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Comparative analysis of different cervical screening methods with special reference to the WHO recommended human papillomavirus detection at a rural tertiary care center - A pilot study

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ABSTRACT

Background: Cervical cancer (Ca Cx) the 4th common cancer has a high mortality rate. Human Papilloma Virus (HPV) is chiefly responsible for a long latent period to progress from preinvasive to invasive Ca Cx. So it can be prevented by screening procedures such as visual inspection with acetic acid (VIA), conventional cervical PAP smear (CP), liquid-based cytology (LBC), and high-risk (HPV-hr) detection along with vaccination. **Aims and Objectives:** The study aims to determine the socio-demographic profile of Ca Cx and the most appropriate cost-effective community screening method. **Materials and Methods:** A prospective observational study with 80 symptomatic women of reproductive and peri/postmenopausal age groups attending the Gynecology Department who underwent VIA, CP, and LBC and thereby detected positive along with screened negative but clinically suspicious women were tested for HPV-hr detection. Results were tabulated into an Excel sheet and screening methods were compared using Chi-square test, gold standard being cervical histopathology ($P<0.5$ significant). Statistical software used - Graphpad Prism 9 (San Diego, CA). **Results:** The mean age was 43.3 years. Multiparity and early marriage were seen in 80% and 45% respectively. Inflammatory lesions were best diagnosed by CP but epithelial cell abnormality and more satisfactory smears by LBC ($P<0.05$). Sensitivity was highest (93.75%) in VIA, positive predictive value, and specificity in CP (91.66%, 75%). negative predictive value was highest in VIA and HPV-hr DNA (50%). **Conclusion:** VIA with CP/LBC is an economically viable and effective screening method for Ca Cx in developing countries along with HPV vaccination.

Key words: Cervical cancer; Visual inspection with acetic acid; Cervical PAP smear; Liquid based cytology; Human papilloma virus-high risk

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INTRODUCTION

Carcinoma cervix (Ca Cx) is the 4th most common cancer worldwide with high mortality.[1] Human Papillomavirus (HPV) is linked to Ca Cx in almost all cases (99%). Age of marriage, parity, use of oral contraception, smoking, and obesity are other risk factors. A long latency period is observed to produce invasive Ca from a preinvasive one. Hence, prevention is important.[1] Primary prevention is presently by HPV vaccination for girls 9–13 years.[2] Secondary prevention is to screen and treat preinvasive carcinomas.[3,4] Screening tests in vogue are visual inspection with acetic acid (VIA), visual inspection using Lugol's iodine (VILI), conventional cervical PAP smear (CP),

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liquid-based cytology (LBC) and HPV-high risk (HPV-hr) DNA detection.[2,5] Cervical cytology screening since long had reduced the Ca Cx-related morbidity and mortality to a great extent. However, paradigm shift from VIA, VILI, and cervical cytology to HPV-hr detection (World Health Organisation [WHO] recommended preferred method) is due to its high sensitivity, specificity, lesser invasiveness, easy collection, and test procedure.[6-9] Young people mostly having self-resolving transient infection and Ca Cx being uncommon under the age of 21 years, the WHO strategy for the acceleration of Ca Cx elimination targets include.[1,2,5]

- 90% of girls should be vaccinated by 15 years of age with HPV-hr
- 70% of women should be screened by the age of 35 years and again by the age of 45 years with high-performance screening methods
- 90% of women with detected cervical disease should be treated/managed accordingly.

Different recommendations[7,10-13]

United States Preventive Services Task Force

- Ages 21–65 years: Pap test only every 3 years
- Ages 30–65 years: Add primary HPV testing every 5 years or
- Ages 30–65 years: Pap plus HPV (co-testing) every 5 years.

American Cancer Society

- Ages 25–65 years: Primary HPV testing every 5 years or
- Ages 25–65 years: Pap test only every 3 years or
- Ages 25–65 years: Pap plus HPV (co-testing) every 5 years.

American Academy of Family Physicians

- Ages 21–65 years: Pap test only every 3 years
- Ages 30–65 years: Primary HPV testing only every 5 years or
- Ages 30–65 years: Pap plus HPV (co-testing) every 5 years.

American College of Obstetricians and Gynecologists

- Ages 21–65 years: Pap test only every 3 years
- Ages 30–65 years: Pap plus HPV (co-testing) every 5 years.

American Society for Colposcopy and Cervical Pathology

- Ages 21–65 years: Pap test only every 3 years
- Ages 30–65 years: Pap plus HPV (co-testing) every 5 years.

