

Original Research Article

Diagnosis of cervical lesion by colposcopy, VIA, pap smear tests, and their correlation with histopathology in a tertiary level laboratory in Chattogram, Bangladesh

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ABSTRACT

Background: Cervical carcinoma is one of the most common causes of mortality among women. This mortality rate can be reduced by early detection of cervical neoplasm by different screening tests. The main objective of this study was to diagnose cervical diseases by colposcopy, by pap smears for cytological examination as well as correlate these findings as screening tests with histopathological diagnosis.

Methods: In this cross-sectional observational study, total 143 cases were included. History was taken and clinical examination was done. Colposcopy and VIA test was done, pap smear sample was collected and reporting was made. Cytological findings were correlated with histopathology.

Results: Mean age of the study cases was 41.2±11.5 years. VIA test was positive in 98 study cases (68.5%). On colposcopy, most of the cases show neoplastic proliferation (80 cases, 55.9%). Among them, most cases were diagnosed as cervical intraepithelial neoplasia-I (CIN-I). Sixty-three (63) cases (44.1%) were non-neoplastic. Most of the biopsies was diagnosed histopathologically as cervical intraepithelial neoplasia-I (CIN-I) (20 cases, 14.0%). Association of colposcopy findings with histopathological diagnosis was done which was significant. Sensitivity of diagnosis of cervical malignancy by colposcopy was 33.33% and specificity was 98.57%.

Conclusions: The study provides good cyto-histopathology correlation in detecting different cervical lesions and malignancy with colposcopy. Although colposcopy sensitivity was low but it can be increased by adequate training and avoiding technical errors. Bethesda system is strongly recommended for adequacy of sampling to minimize inconsistency. Early and regular screening should be advised for reduction of mortality rates from cervical carcinoma.

Keywords: Biopsy, Cervical cancer, Cervical intraepithelial neoplasia, Colposcopy, Pap smear, VIA test

INTRODUCTION

In Bangladesh, we don't have any regular screening protocol to evaluate cervical lesions. But we are getting many cases of cervical malignancy as well as dysplasia in our day-to-day practice. According to International Agency for Research on Cancer, more than 50 million Bangladeshi women are at risk of developing cervical cancer, and 17686 new cases and 10362 deaths occur

annually.¹ Global report published by WHO in 2014, shows that around 2,66,000 women died from cancer of cervix in the year 2012.² Therefore this cancer is taking a toll on the health of women worldwide. Among the South-Asian countries, India has the highest age standardization incidence of cervical cancer at 22, compared to 19.2 in Bangladesh, 13 in Sri Lanka and 2.8 in Iran. So, it is very important to understand the epidemiological pattern to diagnose cervical cancers at an early stage.³

But, if it could be diagnosed at the precursor stage, cervical cancer can be managed and prevented successfully. Screening programs are necessary to identify the disease before it progresses to invasive cancer. Many studies now provide evidence of the feasibility and cost-effectiveness of screening and treatment approaches for cervical cancer prevention. These can be easily adopted for various settings. A significant reduction in cervical cancer mortality was shown following a single round of screening with HPV testing or VIA screening in a randomized trial in India.⁴ Visual inspection with acetic acid (VIA) is a simple, inexpensive test with moderate sensitivity and specificity for screening that can be combined with simple treatment procedures for early cervical lesions. Health workers or nurses can be trained as test providers; the results are available immediately. VIA is feasible in many low-resource areas where it is difficult to sustain high-quality cytology programs. Scaling up and inclusion of VIA-based programs into national programs is already taking place in many low- and middle-income countries. Pap smear is a simple, safe, non-invasive and cost effective method for the detection of pre-cancerous, cancerous and benign lesions of cervix.⁵ It is a sensitive test for early screening of cervical lesions and most widely accepted system for reporting of pap smears is TBS (2001, The Bethesda system).⁶ With the use of pap smear as a screening tool for the detection of abnormal epithelial lesions in cervix, more cases can be diagnosed early and thus the morbidity and mortality of patients can be decreased. This study was conducted to study the pattern of various cervical smear abnormalities in our center, to study the prevalence of epithelial cell abnormalities in our study population and to determine the accuracy of pap test by correlating with histopathology. Periodical cytological screening helps in early detection of cervical lesions and help in reducing the morbidity and mortality associated with cervical cancer. Even the government and NGOs can help in increasing the awareness and educating the people about the screening benefits of pap smear at an early stage.⁷

