Original Article

Cervical Smear Screening by Pap Smear and Surveillance of HPV Infection among Patients Attending Gynaecology OPD in a Tertiary Care Center – A 12-Months Study

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Abstract

Background: Cancer of the uterine cervix stands as the 4th most common cancer in terms of incidence and mortality globally. In India, it is the 2nd most common cancer in 2022. Human papillomavirus (HPV) infection is the most common etiological risk factor for cervical cancer, and HPV-16 and 18 are associated with 70% of all invasive cervical cancers.
Methods: A single-centered descriptive study on 111 women was conducted in collaboration with departments of Obstetrics and Gynecology, Pathology and Multi-disciplinary Research Unit (MRU) of a tertiary care center for a period of 12 months, from September 2023 to August 2024. Cervical Pap smear examination by conventional technique and detection of HPV DNA by Hybrid Capture 2 (HC2) system were performed.
Results: One hundred (90.1%) samples were interpreted as Negative for intraepithelial lesion or malignancy (NILM), two (1.8%) samples as Atypical squamous cells of undetermined significance (ASCUS), one (0.9%) sample as Low grade squamous intraepithelial lesion (LSIL) and samples of eight (7.2%) women as Unsatisfactory. HC2 identified 4 (3.6%) hrHPV positive women, out of which three (2.7%) women belonged to the age group of 36 to 45 years. On comparison to Pap smear study, two (50%) were NILM, one (25%) was ASCUS and one (25%) Unsatisfactory.
Conclusion: HPV testing is a well-established screening module for cervical cancer. There is negligible screening of HPV in our state. This study hopes to lay a foundation stone in early screening and prevention of cervical cancer through HPV vaccination in Manipur.

Keywords: Cervical Cancer; Human Papillomavirus; Pap Smear; HPV DNA Test

Introduction

Cancer of the uterine cervix accounts for 660,000 new cases and 350,000 deaths worldwide in 2022, standing as the 4th most common cancer in terms of incidence and mortality. In India, it stands as the 2nd most common cancer among women with 127,526 new cases and 79,906 deaths in 2022. Ministry of Health and Family Welfare of the Government of India launched population-based screening of cancer in 2017 comprising three preventable cancers, namely oral, breast and cervical cancers [1]. World Health Organization (WHO) recommends DNA-based testing for human papillomavirus (HPV) as a superior method as compared to the rest. The apex body also aims to achieve the following targets by 2030: i) 90% of girls vaccinated with HPV vaccine by age 15; ii) 70% women screened for cervical cancer by age 35 and again by age 45; iii) 90% women with precancerous lesions treated and 90% of women with invasive cancer managed [2]. The age-adjusted incidence rate of cervical cancer in Manipur for the last 17 years is around 6-10/1,00,000 women [3].

Human papillomavirus (HPV) infection is the most common etiological risk factor for cervical cancer. Majority of the sexually active women acquire genital HPV by 18-30 years, which gradually clears within 2 years without any intervention. Less than 1% of HPV infection persist and progress to cervical cancer, more commonly in women >35 years [4, 5]. HPV is a double-stranded, circular, non-enveloped, 8kb sized DNA virus with over 130 identified genotypes. High-risk HPV types are more commonly associated with cervical cancer and include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70. Among the above, HPV-16 and HPV-18 are associated with 70% of all invasive cervical cancers [6].

Visual inspection with acetic acid (VIA) is used most frequently in low-income countries for mass screening. Conventional cytology using Papanicolaou stain combined with colposcopic examination has been the simplest and most feasible technique for screening of cervical cancer. According to ASCUS-LSIL Triage Study (ALTS), HPV testing was earlier used as a reflex testing after cytological evidence for presence of atypical squamous cells [7]. Presently, many testing methodologies are now available such as PCR, ISH, Hybrid capture, NGS, etc. The Hybrid Capture II HPV-DNA Assay (Digene) was the first FDA-approved test for detection of high-risk HPV in 2003. In the later years, 4 other testing modalities were FDA-approved: Cervista HPV HR (Hologic), Cobas HPV test (Roche Molecular Systems), Cervista HPV 16/18 (Hologic) and APTIMA HPV Assay (Gen-Probe) [8]. Co-testing for high-risk HPV testing with cytology increases the sensitivity of a single Pap test for detection of high-grade neoplasia from 50-85% to almost 100%. At present, the guidelines suggest that women aged 21-29 years should be tested with cervical cytology alone and screening for every 3 years. Women aged 30-65 years, co-testing with cytology and high-risk HPV testing are to be performed every 5 years or cytology alone every 3 years or high-risk HPV testing alone every 5 years. However, testing for women above 65 years is not recommended if at least three Pap tests or two HPV tests in the past 10 years, and the test results were normal or negative or not had a cervical precancer in the past or cervix removed as part of a total hysterectomy for non-cancerous conditions, like fibroids [9, 10].

