Cervical cytology in healthy and unhealthy cervices with acetic acid as agent for visual inspection

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ABSTRACT

Introduction: Cervical cancer is the fourth most common cancer among women globally. Though very common, it is a potentially preventable. In developing countries, there is a lack of effective screening programs, like routine Papanicolaou (Pap) smear testing. Also implementation of such programs in low-resource settings is difficult. **Aims :** To determine the best suited screening modality in our hospital setting among Pap smear and VIA. **Materials and Methods:** This was a prospective hospital based study done over a period of three years. It included 200 women who underwent Pap smear test and (Visual inspection with acetic acid) VIA and 120 women who underwent cervical biopsy. **Result:** 120 women were subjected to cervical biopsy. The most common finding on per speculum examination was white discharge followed by erosion. Incidence of epithelial cell abnormalities was more among 4th and 5th decades of life. Incidence of CIN was more in women married at a younger age i.e below 20 years of age. Sensitivity and specificity of Pap test was 80% and 93 % respectively and that of VIA was 93.3% and 80% which are statistically significant. PPV of Pap is 63.15% and VIA is 40% and NPV of Pap is 97.02% and VIA is98.8%.**Conclusion:** VIA is more sensitive than Pap smear but is less specific. The advantages of VIA are that it is simple, rapid, easy to perform, cost-effective, and its result is available immediately so that further investigations can be done in the same sitting. Thus, VIA is accurate screening test and suitable alternative to Pap smear.

Key words: Cervical screening programs, Pap smears, Cervical biopsy.

Introduction

Cervical cancer is the most common cause for cancer related death among women in developing countries[1]. Cervical cancer is the fourth most common cancer among women globally. In Indian women, breast and cervical cancers are the most common of all cancers.[2]Cervical cancer, though very common, is potentially preventable. In developing countries, there is a lack of effective screening programs like routine Papanicolaou (Pap) smear testing, which detects precancerous conditions before they progress to frank invasive stage. Implementation of cervical cytology based screening programs in lowresource settings is difficult. Hence, there is a need to

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look at alternative tests such as Visual inspection with acetic acid application [VIA], Visual inspection with acetic acid application with magnification [VIAM], visual inspection on Lugol's Iodine application [VILI]. These alternative tests should be low cost, easy to perform and at the same time should not compromise on the sensitivity and specificity for detecting the precancerous conditions of the cervix. An analysis of population-based surveys indicates that coverage of cervical cancer screening in developing countries is 19% compared to 63% in developed countries [3]. This further emphasizes the need for an effective and rapid screening method which can cover a larger population in lesser time. This study was done to compare VIA with Pap smear test so as to study the accuracy of VIA, as it is a simpler and easier technique that can be used as screening test in low resource settings and also in our institution.

Materials and methods

This was a prospective hospital based study carried out over a period of three years (2013 to 2015) in the department of Obstetrics and Gynecology, MGMH, Petlaburz, Hyderabad. The study included 200 women attending the Gynecology out- patient and in- patient departments. The study included both symptomatic and asymptomatic women ie, women who had come for primary or secondary infertility issues.

Inclusion criteria

Age : >20 years, Non pregnant women, Sexually active and both nulliparous and multiparous

Exclusion criteria

Age : < /=20 years, unmarried women, pregnant women, women with active vaginal bleeding, women with intra vaginal medications or douching history in the past 48 hours, women having frank cervical growth as seen on per speculum examination.

Women fulfilling the above inclusion criteria were explained about the tests to be conducted and an informed consent was taken. A detailed history including symptomatology, age at marriage, age of menarche, menstrual history, obstetric history, use of contraception, past medical and surgical history, history of sexually transmitted diseases (STDs) and other risk factors for carcinoma cervix were recorded. General physical examination followed by per abdominal examination was done.After placing the woman in lithotomy position, a sterile, lubricated, selfretaining bivalved Cusco's speculum was inserted, the cervix was visualized and naked eye appearance of the cervix was noted. Presence of mucopurulent discharge, erosions, polyp, congestion and hypertrophy were considered unhealthy.Rest of the subjects were considered as having healthy cervix. Following per speculum examination, Pap smear was taken using Aver's spatula with a 360 degree rotation in both healthy and unhealthy cervices. Smear was immediately transferred to a glass slide and placed in Coplin's jar for fixation. Following this, cotton tipped

swab containing 5% acetic acid was used to smear the cervix and result was noted after 60 seconds. The detection of any dense, opaque acetowhite area, well demarcated from the surrounding epithelium was considered positive. If no acetowhite areas were recorded, or if a whitish appearance was doubtful, the test result was considered negative.

