Original Research Article

Comparing effectiveness of COBAS HPV test with VIA/VILI, pap smear and colposcopy for screening of cancer cervix

T V Indirani [1], **Lalitha Subramanian** [2,*], **Igena Sharo Suji I** [1]

[1] Dept. of Obstetrics and Gynaecology, Government Thoothukudi Medical College, Thoothukudi, Tamil Nadu, India
[2] Dept. of Radiotherapy, Government Thoothukudi Medical College, Thoothukudi, Tamil Nadu, India

ARTICLE INFO

ABSTRACT

Introduction: Cancer cervix is the second leading cancer causing 21% of all cancer deaths. Cervical cancer can be prevented if detected by premalignant changes and is curable in its earliest stage. Various procedures including VIA/VILI, pap smear have been routinely used for the purpose of screening cervical cancers. Infection with Human Papilloma virus is the principal cause of cancer cervix. Roche COBAS HPV test was approved by US FDA on April 24, 2014 as one of the first line primary screening method for cancer cervix based on the presence of high risk HPV DNA. The FOGSI guidelines in January 2018 has suggested cobas HPV test for cancer cervix screening.

Materials and Methods: This study was conducted on 100 women with high risk cervical lesions during the period of 18 months. Women attending the NCD clinic of Government Thoothukudi Medical college hospital were taken for the study. The women were to undergo VIA/VILI, Pap smear study and subjected to COBAS HR HPV Test after their consent.

Cobas HPV Test: Cervical specimens collected in PreservCyt solution using an endocervical brush/ spatula or collected in SurePath preservative fluid using a cervical broom were sent for COBAS HR HPV test. Collected sample kits were recruited in Department of Radiation oncology, Government Thoothukudi medical college for storage at low temperature for a short period, after pooling of samples, these were sent through the Department of radiation oncology to Adayar cancer institute, Chennai, with which the department has treated with for COBAS HPV test. The results were analysed and proceeded.

Conclusion: This study has proven that screening for high risk HPV test in cervical specimen can easily pickup the premalignant lesion with high sensitivity, specificity when compared with other screening tests. Hence, this study emphasizes the importance of implementing HPV tests that can provide a 3-year screening free interval, compared to yearly pap screening. The other advantage is that the results are available immediately reducing the frequency of visits to hospital for further follow up on colposcopy/biopsy.

1. Introduction

Cancer cervix is the second leading cancer causing 21% of all cancer deaths. Globally, it is the most common genital cancer occurring in women and 4th commonly occurring malignancy in women (WHO 2012). Around 85% of cervical cancers are reported from developing countries per annum. India bears the burden of having the largest population with cancer cervix. About 1,30,000 new cases with 70,000 cervical cancer related deaths are reported from India. The late presentation and lack of knowledge about the screening methods among Indian female population make them succumb to the disease. A regular screening programme is recommended to prevent the neoplastic

* Corresponding author.
E-mail address: igenasharosuji@gmail.com (L. Subramanian).

progression and mortality.[1] The preinvasive phase of cancer cervix is long, about 10-15 years Screening of women for cancer cervix has greatly reduced the mortality from 80% to 60% due to early detection and effective management. If detected early, pre invasive lesions are nearly 100% curable. Early cancer has 67%. 5 year survival rate while advanced cancers have only 35% survival rate. Various procedures including VIA/VILI, pap smear, liquid based cytology tests, HPV DNA tests has been routinely used for the purpose of screening cervical cancers. The reliability of these tests have become questionable for example, one third of cervical cancers occur in women who have had normal Pap smear results. However this problem has now decreased by combining high risk HPV testing and cytology tests also called co-testing.[1] Infection with Human papilloma virus is the principal cause for cancer cervix though other factors can affect the disease progression after initial viral infection.[1] These include highest risk with HPV 16, 18 and 12 high risk HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. Roche cobas HPV test was approved by US FDA on April 24, 2014 as one of the first line primary screening methods for cancer cervix based on the presence of high risk HPV DNA.[2] This study compares the efficacy of high risk HPV tests with VIA/VILI, pap smear and colposcopy in detecting cervical cancers by comparing sensitivity, specificity and predictive values.

