 DOI: 10.18231/2394-2754.2016.0023

A clinicopathological study of neoplastic and preneoplastic lesions of cervix along with HPV genotyping in biopsy proven preneoplastic lesions

Hemlata Panwar[1], Ujjawal Khurana[2,*], Karuna Gupta[3], Sumita Shrivastava[4], Ayushi Jain[5], Kalpana Sankhla[6]

[1]Assistant Professor, Dept. of Pathology & Laboratory Medicine, AIIMS, Raipur, [2]Scientist C, NIREH (ICMR), Bhopal, [3]Associate Professor, [6]Ex. Professor, SMS Medical College, Jaipur, [5]Assistant Professor, Dept. of Pathology, TMH, Mumbai, [4]PDCC, Dept. of Renal & Transplant Pathology, Apollo, Hyderabad

***Corresponding Author:**
Email: ujjawalkhurana@rediffmail.com

Abstract

Background: Cervical cancer is the commonest cancer in women in developing countries. Cost effective screening methods with optimum diagnostic accuracy can help in reducing the global burden.

Methods: This prospective study was carried out on 100 patients with suspected lesions of the cervix. Cervical biopsy was taken as gold standard to compare the performance of visual inspection with aceitic acid(VIA) and cytology (Conventional pap smear). Various parameters like age, clinical and sexual history were noted. Genotyping of HPV 16 and 18 of preneoplastic cases was done with real time PCR-based method. All statistical analysis was done at alpha-error 5% and power 80%.

Results: Out of 100 cases, 78 were carcinoma cervix (78%), 19% cervical intraepithelial neoplasia (CIN) (CIN I-8, CIN II-3 and CIN III-8), and 3 had inflammation (3%). Accuracy of cytohistological correlation was highest for Carcinoma cervix (94.64%), 72.72% and 75% each for High grade squamous intraepithelial lesion (HSIL) and Low grade squamous intraepithelial lesion (LSIL). The sensitivity and PPV of VIA came out to be 93.32%, 95% respectively. HPV16 genotype was found in 25% cases of CIN I, 66.66% cases of CIN II and 62.2% cases of CIN III. HPV 18 genotype was detected in only one case (12.5%) of CIN III.

Conclusions: Positive VIA formed a good clinical screening method for picking up Carcinoma Cervix and a promising alternative to Pap smear in precancerous lesions. HPV16 integration status has the potential to be a good marker for risk assessment of CIN progression.

Keywords: Cancer of Cervix, CIN, HPV, Pap smear, VIA.

Introduction

Globally, cervical cancer is the fourth most common cancer in women and also the fourth most frequent cause of cancer death.[1] It is the commonest cancer in women in developing countries. The worldwide incidence of cervical cancer is approximately 528,000 new cases annually, with approximately 266,000 deaths (in 2012) worldwide.[2] Almost 70% of the global burden falls in the areas with lower levels of development and more than one fifth of all new cases are diagnosed in India. The current estimates indicate approximately 132,000 new cases diagnosed and 74,000 deaths annually in India, accounting to nearly 1/3rd of the global cervical cancer deaths.[3]

In recent years, the understanding of cervical pathology has progressed and the differential diagnosis of cervical lesions has expanded to include possible premalignant and malignant lesions. Squamo-columnar junction is the most common site for development of carcinoma. Most malignant tumors are squamous (90%) and the rest are glandular. Squamous cell neoplasia is preceded by precancerous lesions that develop through several grades: CIN 1 to 3; or LSIL to HSIL. Adenocarcinoma refers to invasive neoplasms composed exclusively of malignant glandular epithelium. High risk factors found to be associated with squamous cell neoplasia are early age at first coitus, multiple sexual partners, lower socio-economic class, poor genital hygiene, cigarette smoking, oral contraceptives use, a history of abnormal Pap smear, immunosuppression, infection with herpes simplex type-II and HPV.[4]

The use of exfoliative cytology as advocated by Papanicolaou (1941) is a major tool for diagnosis of cervical lesions.[5] VIA, has emerged as an alternative of Pap smear for use in low-resource settings where it can be performed by trained health professionals.[6] Colposcopic directed biopsy also provides better cyto-histological correlation in cervical precancerous and cancerous lesions.