As for Pap smear test, report showing LSIL (low grade squamous intraepithelial lesion) and above were considered positive. Cervix was biopsied in all women with unhealthy cervices and in women with healthy cervices who were screened positive either on Pap smear or VIA or both and the biopsied specimens were sent for histopathological examination. Biopsy showing Cervical Intraepithelial Neoplasia and Carcinoma were considered abnormal.

Pathophysiological basis of VIA

Application of 5% acetic acid causes a reversible coagulation, or precipitation of the cellular proteins. The normal squamous epithelium appears pink and the columnar epithelium red, due to the reflection of light from the underlying stroma, which is rich in blood vessels. If the epithelium contains a lot of cellular proteins, acetic acid coagulates these proteins, which may obliterate the color of the stroma. The resulting acetowhitening is seen distinctly as compared with the normal pinkish color of the surrounding normal squamous epithelium of the cervix, an effect that is commonly visible to the naked eye.

Results

A total of 200 women were included in the study. Informed consent was taken from all the subjects. 153 women (76.5 %) were symptomatic and 47 women (23.5 %) were asymptomatic and were anxious to conceive. On per speculum examination (naked eye appearance) of these 200 women; 119 (59.5 %) cervices were found to be unhealthy (either having white discharge, erosion, congestion, hypertrophy or polyp) while 81 (40.5 %) cervices were healthy.

Table 1: Demographic distribution of study group					
Age group	Unhealthy cervices	Healthy cervices	Total	%	
21-29 yrs	17	29	46	23	
30-39 yrs	44	24	68	34	
40-49 yrs	34	22	56	28	
>=50 yrs	24	6	30	15	
Religion					

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Hindus	75	53	128	64
Muslims	42	26	68	34
Others	2	2	4	2
Parity				
Nulliparous	4	16	20	10
1-2	25	19	44	22
3-4	66	38	104	52
5 and above	24	8	32	16
Socio-economic class				
Ι	-	-	-	-
II	10(8.4%)	10	20	10
III	16(13.44%)	16	32	16
IV	34(28.57%)	30	64	32
V	59(49.57%)	25	84	42
Age at marriage				
10 - 19 years	70	48	118	59
20 - 29 years	48	32	80	40
30 years and above	1	1	2	1

Majority of the women included in the study were in the age groups of 30-49 years. Unhealthy cervices were more in the same age group too. But in the age group 50 years and above; 80% of the included women had unhealthy cervices. When religion was considered, 75 cases (64%) of the women were Hindus, Majority of unhealthy cervices were among para 3 and para 4 women. And maximum percentage (75%) of unhealthy cervices were among para 5 and above. When age at marriage was considered, 118 (59 %) women had consummation of marriage in their teenage. 80 (40 %) women got married in their twenties. 78 (39%) of women were sterilized, 49 (24.5%) women practiced none of the contraceptive methods, 44 (22%) of them used condoms, 19 (9.5%) of the women used oral contraceptive pills while only 10 (5%) used intrauterine contraceptive device.Of the 200 women screened by Pap smear; 40 women had normal smears. Majority of the women (140) had inflammatory smears, i.e; 180 (90 %) women out of 200showed negative for intraepithelial lesion or malignancy while 20 (10 %) women showed dysplasia i.e. LSIL (low grade squamous intraepithelial lesion) and above. Of the 20 cytology positives, 7 were LSIL and 13 were HSIL (high grade squamous intraepithelial lesion). None of the smears showed malignant cells on cytology. So 10% of women included in the study had positive cvtology.Of the 20 women with positive cytology showing dysplasia, 17 women had unhealthy cervices