WHO recommendation

HPV DNA detection in screen and triage and/treat approach from 30 years with follow-up at 5–10 years interval. Human Immunodeficiency Virus females should start the same at the age of 21 years.[4]

India shoulders are 1/5th of the global burden of Ca Cx with an incidence rate 18/100,000; cumulative risk 2.01%; 5-year survival 46% (approximately) and diagnosis at advanced stage 80%.[5] The National Cancer Control Programme of 1976 later integrated with National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Disease and Stroke (NPCDCS) in 2010 recommended Ca Cx screening in the manner depicted in Table 1.[14]

Amidst so many recommendations, some expensive and some requiring high expertise, we from the rural tertiary health center venture to go for a pilot study for comparative analysis of all the screening methods so that we can opt for the most cost-effective one without compromising the statistical part.

Aims and objectives

- To determine the sociodemographic profile of women undergoing screening procedure for Ca Cx.
- To measure the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) and to adopt the most practicable and economically viable Ca Cx screening method in a rural set-up with limited resources.

MATERIALS AND METHODS

Using the prevalence formula, the sample size was estimated for this prospective observational study and the study being a pilot one 1/3rd of the size amounting to approximately 80 women of reproductive and peri or post-menopausal age group, who presented with clinical symptoms or unhealthy cervix on examination at Gynecology Out Patient department and In Patient Department from January 01, 2023, to January 06, 2023, were subjected to screening with VIA (5% acetoacetic acid), CP and LBC (Easyprep sure path) after getting proper consent. Screened positive cases in all methods and screened negative cases with high clinical suspicion of having Ca Cx were sent for HPV-hr DNA Truenat testing against hr strains like 16, 18, 31, and 45. The diagnoses were compared among themselves, the gold standard being histopathological (H/P) examination of biopsy specimens. The data were analyzed using GraphPad Prism 9 (San Diego, CA) ($P<0.05$ significant). The study was done after obtaining institutional ethical clearance (IEC no: IEC/NBMC/M-04/F-02/2022).

RESULTS

The most common age group of the patients under study is 40–49 years (50%). Cases with early age of marriage

Table 1: NPCDCS Ca Cx screening guidelines

Serial Number	Strategy	Essential/limited resource	Optimal/enhanced resource	Optional/high resource
1	Primary screening methods	VIA	HPV DNA	HPV DNA
2	Where: place for screening	Health and wellness centres, Primary and Community Health centre (PHC and CHCs)	Health and wellness centres, PHC, CHCs	Dist. Hospitals, private health care facilities
3	By whom	Trained primary care workers, trained nurse	Trained nurse, physician	Trained nurse, physician
4	Target screening ages	30–65 years	30–65 years	25–65 years
5	Frequency of screening	One to 3 times in a lifetime	10 years if two consecutive negative tests at 5-year intervals	5 years
6	Exiting screening	Resource dependent	65 years of age or older with consistently negative results over the past 15 years	65 years of age or older with consistently negative results over the past 15 years
7	Use triage and diagnostic tests	VIA: See and Treat	Cytology (Quality Assured) VIA	HPV 16/18 Genotyping Cytology: Quality assured
8	After triage		Negative: Follow-up in 12 months Abnormal/positive: Colposcopy if available	Negative: Follow-up in 12 months Abnormal/positive: Colposcopy if available

NPCDCS: National Programme for Prevention and Control of Cancer, diabetes, cardiovascular disease and stroke, VIA: Visual inspection with acetic acid

≤20 years of age (35%) and 21–35 years group (60%) having gynecological problems were more in our study. Symptoms of white discharge were seen in 56% of cases. Oral contraception use was seen in 40%, non-users were 20% and other methods of contraception users were 20%. Out of 80 patients VIA positive cases were 18 (22.5%), CP positive for atypical epithelial cells 12, and LBC positive cases were 15.

Comparative analysis was done between CP and LBC in relation to cytomorphology, cellularity, background of smear, distribution of cells, cellular and nuclear changes, and presence of inflammatory cells (Table 2). Statistically significant P<0.001 is seen in relation to inflammatory cells, clear background, cellular overlapping, uniform distribution, architectural changes, cellular changes with the degree of freedom being 1 while cellularity and nuclear changes being nonsignificant.

Inflammatory (mostly seen under 45 years of age) and unsatisfactory smears were reported more in CP. Negative for intraepithelial lesion/malignancy (NILM) other than inflammation (examples being atrophic smear, smears with reparative atypia, etc.,) and precancerous/cancerous lesions (i.e. epithelial cell abnormality) were more in LBC. Epithelial cell abnormality includes atypical squamous cells of undetermined significance (ASCUS- Figure 1), atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (HSIL) (ASC-H), HSIL, low grade squamous intraepithelial in lesion (LSIL), squamous cell carcinoma (Figure 2), adenocarcinoma, atypical glandular cells not otherwise specified etc. as per the available findings on smears following Bethesda reporting system.

