



## TO STUDY THE INCIDENCE OF VILI +VE CASES IN PATIENTS ATTENDING GYNAECOLOGY O.P.D. OF D.M.C.H. AND TO ASSESS ITS POSITIVE PREDICTIVE VALUE AS COMPARED TO CYTOCERVICAL SMEAR

### Gynaecology

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### ABSTRACT

**Background:** The cervix is the commonest site of female genital tract cancer worldwide. Cancer cervix has a long pre-invasive stage, giving an opportunity for its early detection and treatment.

**Methods:** The present study conducted on 500 patients in OPD and Indoor admitted patients during the period of October 2017 to September 2019. Pap's smears were studied and reported according to the Bethesda classification. On next visit patient attended with cytology report and VIA, VILI was performed and then subjected to colposcopy.

**Results:** In this study, 500 symptomatic patients attending OPD were analysed. Of these, 76 patients were VILI positive, 46 patients were Pap smear positive and 60 patients were colposcopy positive. Those patients who were positive for any of these three tests; VILI, Pap smear, or colposcopy, underwent cervical biopsy to confirm diagnosis of CIN or early carcinoma. Punch biopsies were taken in 79 patients in whom 52 patients were biopsies positive. Adenocarcinoma was confirmed by cone biopsy in one patient. This patient was Pap smear positive, but colposcopy and VILI, negative. Pie chart (1) shows that 447 patients were normal. Abnormal findings were noted in 53 patients; 34 cases were positive for CIN I (6.8%), 9 cases CIN II (1.8%), and 6 cases CIN III (1.2%). Malignancy was found in 4 patients (0.8%).

**Conclusions:** In low resource settings, screening for carcinoma cervix by Pap smear can be replaced by cheaper and easily available visual method like VILI, which has the higher sensitivity to detect any grade of dysplasia, with a reasonable specificity. Even when screening with Pap smear is available, it should be combined with visual screening methods like VILI, as many cases of CIN I are missed by Pap smear may be picked up by visual test and combined testing will reduce the false negative cases. In remote areas VILI should be used as a primary screening tool.

### KEYWORDS

Cancer cervix, Colposcopy, VIA

### INTRODUCTION

Cervical cancer is the common malignancy found among the rural Indians. According to the World Health Organization, cervical cancer is the second most common type of cancer among females, and was responsible for over 250,000 deaths in 2005. Over 500,000 new cases of invasive cervical cancer are diagnosed annually worldwide. Cervical cancer takes a major toll on mankind, society and nation. Annual mortality is about 270,000 each year worldwide and of these, 85% occur in low resource populations. In India alone, nearly 132,000 new cases are diagnosed each year with a mortality rate of 74,000 per year. In spite of the preventable nature of this disease, 12,710 new cases of invasive cervical cancer resulting in 4290 deaths were anticipated in the United States in 2011.

Cancer cervix has been considered preventable because it has a long preinvasive state and availability of screening programmes and treatment of preinvasive lesions is effective. Indeed, the incidence of cervical cancer has decreased in developed countries as a result of screening by cytology in sexually active women of 21-71 years. In developed countries, there are well-established pathology laboratories with qualified pathologists. In addition, supplementary tests for premalignant stage of cervical cancer, including liquid based cytology, computerized Pap smear, colposcopy and HPV test by hybrid capture 2 system, are routinely available.

Approximately 80% of the Indian population resides in rural areas and lack awareness about cervical cancer, that it is a preventable disease. Moreover, poverty and poor access to health care is another road block.

The current mainstay of cervical cancer screening is cervical cytology by Pap smear, which necessitates a well-organized infrastructure: (a) health units to collect cervical material, (b) laboratories to prepare the slides for reading, (c) specialized personnel to render a diagnosis, (d) results typically take days or weeks (e) only moderate sensitivity in detecting the more advanced precursors; therefore, regular rescreening is important for the effectiveness of programmes using this method and (f) trained physicians to treat the cervical cancer. Pap smear is not regularly performed in women of reproductive age in our country, as in developed countries. Moreover, newer approaches such as

computerized Pap, liquid-based Pap, and HPV DNA testing using hybrid capture II (HC II) are time consuming, expensive, and not widely available in India.

The challenges and failure of cytology screening programmes in low-resource countries has stimulated the search for alternative methods of screening that would overcome the many barriers.