Recently, the practice of colposcopy is a cornerstone of cervical cancer prevention. In conjunction with screening and management of precancerous conditions, colposcopy has played a pivotal role in reducing the incidence and mortality from cervical cancer over the past 50 years.^{8,9} The United States, the American Society for Colposcopy and Cervical Pathology (ASCCP), in collaboration with investigators from the US National Cancer Institute, set out to review evidence and develop recommendations for colposcopy practice in the United States.¹⁰ A starting point of this effort was to define the role of colposcopy as a test used in the prevention of cervical cancer. Despite the longevity of colposcopy in clinical practice, there continues to be a lack of standardization of several aspects of the procedure, including terminology and documentation in the medical record.¹¹

Histopathology is the reference standard for diagnosing cervical intraepithelial neoplasia (CIN) and informs clinical management by identifying which women will be treated, followed, or returned to routine screening. In addition, cervical histopathology defines the endpoints in epidemiological studies of cervical carcinogenesis and accuracy of screening tests, as well as population-based surveillance studies on the impact of human papillomavirus (HPV) vaccination. Typically, CIN grade 3 (CIN3) has been considered definitive precancerous, with an approximately 30% risk of progression to cervical cancer; whereas CIN grade 2 (CIN2) is the conservative threshold for treatment in clinical practice. The choice of laboratory would not matter; for any given group of cases, the same distribution of diagnoses would be rendered.¹²

Aim of this study was to evaluate the role of VIA, Pap smear and colposcopy in the diagnosis of precancerous and cancerous lesions of uterine cervix.

METHODS

This was a cross sectional observational study, carried out on the patients attended for cervical screening by VIA, Pap smear, colposcopy and biopsy. Total 143 patients were included in the study.

Study period

This study was conducted in two years, from 1st January 2020 to 31st December, 2021.

Study design and the participants

Total 143 cases underwent VIA, pap test, colposcopy and biopsy were included in this study. Experimentation was done in Epic Health Care, Chattogram, Bangladesh.

Experimentation and data collection

At the beginning of the experimentation, it was confirmed that patient was not on menstruation, passed 10-20 days of her menstrual cycle and had not douched or used tampons or vaginal medication in past 24 hours. Patients were placed in the lithotomy position and the procedure was done by the study investigator Dr. Syeda Rumman Akter Siddiqui, in assistance of a well trained and experienced nurse/midwife. An unlubricated sterile Cusco's bivalve speculum was introduced to observe the cervix, in an ample light condition. Then with the help of endocervical brush/Ayre's spatula, transformation zone was scraped by rotating the spatula in clock wise direction in squamo-columnar junction, and endocervical smears were made on a clean glass slide and fixed with 95% ethyl alcohol for at least 30 minutes. Afterwards the smears were stained by Papanicolaou method. The pap smears were interpreted by two pathologists according to Bethesda 2001 classification and for histology WHO classification was used. Once, the cytology specimen was

collected, the cervix was painted with a cotton wool soaked in 3-5% VIA and examined after 1 minute for aceto-white reaction. Any suspicious or visible lesions were then biopsied. Some patients with abnormal positive pap smear results were also called back for tissue biopsy. These patients were referred for proper follow up and management in the gynecological clinic.

Inclusion criteria

Patients with chief complaints of backache, hypogastric pain, pain during sexual intercourse, postcoital bleeding, discharge from vagina, were included in this study.

Exclusion criteria

Patients who refused to participate; patients having vaginal bleeding other than postcoital and postmenopausal were excluded. Patients with metastatic disease, double primary cancers, and other comorbid conditions such as diabetes, hypertension, ischemia, tuberculosis, autoimmune disorder were excluded for biasness.

Data management and statistical analysis

Data analysis and interpretation was done by descriptive statistics. ANOVA test, Chi-square test were performed to obtain the statistical significance between the variables. Statistical Package for Social Science (SPSS) software was used (licensed version 25).

RESULTS

We studied on one hundred and forty-three (143) patients in Epic Health Care, Chattogram, Bangladesh. Most of these patients came from different areas of Chattogram with different gynecological problems. We took proper history and did clinical examination. Colposcopy and VIA test was done, pap smear sample was collected and reporting was made according to Bethesda 2001 classification. Cytological findings were correlated with histopathological diagnosis.