In Manipur, there is negligible primary prevention including vaccination and an inadequate secondary prevention in terms of timely screening for cervical cancer. This study intends to reach out to all stakeholders and lay a foundation stone in early screening of cervical cancer in this state, along with the hope of prioritizing HPV vaccination.

Materials and Methods

A single-centered descriptive study on 111 women was conducted in collaboration with departments of Gynecology, Pathology and Multi-disciplinary Research Unit (MRU) of a tertiary care center for a period of 12 months, from September 2023 to August 2024. The study was approved by the Institutional Ethical Committee prior to its initiation. Consenting symptomatic women aged $\geq$ 25 years attending the Gynecology OPD were included. Patients below 25 years of age, non-symptomatic patients above 25 years of age, patients unwilling to participate, patients who underwent total hysterectomy or conization and patients receiving or have completed treatment for cervical cancer were excluded from the study.

Clinical parameters of the patients including demographic data, presenting complaints and other relevant information were collected following strict ethical guidelines. Cervical smear samples were collected in a special kit - VTM (viral transport media) for HPV detection and also by Ayre's spatula for smear in the Gynecology OPD and sent to cytology section of Department of Pathology, RIMS for staining by conventional Pap method. Both the cervical smear sample and HPV testing sample were collected at the same time for convenience. Findings were reported using "The Bethesda System for Reporting Cervical Cytology (3rd edition)". The VTM samples were sent to the Pathology laboratory of MRU, RIMS, Imphal and then processed for detection of HPV by Hybrid Capture 2 system (HC2). The HC2 assay (Qiagen Gaithersberg, MD, USA; previously Digene Corp.) which was approved by the FDA in 2003, is a nucleic acid hybridization assay that quantitatively detects HPV high risk and low risk subtypes. The digene R HC2 High-Risk HPV DNA Test using Hybrid Capture 2 (HC2) technology is an in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of thirteen high-risk types of human papillomavirus (HPV) DNA in cervical specimens. The HPV types that can be detected by the assay are the high-risk HPV types 16/18/31/33/35/39/45/51/52/56/58/59/68. The digene HC2 High-Risk HPV DNA Test cannot determine the specific HPV type present in the sample.

Results

A total of 111 symptomatic consenting women, aged above 25 years who attended the Gynecology OPD at the Regional Institute of Medical Sciences, Imphal for a period of 12 months were inducted into the study. One hundred ten (99.1%) out of the 111 women were married. A large proportion (87 women, 78.4%) of the screened women were between the age 36 to 55 years. The predominant presenting symptoms were pelvic pain in 70 (63.1%) women, discharge per vagina in 68 (61.3%) women and bleeding per vagina in 17 (15.3%) women. Additionally, 7 (6.3%) women had history of hormone replacement therapy (HRT).

On cytologic examination, 100 (90.1%) samples were interpreted as Negative for intraepithelial lesion or malignancy (NILM), two (1.8%) as Atypical squamous cells of undetermined significance (ASCUS), one (0.9%) as Low grade squamous intraepithelial lesion (LSIL) and eight (7.2%) were found to be 'unsatisfactory for evaluation' due to reasons such as

low squamous cellularity, obscuring blood, excessive inflammatory cells and thick mucus obscuring the squamous cells. Organisms were found in nine (8.1%) out of 111 samples, that comprised of six (5.4%) samples with bacterial vaginosis and three (2.7%) samples with fungal organisms morphologically consistent with candida. No cases of High grade squamous intraepithelial lesion (HSIL), Squamous cell carcinoma (SCC), Atypical glandular cells (AGC) or Adenocarcinoma (insitu or invasive) were encountered.

The high-risk HPV testing using digene R HC2 High-Risk HPV DNA based on Hybrid Capture 2 (HC2) technology identified four (3.6%) hrHPV positive women, out of which three (2.7%) women belonged to the age group of 36 to 45 years, whereas one (0.9%) woman was aged 48 years. Among the positive women, 2 (50%) were interpreted as NILM, one (25%) as ASCUS and one (25%) as Unsatisfactory by conventional Pap smear examination.

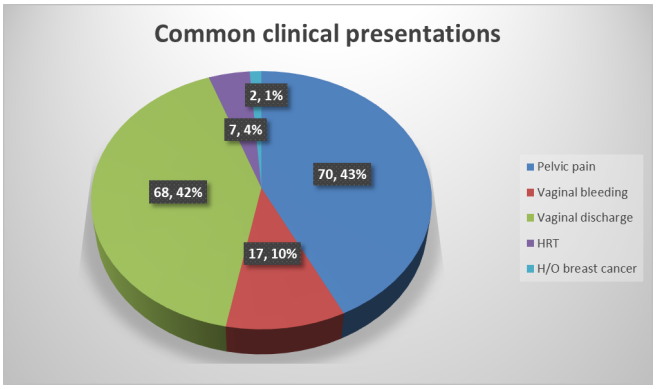

Figure 1: Pie chart showing the common clinical presentations among participant women.