To screen successfully in low-resource settings, the following requirements are essential:

- Screening, diagnosis and treatment provided on-site, or in clinics accessible to the majority of at-risk women;
- Low-cost, low-technology screening test that can lead to immediate treatment of abnormalities;
- Wide coverage of at-risk women;
- Appropriate educational programmes directed towards health workers and women to ensure correct implementation and high participation;
- Built-in mechanism for evaluation of the screening programme.

The two most widely studied alternative approaches to screening of cervical cancer are visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI). Many reports suggest that VIA and VILI closely match Pap smear in performance in detecting cervical cancer precursors. VILI seems to be cheap, highly sensitive, and easily available. It is an alternative screening modality with the advantage that the results are immediately available and the patients can be treated during the same visit. However the sensitivity, specificity and predictive value of VILI in detection of premalignant cervical lesions and early cervical carcinoma are yet to be determined.

The important factors in the genesis of cervical cancer are:

- Infection with high risk HPV
- Early sexual intercourse
- Early age of first pregnancy
- Multiple sexual partners
- Too many and too frequent births
- Low socioeconomic status
- Persistence of HPV infection
- Age > 30 years
- Smoking and

- Compromised host immune defense.

### HUMAN PAPILLOMA VIRUS (HPV):

HPV is epitheliotropic and plays an important role in the development of CIN.

99 per cent of patients with CIN and invasive cancer are found to be positive with HPV DNA. 30 types of HPV out of 130 are primarily infected the squamous epithelium of the anogenital tract of men and women.

### SPECIFIC AIMS

#### We have addressed the following aims:

**Aim # 1:** Incidence of VILI positive cases in OPD patients and prevalence of CIN and early cervical carcinoma in VILI positive cases: To study the incidence of VILI positive cases among patients attending gynaecology OPD with symptoms suggestive of CIN and cervical carcinoma, including whitish vaginal discharge, postcoital bleeding, irregular vaginal bleeding, foul smelling discharge, and postmenopausal bleeding. We will also assess the prevalence of CIN and early cervical carcinoma in VILI positive cases.

**Aim #2:** Predicting value of VILI in diagnosis of CIN and early cervical carcinoma: To assess positive and negative predictive value, sensitivity, and specificity of VILI positivity in diagnosis of CIN and early cervical carcinoma compared to Pap's smear.

**Aim #3:** Risk factors for CIN and early cervical carcinoma: To assess risk factors associated with VILI positive cases, and CIN as well as early cervical carcinoma, including age of patients, socioeconomic group, age of marriage, parity, age of first pregnancy, spacing between children, religion, rural and urban habitant, literacy, use of contraceptives, and others.

If VILI has high sensitivity and specificity as well as high predicting value in the diagnosis of CIN and early cervical carcinoma, this can be used particularly in low resource population and in remote hospitals for screening precancerous and cancerous patients. Moreover, this requires one patient visit for both diagnosis and treatment against pap's smear requiring multiple patient visits.

### MATERIALS AND METHODS

**Patient population:** The present study was performed on patients attending gynaecology OPD at Darbhanga Medical College, Laheriasarai, Bihar. Darbhanga Medical College is a tertiary care center and receives patients from north east Bihar. The study was prospective follow up of patients with the following inclusion and exclusion criteria.

**Inclusion criteria:** Women of 30-70 years age presenting with one or more of the following symptoms: excessive vaginal discharge, postcoital bleeding, irregular vaginal bleeding, postmenopausal bleeding, foul smelling discharge, pain abdomen, and vaginal itching.

**Exclusion criteria:** Women who were never sexually active, underwent hysterectomy for benign uterine lesion, or diagnosed late invasive carcinoma of cervix, were excluded from this study.

A total of 500 patients were enrolled on the basis of the above inclusion criteria in gynaecology OPD between October 2017 and September 2019.

Chief complaints of the patients were excessive vaginal discharge (Leucorrhoea), post coital bleeding, irregular vaginal bleeding, foul-smelling discharge, and postmenopausal bleeding. Significant personal history included present age, religion, habitant, age of marriage, age of first sexual intercourse, age of the first pregnancy, parity, interval between pregnancy, socioeconomic status, personal hygiene, smoking habits, use of Contraceptive methods, human papilloma virus infection, HIV and use of immunosuppressant.