Human papilloma virus has emerged as the main sexually transmitted causative agent in the development of cervical intraepithelial neoplasia and invasive cervical cancer.[7] More than 100 HPV genotypes in humans are identified, of these HPV 16 and 18 are considered to be high risk type and type 6 and 11 are the low risk type in development of cervical cancer.[8] HPV-16 is the most carcinogenic HPV genotype and is associated with approximately 60% of all cervical cancers, while HPV-18 accounts for approximately 10% to 15% of cervical cancers.[9-11] Nowadays HPV genotyping for high risk types has proven to be a very important adjunct for diagnosis of carcinoma cervix in cases where doubt persists even after histopathological

examination, leading to early and confirmatory diagnosis of cervical cancer.[9]

The objective of this project was to study the demographic profile, risk factors of the women suspected of having preneoplastic and neoplastic lesions of cervix. The specific objective was to determine the diagnostic efficacy of Pap smear and VIA. Our study also aimed for detection of high risk-HPV by real time PCR in biopsy proven preneoplastic lesions of cervix.

Materials and Methods

This prospective study was conducted on 100 patients with suspected preneoplastic and neoplastic lesions of cervix coming to the gynaecological OPD of a tertiary care centre located in western India over a period of 9 months. The suspicion was based on either patient's complaints or clinician's cusco speculum examination. The major inclusion criteria were postcoital bleeding, postmenopausal bleeding and presence of growth, erosion and positive VIA test. Pregnant women and women who had undergone hysterectomy were excluded. After having an informed consent the patients were asked a set of questions by the principal investigator about their age, age of menarche, age of marriage, parity, residential area, socioeconomic status and clinical complaints. Patients were added in 20-80 years of age group with all three types of socio-economic category (Upper, middle and lower class). Demographic information was collected from a computer based database.

A gynecological speculum examination was performed for each woman, and a Pap smear was collected with Ayre's wooden spatula scraping all around the cervix including the entire transformation zone. Next, a cotton-tipped applicator soaked in freshly prepared 5% acetic acid was applied to the cervix all over the transformation zone and inspected after one minute. The abnormal epithelium appeared white within transformation zone & this was taken as a VIA positive test. On the other hand if no aceto-white lesion was seen, or a faint and bluish white translucent acetowhite lesion was noted, it was considered as VIA-negative. The patients were biopsied either the same day or on the next visit. The histology of cervical biopsy was taken as gold standard to compare the performance of VIA and cytology. Because of grossly friable growth with bleeding and ethical reasons, the Pap smear and VIA were not possible in 17cases. In another 5 patients the Pap smear procedure was done but VIA was not possible because the lesion had started bleeding after Pap smear. So biopsy was taken in all the 100 cases; out of which VIA and Pap smear were done in 78 and 83 patients respectively.

Another part of this study was HPV genotyping for high risk HPV types 16 and 18 on biopsy proven preneoplastic cases with real time DNA PCR-based method by using Applied Biosystems 7500 thermocycler. The principle is based on generation of amplified DNA from E1-E2 region of genome. DNA was extracted from patient samples (paraffin blocks). Quality and quantity of extracted DNA was checked. 4µl of DNA was added to real time PCR master mix reagent which consisted of *taq* polymerase enzyme, magnesium chloride, dinucleotides (A,T,G,C) and PCR buffer along with distilled water to make up the final volume to 40µl. For each sample two master mixes were prepared. Fluorescent labelled primer against HPV16 was added to one master mix & fluorescent labelled primer against HPV18 was added to another master mix, primers were labelled with Fluorescein amidite (FAM) fluorescent dye. Internal control gene labelled with Joe fluorescent dye was also added to each of the master mix. With each batch of sample, positive and negative controls were also run. The fluorescence signals emitted during amplification were detected in FAM and JOE channels. This instrument monitors the progress of the PCR as it occurs. Data were collected throughout the PCR process. The results were plotted in a graph as Rn vs cycles showing DNA amplification of HPV16 or 18. Reaction (Rn) is the magnitude of normalized fluorescence generated by the reporter at each cycle during the PCR amplification.

All the data were entered in Excel sheets and analyzed by using standard xl stat and primer of biostatistics software. Qualitative data was summarized by proportion and analyzed by chi-square test and relative variate. Quantitative data was summarized in mean and standard deviation. All statistical analysis was done at alpha-error 5% and power 80%.