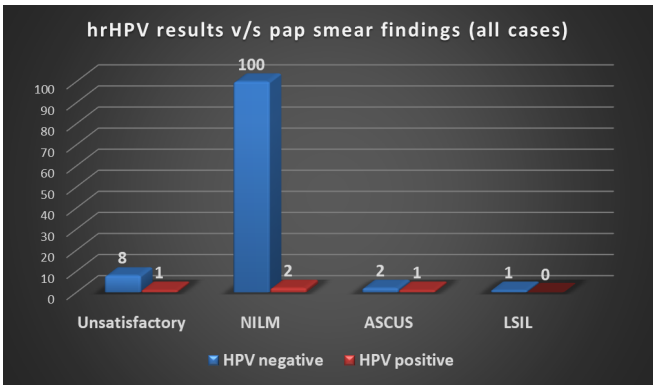

Figure 2: HrHPV testing results among various categories of cervical Pap smear results.

Table 1: Age wise distribution table showing cervical Pap smear examination findings and concurrent HPV testing result. (n=111)

Age in years	Marital status		Cervical Pap smear examination findings		
	Married	Unmarried	Unsatisfactory	NILM	Abnormal (ASCUS+LSIL)
26 to 35	11	1	2 (1.8%)	10 (9.0%)	0
36 to 45	40	0	5 (4.5%)	32 (28.8%)	3 (2.7%)
46 to 55	47	0	0	47 (42.3%)	0
56 to 65	10	0	0	10 (9.0%)	0
>65	2	0	1 (0.9%)	1 (0.9%)	0
TOTAL	**110 (99.1%)**	**1 (0.9%)**	**8 (7.2%)**	**100 (90.1%)**	**3 (2.7%)**

Abbreviations: NILM = Negative for intraepithelial lesion or malignancy; ASCUS = Atypical squamous cells of undetermined significance; LSIL = Low grade squamous intraepithelial lesion; hrHPV = High risk human papillomavirus.

Discussion

The World Health Organization (WHO) issued a call for cervical cancer elimination in May, 2018 for its urgent elimination which was endorsed unanimously at the 73rd congress in August, 2020. Three targets of global strategy that must be met by 2030 were set: i) vaccinate at least 90% of eligible girls against HPV; ii) screen a minimum of 70% eligible women, at least

Table 2: The WHO approved three approaches to HPV testing for cervical cancer screening and future tests (Adapted from WHO guidelines for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention, 2nd edition) [2]

Molecular	Cytologic	Visual inspection
Nucleic acid amplification tests (NAAT)	Conventional Pap smear	Visual inspection with acetic acid and Lugol's iodine (VIA/VILI)
· High-risk HPV DNA/ NAAT	Liquid based cytology (LBC)	· Naked eye
· mRNA	Dual staining to identify p16 and Ki67	· Magnified by colposcope or camera
Future tests		Automated visual evaluation by digital images.
DNA methylation		
Protein biomarkers		
· HPV antibodies		
· Oncoproteins		

Table 3: Comparison of the present study with previous studies.

Study by	Period	Place	Population	N	Setting	Collection	Method	HPV+
Advesh Kumar Rai et al [18]	3 mo	Assam, India	Healthy women	43	Hospital	Clinician	HC2	16.2%
Jung et al [20]	9 mo	Japan	Symptomatic	1983	Hospital	Clinician	PCR	46%
Sowjanya et al [22]	31 mo	Andhra Pradesh	Periurban women >20y	432	Community	Self+Clin	HC2	14.1%(S) 20.1%(C)
Bhatla et al [23]	30 mo	New Delhi, India	Symptomatic >30y	546	Hospital	Self+Clin	HC2	12.3%(S) 13.1%(C)
Anand et al [24]	3 mo	Maharashtra, India	Urban/rural 30-55y	500	Community	Self	HC2	6.4%
Present study	12 mo	Manipur, India	Symptomatic >25y	111	Hospital	Clinician	HC2	3.6%

Abbreviations: mo = months; y = years; N = Sample size; HC2 = Hybrid Capture 2; S = Self-collection; C = Clinician-collection; HPV+ = HPV positive.

twice in their lifetime; and iii) effectively treat at least 90% with positive screening test or cervical lesion, including palliative care whenever needed. Therefore, WHO approved three approaches to HPV testing for screening of cervical cancer.