All cases underwent vaginal examination, Pap smear, VILI and colposcopy. In addition, colposcopic guided biopsies and histological evaluation were done for all positive cases for Pap smear, VILI, and colposcopy, which served as the gold standard in the diagnosis of CIN and early cervical cancer.

**Pap smear:** The Pap test is a procedure used to collect exfoliated cells

from cervix for cervical cytology. Use of a combination of the Ayer's spatula for sampling the ectocervix and a cytobrush for sampling the endocervix is superior technique to conventional Pap smear.

**Visual Inspection with Lugol's iodine (VILI):** In this procedure cervix is inspected after application of Lugol's iodine on it. The principal of this test is based on reactivity of iodine to glycogen (mahogany brown or black) present in the normal cervical squamous epithelium and absence of glycogen in precancerous lesions (non-iodine uptake e.g. mustard yellow). However, inflammatory lesions and cervical metaplasia can result in reduced glycogen in the cervical epithelium. So there is partial iodine uptake. Hence, normal cervical epithelium turns brown-to-black on application of Lugol's iodine and CIN or early cervical carcinoma appear as well defined areas of thick mustard or saffron yellow colour. Interpretation of VILI is depicted in Table M1.

**TABLE-M.1**  
**Categories for VILI test results:**

VILI Category	Clinical Findings
Negative test	Squamous epithelium turns brown (mahogany) and columnar epithelium does not change colour; or irregular, partial or non-iodine uptake areas appear.
Positive test	Well-defined, bright yellow iodine non-uptake areas touching the squamo-columnar junction (SCJ) or close to the os if SCJ is not seen.
Suspicious for cancer	Clinically visible ulcerative, cauliflower-like growth or ulcer; oozing and/or bleeding on touch.

**Colposcopy:** After examination with the standard white light, a green filter may be used on the light source to enhance the contrast of the red blood vessels. This helps to clarify any vascular changes if present. Lugol's iodine solution may also be used to stain the cervix to delineate areas of dysplasia, metaplasia or columnar epithelium.

To evaluate the cervix for abnormal squamous epithelium, the entire cervix was carefully examined. Hence, we insured that the entire squamo-columnar junction (SCJ) was visualized in order to accomplish adequacy of colposcopy. We next performed a careful systematic evaluation of the cervix and upper vagina paying adequate attention to the transformation zone adjacent to the SCJ. Areas of white epithelium, punctation, and mosaic were noted. The severity of any lesions are graded based on the whiteness of the epithelium, the intercapillary distance in vascular lesions, the sharpness of the lesion border, and the surface contour (flat, ulcerated, or raised). The staining characteristics of the lesion with Lugol's iodine have also been used to grade CIN and early carcinoma.

### OBSERVATIONS

#### Classification of patients:

In this study, 500 symptomatic patients attending OPD were analysed. Of these, 76 patients were VILI positive, 46 patients were Pap smear positive and 60 patients were colposcopy positive. Those patients who were positive for any of these three tests; VILI, Pap smear, or colposcopy, underwent cervical biopsy to confirm diagnosis of CIN or early carcinoma. Punch biopsies were taken in 79 patients in whom 52 patients were biopsies positive. Adenocarcinoma was confirmed by cone biopsy in one patient. This patient was Pap smear positive, but colposcopy and VILI, negative. Pie chart (1) shows that 447 patients were normal. Abnormal findings were noted in 53 patients; 34 cases were positive for CIN I (6.8%), 9 cases CIN II (1.8%), and 6 cases CIN III (1.2%). Malignancy was found in 4 patients (0.8%). Biopsies revealing CIN I or worse lesions were considered as positive.

