹² The vaginal discharge in cervical cancer changes from physiological to dirty brown having a particular offensive odor caused by an infection of necrotic tissue with saprophytes.¹³

On speculum examination, 53 (51%) participants had an abnormal looking cervix which is higher than that reported by Dhaubhadel et al.¹ The study conducted by Tamrakar et al. showed the premalignant lesions including LSIL and HSIL in unhealthy cervices.¹⁴ The study conducted by Krishnegowda et al. reported no incidence of SIL noted in healthy cervix, but noted in atrophic cervix.⁷ Our study found 40 (38.5%) of the participants having VIA positive. A study conducted by Dhaubhadel et al. reported VIA screen positive rate as 2.86%.¹ Similar study was conducted by Mohammad et al. and showed VIA positivity rate as 14%.¹⁵ A cross-sectional study conducted by Jumla found 12.4% having VIA positive.¹⁶ The high VIA positive in sexually active age group might be explained by early marriage and repeated infection.

The incidence of cytology positive was 7.7% in our study which was higher than that conducted by Dhaubhadel et al.¹ The study conducted by Shakya et al. reported prevalence of abnormal cytology 12.4%.¹⁷ The reason for better outcome in cytology in our study may be due to use of liquid based cytology instead of Pap smear. Even though the sensitivity and specificity of conventional Pap smear (CPS) and liquid based cytology (LBC) is almost similar in various comparison studies, most western countries have switched over from conventional Pap smear to liquid based cytology. The reason for this is reduced rates of unsatisfactory results on LBC, clarity on microscopy, improved sample processing and small area to be screened.¹⁸

False positive rate was identified higher with VIA in this study which resulted in unnecessary referral for further confirmation. Similar result was noted in study by Consul et al.¹⁹ In this study, sensitivity of VIA was 71.4% and specificity was 32.7%, positive predictive value (PPV) was reported to be 12.5% while negative predictive value (NPV) was 89.5% whereas a multicenter study was done by Sankaranarayanan et al. in 2004 which included 11 cross-sectional studies, the sensitivity of VIA ranged from 56.1% to 93.9% and the specificity ranged between 74.2% and 93.8%.²⁰ The reason for high sensitivity and low specificity

REFERENCES

- Dhaubhadel P, Vaidya A, Choudhary P. Early detection of precursors of cervical cancer with cervical cytology and visual inspection of cervix with acetic Acid. JNMA. 2008;47(170):71–6.
- Pandey RA, Karmacharya E. Cervical cancer screening behavior and associated factors among women of Ugrachandi Nala, Kavre, Nepal. *Euro J Med research*. 2017;22(1):1–9.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians.* 2018;68(6):394–424.
- 4. Kathry R. Nepal: Nepal. The Ecumenical Review. 2012; 64(2):131–2.
- Shastri SS, Dinshaw K, Amin G, Goswami S, Patil S, Chinoy R, et al. Concurrent evaluation of visual, cytological and HPV testing as screening methods for the early detection of cervical neoplasia in Mumbai, India. Bulletin of the World Health Organization. 2005;83:186–94.

in this study could be due to presence of infection and inflammation that take up aceto-white stain, some aceto-white areas might have been interpreted as being positive. Another study by Consul et al. reported sensitivity of VIA as 84.2%, specificity was 55.2%, PPV as 55.2% and NPV as 84.2% which was different from this study.²¹ The high negative predictive values of both tests means that women who are test negative can be reassured with confidence that they are disease free. The sensitivity, specificity, PPV and NPV of cytology screening in this study was 57.1%, 92.3%, 50% and 94.1% respectively which was different from the study done by Consul et al.²¹

Thus single visit approach in cervical cancer screening by VIA then treatment of cervical lesion using cryotherapy can prevent cervical cancer and its related mortality. This single visit approach in cervical cancer screening and treatment is supported by Sankaranarayanan et al. and Rashid et al. as it doesn't require much training and can be done by nurses and midwives.^{15,20}

CONCLUSION

The incidence of positive VIA and cytology screening was 38.5% and 7.7% respectively. Women having unhealthy cervix were identified with more VIA positivity 26 (49.0%) than healthy cervix 14 (27.5%). This study analyzed the effectiveness of VIA, cytology screening by comparing their report with histopathology. For VIA, a positive predictive value was 12.5% and negative predictive value 89.5%. Similarly, the sensitivity of the test was 71.4% and specificity 32.7%. For cytology screening, the positive predictive value was 50.0% and negative predictive value 94.1%. Furthermore, the sensitivity was 57.1% and specificity 92.3%. The VIA was found to be more sensitive than cytology as VIA helped in detection of carcinoma that was missed by cytology screening. Therefore, VIA can be used as a screening tool in a low resource setting. However, high incidence of false positive result produced by VIA cannot be ignored.