Pre-analytical clinical history revealed that the most common presenting complaints in our OPD were pelvic pain in 70 (63.1%) women, followed by discharge per vaginum in 68 (61.3%) women and bleeding per vaginum in 17 (15.3%) women. A wide number of studies found vaginal discharge to be the commonest presenting pattern [11, 12, 13]. Majority of the women screened by conventional Pap smear study were Negative for intraepithelial lesion or malignancy (100 women, 90.1%) which follow a similar dictum globally. However, a significant number of them had superimposing infection (59 women, 53.2%) as was seen by Patel, et al. [12]. Furthermore, smears of 34 (30.6%) women exhibited reactive cellular changes associated with inflammation. Atrophy without inflammation and atrophic vaginitis constituted 3 (2.7%) and 12 (10.8%) cases respectively, within the NILM category. Abnormal cytology (ASCUS & LSIL) on Pap smear examination was found more commonly between age 36 to 55 years. The ASCUS/SIL ratio was 2:1 in our study. In a US based follow up study, Nascimento and Cibas reported ASCUS/SIL ratio entailing a downward trend from 2.05 to 1.73 upon regression analysis done among various cytopathologists [14].

The WHO, American Society of Clinical Oncology (ASCO) recommends HPV DNA detection in "screen and treat" or "screen, triage and treat" approach starting at age of 30 years with regular screening every 5 to 10 years in general population of women. For women living with HIV, WHO recommends HPV DNA detection in "screen, triage and treat" approach starting at age of 25 years with regular screening every 3 to 5 years. Women $\geq$ 65 years of age who have had consistently negative screening results during past $\geq$ 15 years may cease screening [2, 15]. In our study, we also included women living with HIV among those without. Therefore, women aged >25 years were included in the study, irrespective of the HIV infection status in an attempt to harmonize the findings.

According to a large-scale study by ICMR in Manipur, the prevalence of HPV-16/18 was around 3.3% and HPV-18 was the predominant type [16]. According to a monitoring survey of cancer risk factors and health system response in North-East Region, a study conducted by ICMR, there is negligible screening of cervical cancer in urban and rural areas of Manipur [17]. In the present study, hrHPV was found to be positive in only four (3.6%) women with a mean age of 43.8 years (ranging from 37 to 48 years). Being a prima facie hospital-based study, the potential regional or cohort-specific factors that might contribute to this finding seem unclear. Possible environmental and demographic factors may have a role. In a study conducted at a neighboring region, Advesh Kumar Rai et al, found HPV positivity rate of 16.2% with a mean age of 37.5 years among HPV positive women [18]. The HPV positivity rate in various studies varies drastically. In 2009, Castle et al, performed a retrospective analysis of 5 years comprising 797,927 women who underwent co-testing using conventional Pap test and HPV test using Hybrid Capture 2 revealed HPV positivity rate of 6.27% [19]. Whereas, in a Korean study by Jung

et al, done on 1983 women in 2002 found HPV positivity rate of 46% [20].

Jenetschke et al, highlighted the idea of self-collection of cervical samples for HPV testing, elucidating the fact that it would increase participation rate of women to be screened among non-responders [21]. Sowjanya et al, in a community based cross-sectional study among 896 peri-urban women aged >20 years adopted a combined collection methodology, found HPV positivity rate of 14.1% and 20.1% in self and clinician collections respectively [22]. A similar hospital-based study by N Bhatla et al, in 2019 among 546 symptomatic women found HPV positivity rate of 12.3% and 13.1% in self and clinician collections respectively [23]. However, Anand et al, in 2022 a cross-sectional hospital-based study was conducted among 500 rural and urban women who employed self-collection which resulted in a lesser HPV positivity rate of 6.4% [24].

In 2019 ASCCP (American Society for Colposcopy and Cervical Pathology) Risk-Based Management Consensus Guidelines provided a framework for managing abnormal cervical cancer screening tests and cancer precursors, aiming to maximize cancer prevention while minimizing over-testing and overtreatment. These guidelines prioritize a patient's risk of cervical intraepithelial neoplasia grade 3 (CIN 3) or higher (CIN 3+) to determine appropriate management strategies [25].

Conclusion

HPV testing is a well-established screening module for cervical cancer. However, there is negligible screening of HPV in Manipur. A precise understanding of the HPV prevalence may require a larger-scale, population-based, district-wise multicentric study. Furthermore, we need to incorporate HPV genotyping by PCR or other techniques in addition to hrHPV testing in the near future to stratify the commoner genotypes. Identification of the predominant genotypes will help in risk-based management and in undertaking effective HPV vaccination program in our part of the country. Therefore, an entailed unified approach is the need of the hour in converging our efforts to forestall the progression of cervical cancer.

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Competing Interests: All the authors declare that there is no conflict of interests of any sort.

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