Results and Observations

The patients' age ranged from 21-77 years. The maximum number of patients were found in 4th decade (38%) followed by the 3rd decade (25%), 5th decade (18%), 6th decade (9%), 7th decade (6%) and least number of cases were found in the 2nd decade (4%). The ages at menarche varied from 10 to 15 years with 81% of patients having menarche at or below 12 years of age. Almost 71% patients' age at 1st coitus was ≤18years. Maximum number of patients i.e. 73% belonged to lower class followed by middle class (24%) and only 3% belonged to upper class. Most common complaint with which patient presented was excess discharge per vaginam (55%), followed by abnormal bleeding (26%), pain lower abdomen (15%) and dysuria (4%). The cases of abnormal bleeding included post coital bleeding (8 cases), postmenopausal bleeding (8 cases) and 10 cases with intermenstrual bleeding and altered menstrual cycle.

Out of total 78 cases of invasive carcinoma and 19 cases of preneoplastic lesion, 76.92% and 57.89% respectively werein the group with first coitus at less than 18 years showing a significant correlation of early age at first coitus with cervical neoplasm (P<.05). Mean

age at first coitus with cervical cancer was 17.7 yrs in the present study.

VIA positivity was one of the main inclusion criteria. It was done on 78 patients (78%) out of the 100 patients, of which 73 patients were found to be VIA positive and five were VIA negative. These VIA negative were still biopsied because of strong clinical suspicion of cervical lesions.VIA could not be done on 22 patients with complaints of bleed on touch & very bad cervical erosion, but biopsy had been taken in all these 22 cases.

Out of the 100 patients, Pap smear was performed in 83 patients. Pap smear could not be performed in 17 patients due to bleeding-on-touch or cervical erosions. Out of the 83 cytosmears, 78 cases were satisfactory for evaluation, while 5 cases were unsatisfactory due to obscuration of smear by blood. The smears were evaluated according to the Bethesda system 2001. In the category of epithelial cell abnormality, Squamous cell carcinoma(SCC) was the commonest in 54, HSIL was 11, LSIL was 8.Adenocarcinoma constituted only 2 cases and 3 cases were reactive changes associated with inflammation (Table 1). Cyto-histological correlation was performed in 78 cases, of which cytological diagnosis were accurate in 69 cases (Table 2).

Table 1: Details of Cyto-histo correlation

Cytological diagnosis (Pap smear)	No. of cases	Histopathology					
		Infla-mmatory	CIN I	CIN II	CIN III	SCC	Adenocarcinoma
LSIL	8	1	6	1	-	-	-
HSIL	11	-	-	2	6	3	-
SCC	54	-	1	-	2	51	-
Adenocarcinoma	2	-	-	-	-	-	2
Inflammatory	3	2	1	-	-	-	-
Total	78	3	8	3	8	54	2

Table 2: Details of cytohistological accuracy

Grading	Cytodiagnosis	Histopathological diagnosis	Cyto histological accuracy
Inflammatory	3	2	66.66%
LSIL	8	6	75.00%
HSIL	11	8	72.72%
Invasive	56	53	94.64%
Total	78	69	88.45%

Out of 100 cases, Histopathological examination revealed 19% preneoplastic lesions (i.e. cervical intraepithelial neoplasia (CIN I-8, CIN II-3 and CIN III-8)), 78% neoplastic lesions and 3 were inflammatory pathology (3%). Out of the 78 cases of biopsy-proven invasive carcinoma, 54 cases had moderately differentiated non keratinizing squamous cell carcinoma, followed by 10 cases of well differentiated SCC and 5 cases of poorly differentiated SCC, Clear cell carcinoma in 1 case (1.0%), glassy cell carcinoma in 1 case (1.0%), Adeno-squamous carcinoma in 1 case (1.0%) and adenocarcinoma in 6 cases (6.0%).

Mean age for the development of cervical cancer and dysplasia was 48.87±11.75 S.D. Patient with lower socio-economic status had the highest frequency of developing cervical neoplasia. Mean age of menarche in patients who developed preneoplastic and neoplastic cervical cancer was 12.25 years ($p<0.05$). Mean age at first coitus was 17.7 years ($p<0.05$). There was a significant association of dysplasia and cancer cervix with higher parity ($p<0.05$). Clinically, majority of patients with invasive cancer complained of discharge per vaginum, while 47.37% cases of dysplasia had complaints of abnormal bleeding. Maximum cases of invasive carcinoma on clinical examination had an unhealthy cervix &cervical erosions, followed by bleeding on touch.