- Arun R, Singh JP, Gupta SB. Cross-sectional study on visual inspection with acetic acid and Pap smear positivity rates according to sociodemographic factors among rural married women of bareilly (Uttar Pradesh). *Indian J Community Med*. 2018;43(2):86-9.
- 7. Krishnegowda S, Veena MS. Efficacy of colposcopy technique with Pap smear and histology in screening of cervical lesions. *Int J Reprod Contracept Obstet Gynecol*. 2014;3(3):696–702.
- Cronje HS, Cooreman BF, Beyer E, Bam RH, Middlecote BD, Divall PD. Screening for cervical neoplasia in a developing country utilizing cytology, cervicography and the acetic acid test. *Int J Gynecol Obstet*. 2001;72(2):151–7.
- 9. Agarwal S, Gupta R, Agarwal A, Pandey K, Gupta N, Katiyar A. Visual inspection with acetic acid for cervical cancer screening in a tertiary health care centre. *Int J Rep Contracept Obstet Gynecol.* 2016; 5(3):753.

- Ibrahim A, Rasch V, Pukkala E, Aro AR. Cervical cancer risk factors and feasibility of visual inspection with acetic acid screening in Sudan. *Int J Womens Health*. 2011; 4(3):117-22.
- Moreno V, Bosch FX, Muñoz N, Meijer CJ, Shah KV, Walboomers JMM, Herrero R, Franceschi S. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. *The Lancet.* 2002;359(9312):1085– 92.
- Salih MM, Alhag FTES, Khalifa MA, Nabi AH. Cervical cytopathological changes among women with vaginal discharge attending teaching hospital. *Journal of Cytology*. 2017;34(2):90-4.
- 13. Kumar P, Jeffcoate SN, Malhotra N. Jeffcoate's principles of gynaecology. Butterworths; 2008.
- 14. Tamrakar SR, Shrestha S. Study of cervical biopsy over a decade at a tertiary level hospital, Nepal. Nepal Med Coll J. 2019;21(1): 80-5.
- 15. Rashid MHU, Ahmed MM, Chowdhury S, Ahmed S. Effectiveness of visual inspection with acetic acid as a test for cervical cancer screening. *Int J Noncommunicable Diseases*. 2017;2(1):3-7.
- 16. Thapa N, Shrestha G, Maharjan M, Lindell D, Maskey N, Shah R, et al. Burden of cervical neoplasia in mid-western rural Nepal: a populationbased study. *J Gynecol Oncol.* 2018;29(5): e64.

- Shakya S, Syversen U, Asvold BO, Bofin AM, Aune G, Nordbø SA. Prevalence of human papillomavirus infection among women in rural Nepal. AOGS. 2017;96(1):29–38.
- Singh VB, Gupta N, Nijhawan R, Srinivasan R, Suri V, Rajwanshi A. Liquid-based cytology versus conventional cytology for evaluation of cervical Pap smears: experience from the first 1000 split samples. *Indian J Patho Microbiol.* 2015;58(1):17-21.
- Consul S, Agrawal A, Sharma H, Bansal A, Gutch M, Jain N. Comparative study of effectiveness of Pap smear versus visual inspection with acetic acid and visual inspection with Lugol's iodine for mass screening of premalignant and malignant lesion of cervix. *Indian J Med Paedia Oncol.* 2012;33(3):161-5.
- Sankaranarayanan R, Basu P, Wesley RS, Mahe C, Keita N, Mbalawa CCG, et al. Accuracy of visual screening for cervical neoplasia: Results from an IARC multicentre study in India and Africa. *Int J Cancer.* 2004;110(6):907–13.
- 21. Consul S, Agrawal A, Sharma H, Bansal A, Gutch M, Jain N. Comparative study of effectiveness of Pap smear versus visual inspection with acetic acid and visual inspection with Lugol's iodine for mass screening of premalignant and malignant lesion of cervix. *Indian J Med Paediatr Oncol.* 2012;33(3):161-5.