Table 2: Comparative analysis of CP and LBC cytomorphology and smear background

Cytomorphological changes	PAP (%)	LBC (%)	P-value df=1
Cellularity	72 (90)	75 (93.75)	NS
Clear background	8 (10)	72 (90)	S
Uniform distribution	16 (20)	56 (70)	S
Cellular overlapping	64 (80)	20 (25)	S
Architectural changes	52 (65)	12 (15)	S
Cellular changes	60 (75)	12 (15)	S
Nuclear changes	16 (20)	20 (25)	NS
Inflammatory cells	72 (90)	20 (25)	S

LBC: Liquid-based cytology

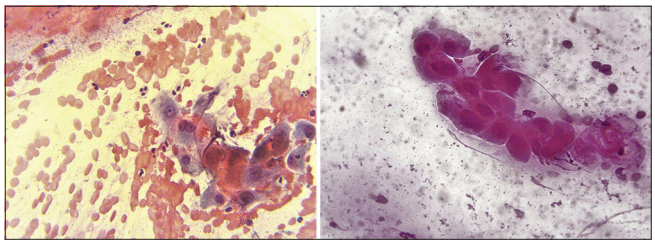

Figure 1: Atypical squamous cells of undetermined significance - conventional versus liquid-based cytology

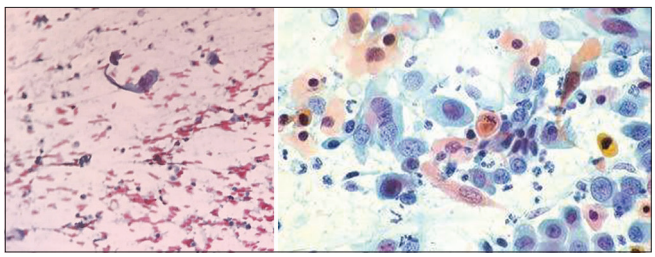

Figure 2: Squamous cell carcinoma - conventional versus liquid-based cytology

Total epithelial cell abnormality in CP was 12 and in LBC was 15. Inflammatory smears are generally categorized as NILM but for the purpose of better comparison, inflammatory smears are separated from other NILMs (Table 3 and Figure 3).

All VIA (18 in number), CP (12 in number), and LBC (15 in number) positive cases along with screened negative but clinically highly suspicious patients underwent HPV-hr DNA screening, total patient being 20 in number. The gold standard was H/P examination of all these 20 patients. HPV-hr DNA positivity was in 16 cases (mostly against HPV-hr 16 and 18 strain), rest 4 being negative while H/P reports were positive in 15 cases and negative in 5 cases. 18 positive VIAs when compared with H/P, 15 cases were true positive (TP), 3 cases were false positive (FP) and 1 each came as true negative (TN) and false negative (FN). CP showed 11 TP, 1 FP, 3 TN, and 5 FN. LBC had 13 TP cases, 2 FP cases, 2 TN cases, and 3 FN cases. And the last one, i.e., HPV-hr DNA resulted in 14 TP, 2 FP, 2 TN, and 2 FN.

Sensitivity, specificity, PPV, and NPV were calculated accordingly and compared (Table 4).

Sensitivity was highest in VIA (93.75%) followed by HPV-hr DNA (87.5%) and LBC (81.25%), the lowest being CP (68.75%). Specificity is lowest in VIA (25%) followed by in ascending order as 50% both in LBC and HPV-hr DNA, highest being CP (75%). CP also showed the highest value in relation to PPV (91.66%) followed by HPV-hr DNA (87.5%) and LBC (86.66%). PPV showed lowest value in VIA (83.33%). HPV-hr DNA and VIA showed shared highest value of 50% in NPV, lowest being CP (37.5%). NPV of LBC is 40%.

All the screening tests mostly have values not wide apart from each other and when compared with H/P results individually and also amongst themselves statistically significant P-value did not surface.

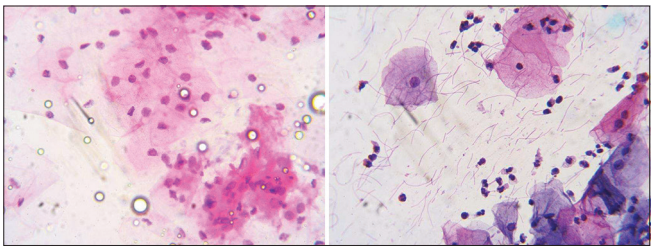

Figure 3: Liquid based cytology -reactive and fungal

DISCUSSION

The sociodemographic profile of the patients found in our study is mostly concordant with other studies such as Aboobacker and Shariff; Taylor et al., Raj and Srivastava; Alta et al., in relation to age, parity, early age of marriage, presenting symptoms and use of contraception,[15-18] but having partial discordance with Raj and Srivastava; Alta et al., in relation to presenting symptoms of white discharge and oral contraception.[17,18]