Out of the 3 inflammatory lesions reported in surgical biopsies, one was reported as LSIL in Pap smear and another case of Squamous cell carcinoma reported in Pap smear turned out as CIN I in biopsy (False positive case). One case of inflammatory smear in Pap smear was given out as CIN I in biopsy (False negative case). In rest all 75 cases there was hundred percent correlation. The Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of Pap smear came out to be 98.64%, 50%, 97%, 66% respectively.

On histopathology, out of these 73 VIA positive cases, three cases showed only inflammation, hence were considered to be false positive. Five VIA negative cases were found as preneoplastic (1 case of LSIL and 2 cases of HSIL) and neoplastic lesion (2 cases of SCC) on histopathology and thus were false negative. These patients with SCC aged 58 and 75 years respectively. As we selected patients with strong clinical suspicion of malignancy and those with positive VIA test, we could not comment on true negative patient (Specificity) and NPV. The sensitivity and PPV of VIA came out to be 93.32%, 95% respectively.

HPV genotyping was done only in the 19 preneoplastic cases to detect the presence of high risk type HPVs. HPVpositivity was found in 10 (52.63%) cases with 25% in CIN I(2/8), 66.6% in CIN II(2/3), 75% in CIN III (6/8)cases. Out of 10 cases found to be positive, HPV type 16 was found in 9 cases. It is evident that there is strong association of HPV 16 positivity with higher grade of CIN. HPV type 18 was found only in one case of CIN III(Table 3).

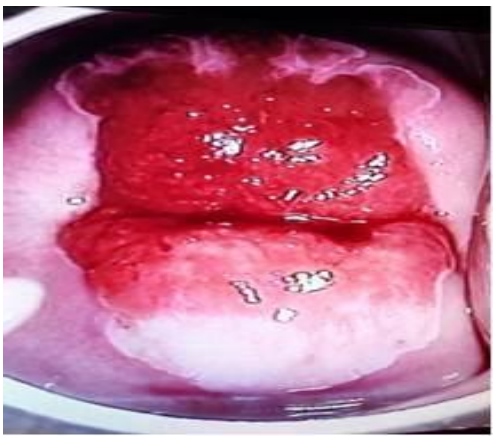

Fig. 1: Aceto grey-white lesion at 6 o'clock (VIA)

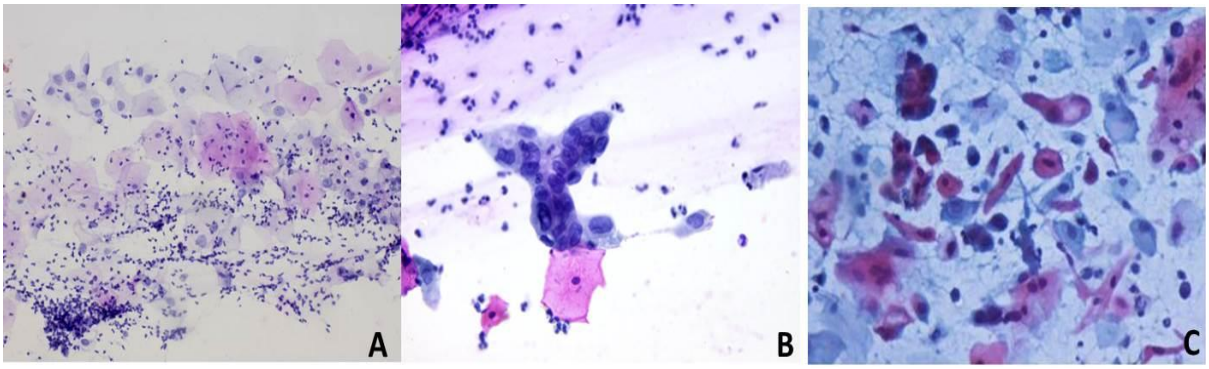

Fig. 2: (A) VIA negative, Low grade squamous intraepithelial lesion (LSIL) in pap smear of a 35 years old female (Pap; X400) (B) High grade squamous intraepithelial lesion (HSIL) showing variation in nuclear size and shape with crowded hyperchromatic cell group (Pap; X400) (C) Keratinizing squamous cell carcinoma showing elongated cell and hyperchromatic nuclei (Pap; X400)