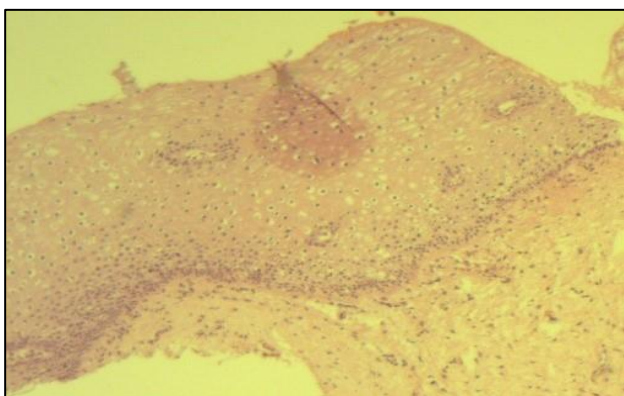


Figure 1: Chronic cervicitis, hematoxylin and eosin stain, 10X.

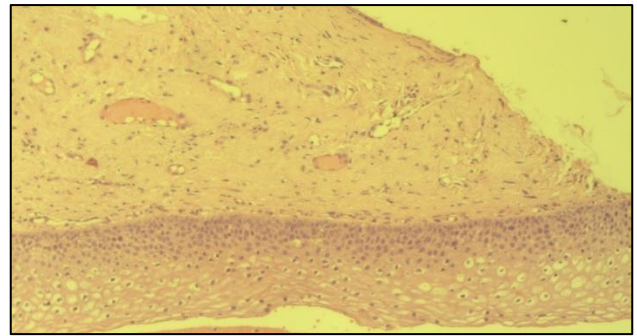


Figure 2: Cervical intraepithelial neoplasia-I (CIN-I), hematoxylin and eosin stain, 10X.

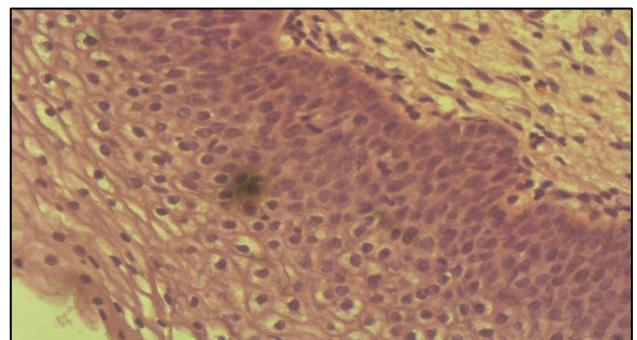


Figure 3: Cervical intraepithelial neoplasia-II (CIN-II), hematoxylin and eosin stain, 40X.

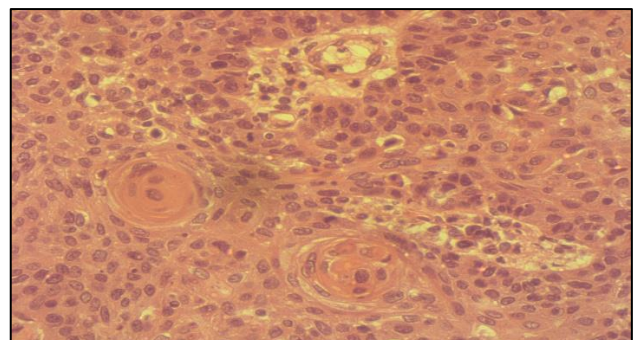


Figure 4: Cervical invasive squamous cell carcinoma, grade-I, hematoxylin and eosin stain, 40X.

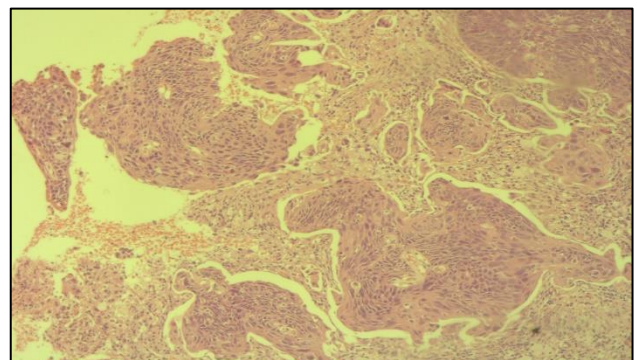


Figure 5: Cervical invasive squamous cell carcinoma, grade-II, hematoxylin and eosin stain, 10X.