#### Incidence of cases positive for Pap smear, VILI, and colposcopy:

The incidence of cases, which were positive for Pap smear, VILI and colposcopy. Of 500 cases, 46 patients were Pap smear positive. Histopathological evaluation of Pap smear positive cases revealed that 33 (71%) cases were positive for CIN or early cervical carcinoma. Pap smear cases were classified into 5 categories: normal (144), inflammatory (310), LSIL (23), HSIL (19), and malignancy (4). Of LSIL positive cases (23 patients), 7 patients were biopsy negative (31%). Likewise, 13 (68%) patients in HSIL group were biopsy positive and 6 (32%) patients were biopsy negative. Similarly, 4(100%) cases were positive for malignancy. Of 20 patients which were pap negative but biopsy positive. Of 19 out of the 310 cases reported as inflammatory smears were under-diagnosed by Pap smear. The cases missed by inflammatory Pap smears were 17 cases of CIN I

and 2 cases of CIN II. One case of CIN I positive in biopsy was found in normal Pap smear report. Reports of Pap smear. We next evaluated VILI positive cases. Of 76 VILI positive cases, 51 (67%) were biopsy positive for CIN and early carcinoma of cervix. Biopsy negative cases (25) included patients with cervicitis (24), and patient with metaplasia (1). In false negative cases (2), one case of CIN I was missed and also one case of adenocarcinoma of cervix was missed. VILI positive cases. We then analyzed colposcopy positive cases. Of 60 patients positive for colposcopy, 51 (85%) patients were biopsy positive. Notably, colposcopy missed one case of adenocarcinoma and one case of CIN I. Colposcopy positive cases. Out of 80 biopsies, one case of metaplasia, 26 cases of cervicitis, 34 cases of CIN I, 9 cases of CINII, 6 cases of CINIII and 4 cases of early carcinoma of cervix.

## DISCUSSION

500 symptomatic patients were first subjected to Pap smear followed by VILI and colposcopy. The patients, in whom either of screening tests or colposcopy was positive, underwent colposcopy guided biopsy (No-79) as a gold standard. In case of adenocarcinoma (No-1) cone biopsy was done for confirmation.

In the present study, amongst the patients who underwent cervical biopsy, 10.6% showed CIN or early carcinoma. Of these 9.8% had CIN and 0.8% had early invasive cancer of the cervix.

Pap smear was positive 9.2% for LSIL (cut off point) and 4.6% for high grade lesion. Sensitivity, specificity, PPV and NPV of Pap smear were 62.26%, 97%, 71.70% and 95.59% (cut off point LSIL). VILI was positive 15.2%. VILI positive cases were more common among those, who presented with LSIL, HSIL and cervical carcinoma in Pap smears compared with those women with normal cytology.

Sensitivity, specificity, PPV and NPV of VILI were 96.22%, 94.40%, 67.10% and 99.52% respectively. Colposcopy was positive 12%. Sensitivity, specificity, PPV and NPV of colposcopy were 96.22%, 97.98%, 85% and 99.54% respectively.

In North eastern parts of India there are barriers to cytocervical screening, HPV test (Hybrid capture 2 systems) and colposcopy despite the great burden of cervical carcinoma.

So, visual inspection of cervix with Lugol's iodine (VILI) becomes an appropriate alternative screening method.

It has low cost, easy availability, can be easily performed by paramedical staffs or doctors, test results are immediately available and hence 'see and treat' policy can be used.

### Risk factors of cervical carcinoma:

Over 99% of patients with CIN and invasive cancer are found to be positive for HPV DNA. Other non-viral cofactors are smoking, poor diet, oral contraceptive pills, immunosuppression and chlamydia infection.

The most common age of cervical carcinoma was in the 60s and the distribution of case was bimodal with earlier peak was 35 to 39 years. Cases of CIN were found 10 to 15 years earlier than cervical carcinoma.

Therefore, screening for cervical carcinoma should start ideally at the onset of sexual activity and all women should be screened at least once by the age of 30-35 years. Patient married earlier than 18 years of age had 3 times more incidence of CIN and early carcinoma than those married after 20 years.

In low socio-economic group the incidence of CIN and early carcinoma of cervix was more than 1.5 times commoner than in high socio-economic group.

In those women with more than 4 children, the incidence of CIN and early carcinoma was 2 times more common than in those women with 2 children. In more than four children incidence of early cervical carcinoma was 1.2% and in two children mother had no cervical carcinoma.

In women, who became pregnant at less than 18 years of age, the incidence of CIN and early cervical carcinoma was two times more common than those who become pregnant after 18 years of age. It is

due to the most observed active metaplastic process at the time of menarche and during and after the first pregnancy. In this group the interval between menarche and first pregnancy is less, and hence metaplastic changes are more common.

Prevalence of CIN and early carcinoma was more common when interval between two children was less than 2 years.

In rural population incidence of CIN and early carcinoma was twice as common as in the urban population.