while 3 women had healthy cervices. Among the 3 women with healthy cervices, two presented to gynaecology OPD with primary infertility and they belonged to the age group 21-29 years. And one woman with healthy cervix showing HSIL belonged to the age group 50 years and above. Among all the women screened by VIA, 38 women showed distinct acetowhite areas; i.e. 19% of women were VIA positive. Of the 38 women, 6 had healthy cervices while 32 were unhealthy. Of the 200 women screened; only Pap smear was positive in 5 women and only VIA was positive in 23 women; while both were positive in 15 women.Among the 200 women included in the study, 119 had unhealthy cervices and 9 women with healthy cervices were screened positive. But only 120 women contributed to cervical biopsy sample. Of the 8 women who refused, 1 was pap smear positive with a healthy cervix (nulliparous woman with primary infertility belonging to 21-29 years age), 3 were VIA positive(1 with unhealthy cervix and 2 with healthy cervices) and 4 of them had unhealthy cervices with both Pap smear and VIA negative.Of the 120 women who underwent cervical biopsy, 15 women showed abnormality, i.e; positive for malignancy or CIN.The incidence of dysplasia on cytology, acetowhite areas on VIA and the incidence of malignancy and CIN on biopsy in comparison with different age groups is shown.

Age group	Cytology positive	VIA positive	Biopsy positive
21-29 years	2(10.5%)	2(5.7%)	-
30-39 years	3(15.7%)	7(20%)	2(13.3%)
40-49 years	6(31.5%)	10(28.5%)	2(13.3%)
50 years and above	8(42.1%)	16(45.7%)	11(44%)
	19	35	15

 Table 2: Comparison of tests (Cytology, VIA and biopsy)

In the age group of 21-29 years, only two women showed positivity with VIA and 2 women showed positive cytology but none of them revealed abnormality on biopsy. Incidence of dysplasia on cytology, VIA positivity and incidence of malignancy on biopsy increased with age with the maximum incidence in the age group of 50 years and above. When dysplasia was compared with parity; of the 19 cytology positives, two were nulliparous, three women were of para 1 and 2, six women were of para 3 and 4 and eight women were of para 5 and above. On comparing VIA with parity, three were nulliparous, five were para 1 and 2, ten were para 3 and 4 while 17 women were para 5 and above. Biopsy revealed CIN III (cervical intraepithelial neoplasia) in a single case who was paral. 3 women of para 3 and 4 revealed malignancy on biopsy while 1 woman of para3 showed CIN II.Ten women of para 5 and above showed malignancy on biopsy. Of the 10 women with abnormal biopsies, 9 were squamous cell carcinomas while 1 was adenocarcinoma. Thus, it is evident that incidence of dysplasia and malignancy increases with parity.When socioeconomic status is considered, it was observed that incidence of dysplasia on cytology, positivity with VIA and malignancy on biopsy was highest in the lower socioeconomic class.11 of 19 cytology positives, 20 of 35 VIA positives and 10 of 15 biopsy positives were from the lower socioeconomic class.Of the 19 positive pap smears, 11 women were married in their teenage and the remaining 8 women got married in their twenties. Of the VIA positives, 24 of the 35 women had consummation of marriage in their teenage and the rest of the 11 women in their twenties. 7 out of 15 women with malignancy on biopsy and 2 women with high grade CIN got married in their teenage while 6 women positive for malignancy married in their twenties. Earlier the age at marriage, more is the incidence of dysplasia and malignancy.

	Biopsy positive	Biopsy negative	
Cytology positive	12	7	19
	TP	FP	
Cytology negative	3	98	101
	FN	TN	
Total	15	105	
VIA positive	14	11	35
_	TP	FP	
VIA negative	1	84	85
- The second sec	FN	TN	
Total	15	105	

Table 3: Sensitivity and specificity of Cytology and VIA

Sensitivity and specificity of Cytology

Sensitivity = TP/TP+FN * 100 = 12/15 * 100 = 80%Specificity = TN/TN+FP * 100 = 98/105 * 100 = 93%PPV = TP/TP+FP * 100 = 12/19 * 100 = 63.15%NPV=TN/TN+FN * 100 = 98/101 * 100 = 97.02%Statistical test of significance : chi square test; "p value" : <0.0001(significant)