Table 1: Age distribution of the study patients (n=143).

Age group (years)	Frequency	Percentage
<30	27	18.9
31-40	53	37.1
41-50	38	26.6
51-60	22	15.4
>70	3	2.1
Total	143	100.0
Mean±SD	41.2±11.5	
Median	40.0	
Range	(22-80) years	

Out of 143 study cases, most of the study patients were within at 31-40 years age group (53 cases, 37.1%). Mean age was 41.2±11.5 years. Range was 22-80 years (Table 1).

Table 2: Distribution of the study patients according to cervix type (n=143).

Cervix type	Frequency	Percentage
Nulliparous	2	1.4
Normal looking	3	2.1
Tubular	4	2.8
Broad hypertrophied	39	27.3
Atrophic	12	8.4
Erosion	35	24.5
Unhealthy	44	30.8
Prolapsed	2	1.4
Growth	2	1.4
Total	143	100.0

Squamocolumnar junction was clearly visualized in most of the study patients. It was in 121 cases (84.6%). Among the 143 study patients, most of the cases were presented with unhealthy cervix (44 cases, 30.8%) followed by broad hypertrophied cervix (39 cases, 27.3%). In 35 cases (24.5%), cervix was eroded, visual growth was found in only 2 cases (1.4%) (Table 2).

In this study, acetowhite area on the cervix was clearly distinguished in 93 cases (65%). In 42 cases (29.4%), acetowhite area was not found. Punctation was absent in most of the cases (129 cases, 90%). Only 14 patients (9.8%) showed punctation on the cervix. Most of the cases did not show any mosaicism (139 cases, 97.2%). Only 4 cases show mosaicism (2.8%). VIA test was positive in 98 study cases (68.5%) and negative in 45 cases (31.5%). Iodine was up taken in 80 cases (55.9%). 63 cases (44.1%) did not show any iodine uptake (Table 3).

On colposcopy examination, most of the cases show neoplastic proliferation (80 cases, 55.9%). Among them, most cases were diagnosed as cervical intraepithelial

neoplasia-I (CIN-I). 63 cases (44.1%) were non-neoplastic (Table 4).

Table 3: Distribution of the study patients according to punctation, mosaicism, VIA test and iodine uptake (n=143).

Topics	Findings	Frequency	Percentage
Punctation	Present	14	9.8
	Absent	129	90.2
	Total	143	100.0
Mosaicism	Present	4	2.8
	Absent	139	97.2
	Total	143	100.0
VIA test	Positive	98	68.5
	Negative	45	31.5
	Total	143	100.0
Iodine uptake	Taken	80	55.9
	Not taken	63	44.1
	Total	143	100.0

Table 4: Distribution of the study patients according to colposcopy findings (n=143).

Colposcopy findings	Frequency	Percentage
Non-neoplastic	63	44.1
CIN I	56	39.2
CIN II	15	10.5
CIN III	4	2.8
Malignant	3	2.1
Benign (polyp)	2	1.4
Total	143	100.0

Table 5: Comparison of SWEDE score among different type of colposcopy diagnosis (n=143).

Colposcopy findings	No. of patients	SWEDE score		P value
		Mean±SD	Range (min-max)	
Non-neoplastic	63	2.02±0.89	1.00-4.00	<0.001*
CIN I	56	5.02±0.75	3.00-7.00	
CIN II	15	8.07±0.26	8.00-9.00	
CIN III	4	9.50±0.58	9.00-10.00	
Malignant	3	10.00±0.00	10.00-10.00	
Benign (Polyp)	2	4.00±0.00	4.00-4.00	
Total	143	4.23±2.46	1.00-10.00	

P value reached from ANOVA test, *significant

One of the most popular procedures to predict the accuracy of colposcopy is SWEDE score. In our study,

among 143 study cases, SWEDE score was done. P value reached from ANOVA test which was significant (<0.001) (Table 5).