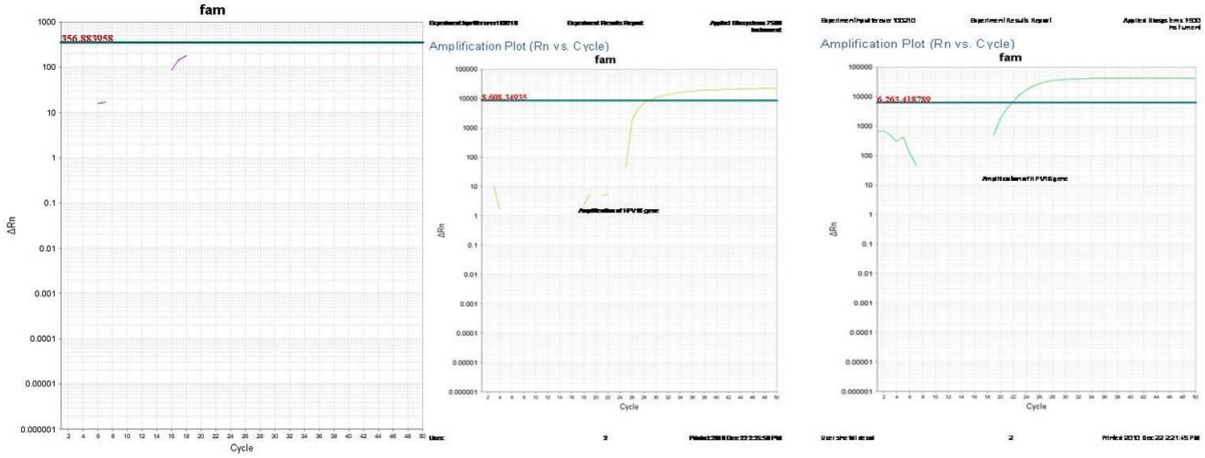

Fig. 3: (A) Amplification plot (Rn vs cycle) of HPV 16 and 18 negative graph (B) Amplification plot (Rn vs cycle) of HPV 16 Positive graph (C) Amplification plot (Rn vs cycle) of HPV 18 Positive graph (Rn is the magnitude of normalized fluorescence generated by the reporter at each cycle during the PCR amplification)

Table 3: HPV genotyping of Preneoplastic cervical lesions

Diagnosis	Total no. of cases	HPV 16 detection	HPV 18 detection	HPV not Detected
Cervical intraepithelial lesion (CINI)	8	2 (25%)	-	6
Cervical intraepithelial lesion (CINII)	3	2(66.6%)	-	1
Cervical intraepithelial lesion (CINIII)	8	5(62.5%)	1(12.5%)	2
Total	19	8	1	10

Discussion

Visual inspection with 5% acetic acid has been explored as an adjunct to Pap smear and as a more promising alternative to Pap smear, for identifying women with precancerous lesions. VIA has been found to be an effective screening approach in less-developed countries according to several studies.[12,13]

Sankaranarayanan et al studied 3000 women; the sensitivity and specificity of VIA was 90% and 92% respectively as compared to 86% and 91% for that of Papsmear.[6] Gaffkin in 2003, studied over 34,000 women with the range of estimated VIA sensitivity values being 66-96% and specificity rates from 64% to 98%.[14] In this study, he published test qualities of Pap smears ranging from 11-99%. In another study screening of 1,200 women between the ages of 18 and 70 from Tehran, Iran was done by VIA and Pap smear and the sensitivity of VIA was found to be 74.3% compared with 72% for pap smear.[15] The specificity of VIA was 94% compared to 90.2% for Pap. Rana et al found the sensitivity of VIA to be 93% and of Pap smear 83%. Corresponding specificities were 90% and 97% respectively.[16] The PPV of VIA was 62.5% versus 83% for Pap smear which is statistically significant (P value < 0.001). The NPV of VIA was 98% versus 97% for cytology. Overall, VIA demonstrated an accuracy of 90% as compared to 96% for cytology.

In our study, Pap smear was found to be more sensitive than VIA in detection of epithelial cell abnormality, possibly due to this being a hospital based study, as well as being targeted towards symptomatic and very high risk women attending the gynaecology out-patient department. Also, the sample size is smaller as compared to other studies.