VIA positivity in our study was 22.5% being discordant with studies of Raj and Srivastava (acetowhite areas 60.81%) and Vahedpoor et al., (50.5%).[17,19]

In our study unsatisfactory and inflammatory smears were diagnosed more in CP, NILM more in LBC as well as epithelial abnormality too. This is concordant with a study conducted by Hegde et al.,[20] and discordant with Ilter et al.[21]

Cytomorphological comparison of CP and LBC in our study showed better performances in LBC regarding cellular overlapping, architectural changes, uniform distribution, inflammatory cells, cellular changes, and clean background having statistically significant P<0.001. Similar results were documented in studies by Nandini et al.[22]

Table 3: Comparison of diagnoses in CP and LBC

Diagnoses	ASCUS	ASC-H	LSIL	HSIL	SCC	Adeno CA	AGNOS	Unsatisfactory	NILM	Inflammatory
CP	2	2	3	1	2	1	1	8	40	20
LBC	3	2	3	2	3	1	1	4	49	12

LBC: Liquid-based cytology, NILM: Negative for intraepithelial lesion/malignancy, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial in lesion, AGNOS: Atypical glandular cells not otherwise specified

Table 4: Comparative analysis of screening tests

Screening tests	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
VIA	93.75 (highest)	25 (lowest)	83.33 (lowest)	50 (shared highest)
CP	68.75 (lowest)	75 (highest) 1	91.66 (highest)	37.5 (lowest)
LBC	81.25	50	86.66	40
HPV-hr DNA	87.5	50	87.5	50 (shared highest) 100

LBC: Liquid-based cytology, PPV: Positive predictive value, NPV: Negative predictive value

Early cancers detection in our study in relation to ASCUS (LBC>CP) is the same as Aboobacker and Shariff; Budak et al.[16,23] LSIL (LBC>CP) reporting showed concordance with Budak et al.,[23] but discordance with Aboobacker and Shariff (LBC=CP).[16] HSIL (LBC=CP) detection here is discordant to Aboobacker and Shariff; Taylor et al.[15,16]

VIA having the highest sensitivity in our study is quite comparable to other studies done by Aggarwal et al.,[24] in low resourced region that showed reduced Ca Cx incidence and mortality with VIA, thus supporting our national NPCDCS guidelines and Federation of Obstetric and Gynaecological Societies of India recommendation. VIA has a comparable sensitivity to cytology as the study of Bhatla et al., stated and our study is not discordant in this regard.[5]

VIA with lowest specificity in our study is similar to Vahedpoor et al., study[19] stating cytology having higher specificity than VIA.

PPV best in CP in our study is discordant with Taylor et al., while NPV with shared highest in VIA and HPV-hr DNA is partially concordant with the same study.[16]

Thus different studies, some similar and some not similar to our results, have wide range of sensitivities, specificities, PPVs, NPVs for VIA for cervical cytology. Many factors such as lack of expertise, observers' bias, lack of standardization, and sample size variation might contribute to these wide variations.

Hence, HPV-hr DNA paved its way into the scenario and due to its less invasive collection procedure including self-collection comfort and commendable sensitivity, specificity, PPV, and NPV it is preferred and considered to be the best method of cervical screening as of now.[4] In this regard our study showed sensitivity and specificity detection quite comparable to the study done by Raj and Srivastava but PPV and NPV are different from the same study.[17]

In a study by Gravitt et al., sensitivity, specificity of VIA were 31.6%, 87.55; cytology showed sensitivity and specificity as 78.2%, 86%, and HPV-hr DNA came as 100%, 99.65%. HPV-hr DNA results of our study though quite comparable, rest show differences with the study.[25]

All the screening tests when compared with H/P results individually and also amongst themselves statistically significant P-value did not surface. This result is though partially discordant to Raj and Srivastava which is more in favor of HPV-hr DNA screening test.[17] Our study results of different cervical screening methods showed no statistically significant difference when compared with gold standard of H/P results. And wide range of variations are there in statistical measurements in different studies. These bar us from unequivocal consideration of HPV-hr DNA test as the single best method of cervical screening, especially in poverty-stricken rural communities with limited resources.

Limitations of the study

Being a pilot study small sample size did not yield satisfactory statistical analysis in some areas and HPV-hr DNA tests were done in VIA and cytologically positive cases along with some negative but clinically suspicious cases only. Hence, carrier state of the cases was not identified properly.

CONCLUSION

Considering the huge cost of HPV-hr DNA test and present scenario of NPCDSC programme at its neonatal state VIA in low resourced areas to VIA with cervical cytology (LBC preferred) in moderately resourced areas should be continued along with HPV-hr vaccination till HPV-hr DNA kits are widely available in all corners of India just like highly resourced institutions.

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