Sensitivity and specificity of VIA

Sensitivity = TP/TP+FN * 100 = 14/15 * 100 = 93.3%Specificity = TN/TN+FP * 100 = 84/105 * 100 = 80%PPV = TP/TP+FP * 100 = 14/35 * 100 = 40%NPV = TN/TN+FN * 100 = 84/85 * 100 = 98.8%Statistical test of significance : chi square test; "p value" : <0.0001(significant)

Discussion

Cervical cancer is more common in developing countries as compared to developed countries. This can be attributed primarily to the effective screening programs that are present in the developed countries. According to the literature, a large number of studies (Bhattacharya et al,[4] Hegde et al [5]) were done in various parts of our country and across the world to assess the effectiveness of VIA and Pap smear as a screening tool. Majority of them concluded that VIA is a more effective screening tool than Pap smear in decreasing the incidence and mortality related to cervical cancer.In the present study, 200 women attending the gynecology OPD and those admitted in wards at MGMH, Petlaburz and who fulfilled the inclusion criteria were included. Similar hospital based study was first performed in 1982.[6] In 1997, University of Zimbabwe/JHPIEGO Cervical Cancer Project involved around 8731 women screened by VIA.[7] Recent studies that were performed using a hospital-based population were conducted in 2005 by Goel et al [8] involving 400 patients and in 2007 by Khan S et al [9] involving 300 women.Women aged >20 years were included in this study. Most of the participants belonged to the age group 30-39 years. In a study done by Goel et al[8] most subjects belonged to the age group of 30-34 years. Whereas Khan et al[9] in 2007 studied this screening method in the age range of 25-65 years.In our study, comparison was made between VIA and Pap smear with cervical biopsy as gold standard. Cervical biopsy was done in all unhealthy cervices and in those healthy cervices that were screened positive. Bhattacharya et al [4] conducted a similar study among 300 women over a span of one year, where Pap and VIA were done in all women and those who were screened positive were subjected to cervical biopsy. In a study by Goel et al [8] comparison was made between VIA, pap smear and colposcopy directed LLETZ (large loop excision of transformation zone) instead of cervical biopsy. In a study by Khan et al[9] visual inspection with Lugols iodine (VILI) in addition to VIA and cytology was done and patients with positive findings were scheduled for colposcopy-guided biopsies. A much larger study was conducted by Satyanarayana et al[10]

including 4988 women of north India, who were subjected to VIA, Pap and VILI and followed with cervical biopsy in positively screened women.In the present study, 34% belonged to the age group 30-39 years, while 43% belonged to the 4th and 5th decades of life. Incidence of dysplasia among 3rd decade was found to be 15.7% in our study. Dysplasia was found to be maximum (47.3%) in 50 years and above age group. But dysplasia constituted 84.1% combined in women of 4th and 5th decades. A similar study was conducted in Gauhati Medical College and Hospital by Bhattacharya et al[4]among 300 women attending the Gynecology OPD over a span of 1year from 2012-2013. In their study too, 34% women belonged to age group 30-39 years and 52% belonged combinedly to 4th and 5th decades. And dysplastic changes noted were 6.25% among 3rd decade and 87.5% in the 4th and 5th decades. This finding is in contrast with the usual incidence of precancerous lesions of the cervix at the age of 25-30 years of life. This could be explained by the fact that, in India, the type of cervical cancer screening is opportunistic and though three fourths of the population knew that cervical cancer could be detected early by a screening test, only 6.9 per cent had ever done the Pap test.[11] The women included in the study had never undergone any previous pap smear test as majority of them now presented to OPD with complaints. Had they undergone screening as per guidelines, these precancerous lesions would have been detected at an earlier age group.Our study included 59.5% of the patients with abnormal appearing cervix on speculum examination. It was found that 85% of epithelial cell abnormalities were noted in the patients with findings of abnormal appearing cervix. And 86.6% of CIN and carcinomas were noted in unhealthy cervices. 96.19% of normal findings were also accompanied by unhealthy cervices. Sehgal et al [12] study found that 40-50% of cancers were accompanied by abnormal appearing cervices. But 85.7% of the normal or benign lesions were also noted in the patients with the findings of abnormal appearing cervix. Hence findings of cervix on speculum examination like congestion, erosion, and hypertrophy cannot be attributed completely topremalignant lesions. These