Among 143 cases, cervical biopsy was taken in 48 study cases. Most of the biopsies was diagnosed histopathologically as cervical intraepithelial neoplasia-I (CIN-I) (20 cases, 14.0%). Three cases were diagnosed as malignant neoplasm (2.1%), among them 2 cases were squamous cell carcinoma (1.4%) and 1 case was adenocarcinoma (0.7%) (Table 6).

In this study, sensitivity of diagnosis of cervical malignancy by colposcopy was 33.33% and specificity was 98.57% (Table 8).

Table 6: Distribution of the study patients according to histopathological diagnosis (n=143).

Histopathological diagnosis	Frequency	Percentage
Non-neoplastic	18	12.6
CIN I	20	14.0
CIN II	4	2.8
CIN III	2	1.4
Malignant		
SSC	2	1.4
Adenocarcinoma	1	0.7
Benign (polyp)	1	0.7
Not taken	95	66.4
Total	143	100.0

Table 7: Association of colposcopy findings with histopathological diagnosis (n=143).

Colposcopy diagnosis	Histopathological diagnosis	Histopathological diagnosis							Total	P value
		Non-neoplastic	CIN I	CIN II	CIN III	Malignant	Benign (polyp)	Not taken		
Colposcopy diagnosis	Non-neoplastic	4	2	0	0	0	0	57	63	0.001*
	CIN I	7	13	1	1	1	1	32	56	
	CIN II	5	1	3	0	1	0	5	15	
	CIN III	0	3	0	0	0	0	1	4	
	Malignant	1	0	0	1	1	0	0	3	
	Benign (polyp)	1	1	0	0	0	0	0	2	
Total		18	20	4	2	3	1	95	143	

P value reached from Chi-square test, *significant

Table 8: Diagnostic performance of colposcopy to predict malignancy.

Test	Disease Present		Disease Absent		Total
	n	a=	n	c=	
Positive	True positive	a=	False positive	c=	a + c = 3
Negative	False negative	b=	True negative	d=	b + d = 140
Total		a + b = 3		c + d = 140	
Statistic		Value		95% CI	
Sensitivity		33.33%		0.84-90.57%	
Specificity		98.57%		94.93-99.83%	
Positive predictive value (*)		33.33%		5.71-80.49%	
Negative predictive value (*)		98.57%		96.87-99.35%	
Accuracy (*)		97.20%		92.99-99.23%	

DISCUSSION

Cervical cancer is the second most common cancer worldwide next to breast in women. It is considered as a vital reproductive health problem and one of the major causes of mortality in women. Early detection by

screening program by colposcopy, VIA and pap test can dramatically reduce the mortality rate.¹³ The current study was planned to find out the correlation between the colposcopy, VIA test and pap test with histopathological diagnosis and to find out the sensitivity and specificity of colposcopy in detecting cervical malignancy.

Total 143 study cases were included in this study, most of the study patients were within at 31-40 years age group (53 cases, 37.1%). Mean age was 41.2±11.5 years. Range was 22-80 years (Table 1). Patel et al in 2011 observed premalignant cervical lesion in patients with 30-50 years age group which is similar to our study.¹⁴ Naik et al in 2015 showed that most vulnerable age group for cervical malignancy was 35-55 yrs.¹⁵ Usha et al also observed that, majority of the cervical abnormalities (85%) in India were detected in women <40 years of age.¹⁶

In our study, all the 143 study cases were grouped by visualization of squamocolumnar junction (SCJ or TZ). Squamocolumnar junction was clearly visualized in most of the study patients. It was in 121 cases (84.6%). The junction was partially visualized in 9 cases (6.3%) and not visualized in 13 cases (9.1%). By colposcopy, cervixes of most of the study cases were found to be unhealthy (44 cases, 30.8%). Broad hypertrophied cervix

was found in 39 cases (27.3%), cervical erosion was found in 35 cases (24.5%) and atrophied cervix was found in 12 cases (8.4%). Grossly growth was found in 2 cases (1.4%). Patel et al in 2011 found inflammatory changes in cervix in 57.48% cases, atrophy in 2.2% cases and squamous cell carcinoma in 0.7 % cases.⁶ Naik et al in 2015 found 53.8% cases of cervical inflammation and 5.8% cases of cervical cancer. These two studies have similar result with our study. Among 143 study patients, acetowhite area on the cervix was clearly distinguished in 93 cases (65%). In 42 cases (29.4%), acetowhite area was not found. Acetowhite areas are shady in 8 cases (5.65%). Punctations are capillary projections appears as tiny red dots, commonly found in inflammation, immature metaplasia and sometimes visible in cervical intraepithelial neoplasia-I (CIN-I).