Incidence of CIN and early carcinoma was more in illiterate people than in educated people.

In Hindus the incidence of CIN and early carcinoma was three times more common than that in Muslims. The custom of circumcision may be responsible for reducing the chance of HPV infection.

Incidence of CIN and early carcinoma was 8.6% in leucorrhoea, 14% in post-coital bleeding, 12% in irregular vaginal bleeding, 13.31% in foul smelling discharge and 15% in postmenopausal bleeding. Incidence of early carcinoma of cervix in postmenopausal bleeding was 2.5%.

CIN and early carcinoma were common in unhealthy cervix. But it was also found in healthy cervix. In healthy cervix incidence was 6% and in unhealthy cervix it was 11.75%.

## CONCLUSION

In north eastern India poverty, illiteracy, early marriage, frequent child births, early pregnancy, unhygienic conditions of living, ignorance of early symptoms of disease, lack of proper screening by government and private sectors are responsible for high incidence of CIN and cervical carcinoma. Ultimately these are responsible for increased HPV infection. Late clinical diagnosis and difficulty in treatment of advanced cervical carcinoma are responsible for high incidence of mortality from the cervical carcinoma.

Cervical carcinoma is a preventable disease because it has a long preinvasive state and availability of screening programmes and treatment of preinvasive lesions is effective.

Organised and quality assured cytology-based screening programs have substantially reduced the incidence of cervical carcinoma in many developed countries. There are also advanced tests for screening of cytology example are liquid based cytology and computerized Pap smear. There are tests for HPV, for example - Hybrid Capture 2 System and Care HPV test. Now-a-days colposcopy examinations and colposcopy guided biopsies are most widely used tools for diagnosis of cervical cancer precursors and early cervical cancer.

However, there are considerable barriers to setting up cytology based programmes and other high profile tests particularly in developing countries. These tests are costly, time consuming, need equipped pathology lab, need trained technicians and qualified pathologists. The demerits of Pap smear are, the results typically take days or weeks to become available and it has moderate sensitivity in detecting the more advanced precursors, therefore, regular screening is important for the effectiveness of programs using this method. Colposcopy is available only in tertiary centers as colposcopy can be done only by trained gynecologists. These barriers stimulated the search for novel and alternative approaches to cytology for cervical cancer prevention.

To screen successfully in low resource settings following criteria should be fulfilled:

- Screening, diagnosis and treatment provided on-site accessible to the majority of at risk women;
- Low cost, low technology screening test that can lead to immediate treatment of abnormalities
- Appropriate educational programmes directed towards health workers and women to ensure correct implementation and high participation.
- Built-in mechanism for evaluation of the screening programmes.

The VILI is a suitable alternative for early detection of premalignant cervical carcinoma.

Therefore in the present study, an attempt has been made to analyse efficacy of Pap smear, VILI and colposcopy.

We found that the sensitivity, specificity, positive predictive value and negative predictive value of VILI are 96.22%, 94.40%, 67.1%, 99.52% respectively. Similarly, sensitivity, specificity, positive predictive value and negative predictive value of Pap smear are 62.26%, 97.09%, 71.73% and 95.59% respectively. And sensitivity specificity, positive predictive value and negative predictive value of colposcopy are 96.22%, 97.98%, 85% and 99.54% respectively.

In our study the sensitivity and NPV of VILI were more than of Pap smear. Therefore, VILI is a suitable potential alternative / adjunctive screening test not only in resource poor setting but also in well-equipped centers.

The slight low specificity and positive predictive value may be due to the fact that only mustard yellow areas are VILI positive but partial iodine uptake in cervicitis and non-uptake by columnar epithelium (lack of glycogen) may be misinterpreted as positive. The very high NPV of VILI, which means that when a test is negative, the women can go home reassured that she is not likely to have a neoplastic cervical lesion; eliminating the need for follow up visits.

In conclusion, in low resource settings, screening for carcinoma cervix by Pap smear can be replaced by cheaper and easily available visual method like VILI, which has the higher sensitivity to detect any grade of dysplasia, with a reasonable specificity. Even when screening with Pap smear is available, it should be combined with visual screening methods like VILI, as many cases of CIN1 are missed by Pap smear may be picked up by visual test and combined testing will reduce the false negative cases. In remote areas VILI should be used as a primary screening tool.

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