findings could be due to the process of inflammation also. Therefore it warrants universal screening for all the patients irrespective of cervical findings on visual inspection.In our study, majority of the women included were of parity 3 and above, who, together constituted 68% of the total study group. And the rest 32% were either nulliparous or of parity index 1 and 2. Maximum number of women in the study with unhealthy cervices was of parity 3 and above.66.9% of those with parity 3 and above and 45.3% of those with parity index less than 3 had unhealthy cervices. This finding is concordant with the study done by Pradhan et al,[13]in 2007 which showed that unhealthy cervix was more common finding in women of higher parity (>=3 children) than in women of lower parity (63% vs 46%) and vice versa showing healthy cervix less common in higher parity women compared to lower parity ie, 37% vs 54%. In the present study, dysplasia on cytology was more in women with para 3 and above than in those with parity less than 3. Dysplastic changes were seen in 7.8% of women with parity below 3 and in 10.2% with parity 3 and above.Similar findings were obtained in studies conducted by Bhattacharya et al [4] and Pradhan et al.[13]Of the women included in our study, 84 women (42%) were of the socioeconomic class V and 32% were of class IV. 49.57% of class V women had unhealthy cervices. On cytologic screening, 11 of the 19 women with dysplastic changes belonged to class V.This accounted to 57.8% dysplastic changes in class V women. While 31.5% dysplasia was noted in women of class IV and 10.52% dysplastic changes were in class III women. No dysplasia was noted in class I and II in the present study. This finding of highest incidence of dysplasia in the lowest socioeconomic class is similar with the findings of other studies done by Mhaske et al [14], Kashyap et al[15] and Bhattacharya et al.[4]In the present study, considering the age at marriage, 59% study group got married at and below the age of 20 years while 41% got married at an age above 20 years. Among these women, 58.8% of unhealthy cervices were in those women who got married before 20 years age. And 41.2% of unhealthy cervices in those married at 20 years and above. Pradhan et al.[13]study also stated that women having unhealthy cervix were more likely to get married before the age of 20 years than the women with healthy cervix. On cytological screening, 63.15% of dysplasia was among women who got married at and before 20 years and 36.8% of dysplasia noted in women married above 20 years age. This correlation of dysplasia with early consummation of marriage was similar to the study by Pradhan et al [13] which showed that 64.2% of dysplasia was noted in women married below 20 years and 42.8% dysplasia in

those married later. Study by Mhaske et al [14] showed a much higher incidence of dysplasia among women married at a younger age. Their study showed that 93.8% dysplasia occurred in women married at an age of 17 years and below and only 6.17% in women married after 17 years age. This disparity was probably due to higher sample size and a higher percentage (86.38%) of women married at an age of 17 years and below compared to the present study.In the present study, considering n=120 (net study group who were subjected to PAP, VIA and biopsy); it was noted that 15.8% of smears were abnormal. Bhattacharya et al[4] reported a cytology positive rate of 7.3%. Patil et al [16] found that 17.5% of the study group had abnormal smears. Study by Hegde et al [5] showed 11.7% abnormal smears. University of Zimbabwe/JHPIEGO Cervical Cancer Project [7]found that 14.6% of the women in their study had an abnormal Pap smear. Megevand et al [17] noted an abnormal Pap smear in 13% of their study population. The VIA positive rate in our study was 29.16% (n=120). Cecchini et al [18] reported positive VIA in 25.4% in their study. Bhattacharya et al [4] study showed VIA positivity in 17.3%. But study by Hegde et al[5] and Goel et al[8] showed VIA positive rates of 12% and 12.5% respectively. Megevand et al [17] reported an incidence of abnormal smears in 3.13% in their study. This wide range of variation is due to the difference in interpretation since some of the studies used nurses or paramedical workers to do the test. It also depends on the study population since few studies were done on symptomatic hospital-based population and others as a mass screening test. The VIA test is also affected by the quality of acetic acid, concentration of the acetic acid, lighting, and visualization. In the present study, biopsy positivity was noted in 12.5% women. Hegde et al[5] study showed a biopsy positivity rate of 10.5%. Goel et al [8] study revealed a dysplasia rate confirmed on histopathology in 7.5% of their population. Bhattacharya et al [4] revealed biopsy positive rate of only 6.3%. Singh K N et al [19] confirmed positive biopsy in 3.6%. Since these studies involved a larger number of patients, their incidence of dysplasia was less in comparison to ours. In our study, majority of the women screened presented to Gynecology OPD with complaints and hence would belong to high risk group.That would also be another contributory factor to the high incidence of biopsy-confirmed dysplasia in our study.In our study, VIA could detect all cases of CIN and carcinoma. Two cases of CIN II and III were missed by Pap smear but were detected by VIA. Only Pap smear was positive in 5 women while only VIA was positive in 23 women, while both Pap and VIA were positive in 15 women. In the study conducted by