In our study, among 143 cases punctation was absent in most of the cases (129 cases, 90%). Only 14 patients (9.8%) showed punctation on the cervix. Mosaicism in colposcopy is a pattern seen which is due to abnormal vessels and which resembles mosaic tiles. This mandates a biopsy of that region of cervix as it may be associated with cervical intraepithelial neoplasia. In our study, most of the cases did not show any mosaicism (139 cases, 97.2%). Only 4 cases show mosaicism (2.8%).

VIA (visual inspection by acetic acid) is a visual examination of the uterine cervix after application of 3-5% acetic acid. If the cervical epithelium contains an abnormal load of cellular proteins, the acetic acid coagulates the proteins conferring an opaque and white aspect of the concerned area. In our study, among 143 cases, VIA test was positive in 98 study cases (68.5%) and negative in 45 cases (31.5%). Usha et al showed that total VIA test was positive in 10.75% cases only which is far lower than our study. They ran a screening program on random population from 2006 to 2012 in India but our study population was included with definite abnormalities in cervix. So, most of our cases was VIA positive.¹⁶

Squamous epithelium contains glycogen, whereas precancerous lesions and invasive cancer contain little or no glycogen. Iodine is glycofytic and is taken up by the squamous epithelium, staining it mahogany brown or black. Columnar epithelium does not change colour, as it has no glycogen. Immature metaplasia and inflammatory lesions are at most only partially glycogenated and, when stained, appear as scattered, ill-defined uptake areas. Precancerous lesions and invasive cancer do not take up iodine (as they lack glycogen) and appear as well-defined, thick, mustard or saffron yellow areas. Thus, iodine can be used to identify precancerous and cancerous lesion of the cervix. We have done iodine uptake test in our study and iodine uptake was showed in 80 cases (55.9%). 63 cases (44.1%) did not show any iodine uptake (Table 3).

On colposcopy examination, among 143 cases, most of the cases show neoplastic proliferation (80 cases, 55.9%).

Among them, most cases were diagnosed as cervical intraepithelial neoplasia-I (CIN-I). CIN-II was found in 15 cases (10.5%), CIN-III was found in 4 cases (2.8%). Malignancy was seen in 3 cases (2.1%). 63 cases (44.1%) were non-neoplastic (Table 4).

One of the most popular procedures to predict the accuracy of colposcopy is SWEDE score. In our study, among 143 study cases, SWEDE score was done. P value reached from ANOVA test which was significant (<0.001) (Table 5).

Among 143 cases, cervical biopsy was taken in 48 study cases. Most of the biopsies were diagnosed histopathologically as cervical intraepithelial neoplasia-I (CIN-I) (20 cases, 14.0%). Three cases were diagnosed as malignant neoplasm (2.1%), among them 2 cases were squamous cell carcinoma (1.4%) and 1 case was adenocarcinoma (0.7%) (Table 6). Naik et al in 2015 found 8.6% cases of LSIL, 1.9% cases of HSIL, 5.8% cases of cancer and 53.8% cases of inflammation. Patel et al in 2011 in their study found 4.12% ASCUS, 0.1% LSIL, 0.1% HSIL, 0.7% squamous cell carcinoma.¹⁴

Association of colposcopy findings with histopathological diagnosis was done in our study. P value was reached through Chi-square test which was significant (0.001) (Table 7).

Diagnostic performance of colposcopy to predict malignancy was evaluated by sensitivity and specificity test. The sensitivity was 33.33% and specificity was 98.57% (Table 8).

There are few limitations of this study. Biopsy and excised specimens were not available for all the cases. The study was done in a single centre of a city which may not reflect the exact scenario of the total population of the country.

CONCLUSION

Colposcopy and pap smear test is widely accepted screening method. These are cost-effective non-invasive test for early detection of cervical malignancy and may be practiced in rural areas effectively. These may be highly effective to reduce the mortality and morbidity from cervical malignancy in low-income country like Bangladesh. Though the sensitivity is low, it may be overcome by proper training of health personal. Government and NGOs can take a prime role by health education, advertisement, campaigning and implementation of these screening programs at an early stage.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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