In our study two out of five VIA negative cases were found as SCC on histopathology. This is in concordance with observation seen in elderly women that VIA may be falsely negative due to tendency of the transformation zone and any lesion within it, to recede into the endocervical canal.[17] Also VIA has been stated to be less efficient for detection of endocervical carcinoma however the two cases of endocervical carcinoma in this study were VIA positive.[18]

Our study showed a higher percentage of invasive carcinoma, possibly as it was an institution based study, where high risk cases and patients in late stages were referred, rather than community based cervical screening. Mean age of LSIL, HSIL & invasive carcinoma has been reported as 32.9, 36.3 & 48.3 years, which was comparable with our findings.[19] In a study conducted by Mishra et al (2002) the prevalence rate of SIL was noted being highest in the low socio-economic group (8.3%) followed by middle class (4.3%) and lowest in upper class which was comparable with our findings.[20] This appears to be related to poor genital hygiene, illiteracy and high parity being prevalent in this group. In our study; the mean age of menarche in cases of dysplasia was 12.25 yrs±0.90, (P <.05) indicating an early age at menarche as a significant risk factor for development of cervical neoplasm. Increased association of HPV infection has been described with early age at first intercourse.[21,22] In a study by Parazzini F et al risk of cervical cancer increases with first intercourse at <12 yrs of age however this cannot be proved in this study because we took age of marriage as age of first coitus, since the incidence of premarital sex is very less in India.[23]

In this study, abnormal bleeding was the chief complaint in cases of preneoplastic lesions which is comparable with a study stating menstrual disturbances to be the chief symptoms in cases of dysplasia and cervical carcinoma.[24] Chabra et al (2003) found overall cyto-histological accuracy to be 80%, comparable with the present study (88.46%).[19] The false negative rate was 1.35%, which is below the range of reported results, 5-15% (Joseph et al).[25] These variations were possibly due to high risk targeted population and our study is tertiary care hospital based rather than a screening purpose study. There is scant literature available for evaluating presence of HPV in cervical lesions, especially from India. In a study by Das et al, they observed gradual increase in the rate of high risk type HPV positivity with increase in grade of CIN.[26] They also found the prevalence of HPV 16 and 18 to be 54.28%, 52.94% and 27.08% in severe dysplasia, moderate and mild dysplasia respectively. In our study, HPV16 was detected in just 25% of LSIL, which could be due to pre-analytical errors. We did not evaluate the HPV genotyping in invasive cancer because of cost effectiveness.

Carter et al found the relative frequency of HPV-16/18 increases with the severity of the lesion which is in concordance with our results. It is evident that there is strong association of HPV positivity with higher grade of CIN.[27]

De Vuyst studied 653 women for concurrent screening methods: pap smear, VIA, PCR for high risk HPV and cervicography.[28] The gold standard for affirmative diagnosis was biopsy. Sensitivity and

specificity were 83.3% and 94.6% for Pap smear, 73.3% and 80.0% for VIA, 94.4% and 73.9% for HR HPV PCR, and 72.3% and 93.2% for cervicography. Although pap smear had the highest sensitivity and HPV PCR the highest specificity, the visual inspection showed an accuracy between the two which was comparable with our findings. He also concluded that in poor resource countries VIA is effective as a primary screening tool.

To conclude, Visual inspection using acetic acid (VIA) is an alternative to Pap smear in a low-resource setting for screening of cervical precancerous and cancerous lesions. It is a suitable low cost test and also gives the facility of seeing & treating at the same time due to immediate results. On the other hand, VIA also has the disadvantage of higher number of referrals and potential for over-treatment. Also some cases may be missed on VIA. So, combined screening of Pap smear with VIA is a more effective approach for detection of cervical precancerous and cancerous lesions.

Our findings suggest that selective genotyping for the oncogenic HPV 16 and 18 types might be useful in separating women with a higher risk of CIN progression from those with a minimal risk. We also conclude that the HPV16 integration status has the potential to be a marker for risk assessment of CIN progression.