Hegde et al [5] VIA did not detect six cases of mild dysplasia but did not miss any case of moderate and severe dysplasia. Two cases of mild dysplasia and one case of severe dysplasia that were not detected by Pap smear were detected by VIA. In a study done by Singh et al [19] out of 27 cases, 25 were detected by VIA, and only 20 cases were detected by Pap smear. In a study done by Goel et al[8] out of 30 dysplasia cases, Pap smear was positive in only 15 and VIA in 29 cases. All the compared studies show that VIA could detect more number of cases when compared to Pap smear and could even detect those cases missed by Pap smear.In the present study, sensitivity of Pap smear is 80% which is less than that of VIA which is 93.3%. While specificity of Pap smear is higher than VIA which is 93% and 80% respectively. Similar results were obtained in the study conducted by Bhattacharya et al [4]where sensitivity of VIA was higher than that of Pap smear, accounting for 89% and 52% respectively. And the specificity was noted to be more with Pap than VIA, i.e.; 95% vs 87%.Singh et al [19] study showed sensitivity of VIA to be 93.1% while that of Pap smear was 70.02%. While specificity of VIA was 86.8% and that of Pap smear was 97.2%. According to Goel et al [8] VIA was more sensitive than Pap smear (96.7% vs 50%) but VIA had lesser specificity than Pap.(36.4% vs 97%) University of Zimbabwe/JHPIEGO [7] study also showed that VIA is more sensitive than Pap smear but less specific than Pap smear. Hegde et al [5] also agrees that cytology as a screening test has 98-99% specificity but as far as the sensitivity is concerned, it drops to around 50 %.

Study	Screening test	Sensitivity	Specificity
Present study	Pap	80%	93%
	Via	93.3%	80%
Bhattacharya et al(2015)[4]	Pap	52%	95%
	Via	89%	87%
Hegde et al(2011)[5]	Pap	83%	98%
	Via	70.8%	95%
Singh et al(2010)[19]	Pap	70.02%	97.2%
	Via	93.1%	86.8%
Goel et al(2005)[8]	Pap	50%	97%
	Via	96.7%	36.4%
University of zimbabwe/	Pap	44%	91%
jhpiego(1999)[7]	Via	77%	64%

Our study revealed a PPV of 63.15% for Pap smear while it was 40% for VIA. This is explained by the high incidence of false positives with VIA. False positives with Pap smear were 7 and with VIA were 11. NPV of Pap smear is 97.02% and that of VIA is 98.8%; which are comparable. In the study by Bhattacharya et al [4] Positive predictive value is 45% in Pap smear as compared to 32% in VIA while Negative predictive value of VIA is 99% as compared to 96% in Pap smear. Most of the women who undergo screening with Pap smear in developing countries do not come for follow-up or do not collect their report on time thereby leading to delay in diagnosis and treatment. The advantage of VIA is that it is a real-time screening test where results are immediately known and appropriate counselling and referral for treatment can be immediately started.

Conclusion

From the present study, it is evident that VIA is more sensitive than Pap smear test. Specificity of VIA is less than that of Pap smear and the false positivity could be overcome by subjecting all VIA positive cases to colposcopy and directed biopsies. The advantages of VIA are that it is simple, rapid, easy to perform, does not require much infrastructure, it is cost-effective, and its result is available immediately. If the test result is positive, in the same setting, further investigations can be carried out and treatment can be planned, ensuring a better patient compliance. It can be done even by health care personnel even in remote areas with minimum facilities. Thus VIA is an accurate screening test and a suitable alternative to Pap smear testing.

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