References

1. Cervix cancer screening IARC handbooks of cancer. Vol. Lyons (France):IARC, 2013.
2. Sankaranarayanan R, Ferlay J. Worldwide burden of gynecological cancer: The size of the problem. Best Pract Res Clin Obstet Gynaecol 2006;20:207–25.
3. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). Summary report on HPV and cervical cancer statistics in India 2007.
4. Murthy NS, Methaw A. Risk factor for precancerous lesions of cervix. Eur J Cancer Prev 2000 Feb;9(1):5-14.
5. Papanicolaou GN and Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. Am J Obstet Gynecol 1941;42:193-206.
6. Sankaranarayanan R, Gaffikin L, Jacob M, Sellors J, Robles S. A critical assessment of screening methods for cervical neoplasia. Int J GynaecolObstet 2005;89:S4–S12.
7. Schiffman M, Castle PE, Jeronim J, Rodrigue AC, Wacholde S. Human papillomavirus and cervical cancer. Lancet 2007;370:890–907.
8. Munoz N, Bosch FX, De Sanjose S, Herrero R, Castellsague X, Shah KV. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Eng J Med 2003;348:518–27.
9. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain JM, et al. American Cancer Society, American society for colposcopy and cervical pathology, and American society for Clinical Pathology screening Guidelines for the prevention and early detection of cervical cancer. J Low Genit Tract Dis 2012;16(3):175-204.
10. Walboomers JM, Jacobs MV, Manos MM,et al. Human papilloma virus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-19.
11. De Sanjose S, Quint WG, Alemany L, et al. Human papilloma virus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048-56.
12. Cronje HS. Screening for cervical cancer in the developing world. Best Practice Res Clin Obstet Gynaecol 2005;19:517–29.
13. Szarewski A. Comment: Cervical screening by visual inspection with acetic acid. Lancet 2007;370:365–6.
14. Gaffikin L, Lauterach M, Blumenthal P D. Performance of Visual Inspection with Acetic Acid for Cervical Cancer Screening: A Qualitative Summary of Evidence to Date. Obstetrical and Gynecological Survey 2003;58:543-50.
15. Ghaemmaghami F, Behtash N, Modares Gilani M, Mousavi A, Marjani M, Moghimi R. Visual Inspection with acetic acid as a feasible screening test for cervical neoplasia in Iran. Int J Gynecol Cancer 2004;14(3):465-9.
16. Rana T, Zia A, Sher S, Tariq S, Asghar F. Comparative evaluation of pap smear and visual inspection of acetic acid (VIA) in cervical cancer program in Lady Wilingdon Hospital, Lahore. Special edition annals vol 2010;1:104-107.
17. Society AG. Screening for Cervical Carcinoma in Older Women. Journal of the American Geriatrics Society2001,49:655–657.
18. Goel A, Gandhi G, Batra S, Bhambhani S, Zutshi V, Sachdeva P. Visual inspection of the cervix with aceitic acid for cervical intraepithelial lesions. Int J Gynaecol Obstet 2005;88;25-30.
19. Chhabra Y, Behera BG, Khalkho J, Pati N. Cytomorphological study of cervical PAP smears for pre-cancerous and cancerous lesions. Journal of Cytology 2003;20:64-67.
20. Mishra J, Das H. Study of vaginal cytology in erosion cervix and cervicitis. Indian J Obstet Gynaecol 1979;20:669.
21. Datta P, Bhatla N, Dar L, Patro A R, Gulati A, Kriplani A, Singh N. Prevalence of human papillomavirus infection among young women in North India. Cancer Epidemiol 2010;34:157–61.
22. Flores YN, Bishai DM, Shah KV, Lazcano-Ponce E, Lorincz A, Hernández M, et al. Risk factors for cervical cancer among HPV positive women in Mexico. Salud Publica Mex 2008;50:49–58.
23. Parazzini F, La Vecchia C, Negri E, Fedele L, Franceschi S, Gallotta L: Risk factor for cervical intraepithelial neoplasia. Cancer 1992;69:2276-82.
24. Rathee S, Jaiswal TS, Mohan PS, Mohan H. Detection of uterine cervical dysplasia and carcinoma cervix by cervical smears. A clinicopathological analysis of 1181 cases .Indian J. Obstet Gynaecol 1983;863-67.
25. Joseph MG, Cragg F, Wright VC, Kontozoglou TE, Downing P, Marks FR. Cytohistological correlates in a colposcopic clinic: A 1 year prospective study. Diagn Cytopathol 1991;7:477- 81.
26. Das BC, Sharma JK, Gopala Krishna V, Luthra UK. Analysis by Polymerase chain reaction of the physical state of human papilloma virus type 16 DNA in cervical preneoplastic and neoplastic lesions. J Gen Virol 1992;73:2327-2336.
27. Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. J Infect Dis 2000;181:1911–9.
28. DVuyst H, Claeys P, Njiru S, Muchiri L, Steyaert S, De Sutter P, VanMarck E, Buvayo J, Temmerman M. Comparison of pap smear, visual inspection with aceitic

acid, human papilloma DNA-PCR testing and cervicography. Int J Gynaecol Obstet 2005;89;120-6.