2. Aim of the Study

1. To screen patients with cervical cytology test results to determine the need for referral to colposcopy
2. To guide patient management by detecting high risk HPV genotypes 16 and 18 together with gynecologist's assessment and other risk factors
3. To determine the pattern of occurrence of HPV genotypes for preventive vaccination
4. To reduce the frequency of pap tests by determining the high risk subtypes by cobas HPV test in women more than 20 years as the first line screening test
5. To compare the effectiveness of cobas HPV test with VIA/VILI, pap smear and colposcopy with histopathology as gold standard.
6. To correlate between the findings of cobas HPV test, VIA/VILI, pap smear and colposcopy findings.
7. To compare the sensitivity and specificity of the above said screening methods.
8. To determine a definitive screening tool to reduce time yet effective in massive screening in government hospital settings.

2.1. Human papilloma virus

Nearly all cervical neoplasia and neoplasia of lower genital tract- vulva, vagina, anus are caused by human papilloma virus. This has been proved by various studies. The cells most commonly prone for HPV infection are cervical squamous and the cells that have undergone metaplastic changes. Human papilloma virus is a double stranded DNA virus that has a protein capsid which is unique to each type. Research has identified more than 150 unique HPV types. Among these, 40 types have known to infect the lower genital tract. The HPV genome is circular and its genome contains nine ORF(open reading frame). The six early genes (E) genes together with a regulatory region control viral functions like the life cycle, DNA maintanence, replication and transcription. The lower layers of squamous epithelium contain the early genes. The late genes- L1 and L2 genes also called the major and minor capsid proteins are found in the superficial layers of the epithelium. These genes are expressed late in the life cycle and assembly of the new viral particles. The genetic expression of Human papilloma virus is synchronous with the differentiation of the squamous epithelial cells of the cervix. Therefore the life cycle of virus can be completed only within a fully differentiated epithelium. This reasons out the impossibility in invitro culture of human papilloma virus. Human papilloma virus resists lysis and the desquamation of the epithelial cells is required for the infection to occur. The initiation of viral infection occurs when the viral L1 protein and L2 protein bind to the basement membrane of the squamous epithelial cells or the basal cells which inturn permits the entry of the viral particles into the host cell. Women aged below 25 years are reported to have most of the initial HPV infection. When comparing the point prevalence of HPV infection, women aged 14 to 59 years have 27% of infection while the highest prevalence -45 percentage is seen with women aged 20-24 years. The prevalence decreases with increasing age. Based on the strength of association with cervical cancer, HPV has been classified as high risk HPV (HR) and low risk HPV (LR). HPV 6 and 11 comprise low risk HPV. Low risk HPV causes almost all genital warts, papillomas of laryngx and a few subclinical infection. Infection with low risk HPV rarely cause malignancy. Various studies and research work have emphasized the definitive role of HR HPV viruses in the development of cancer cervix. High risk HPV, includes -16, 18, 31, 33, 35, 45, and 58, and a few less common types, and are responsible for more than 95% of new cervical cancer case globally. Among these, HPV 16 is the most oncogenic, responsible for more than 45% of CIN 3 lesions and more than 55% of cervical carcinomas. These viruses are also responsible for most of the HPV related anal and oral cancer. The prevalence of HPV 18 is very much lower than HPV 16. These viruses are known to cause a higher rate- (40 percentage) of cervical adenocarcinomas or adenosquamous carcinomas when compared to 13 percentage of cervical squamous cancers. Globally, HPV 16 and HPV 18 together are responsible for 70 percentage of cancer cervix, 68 percentage of squamous neoplasm and 85 percentage of adenocarcinomas.[1] After HPV 16 and 18, HPV 45 is the

third most common virus causing cervical cancers. HPV 16 is the most common papilloma virus causing low grade lesions and causes more than 1 in 5 cervical HPV infection.

2.2. HPV tests

2.2.1. Primary HPV testing

Growing evidence supports only HR HPV testing without cytology as an option for primary screening of cervical cancer. In 2014, the cobas HPV test was the first HPV test approved by FDA for primary screening of cervical cancer in women aged 25 years or older.[1] This test gives simultaneous results on the presence or absence of HPVs 16 and 18 (highest risk) and a group of 12 other high risk oncogenic HPV types. This lead to a profound shift in cervical cancer screening, where Pap testing takes only a secondary role.

The sensitivity of HPV testing alone (>90%) is twice than that of single pap test and this leads to earlier detection of high grade cervical neoplasias. Sankaranarayanan[3] in 2009 showed that a single negative HPV test had a very high negative predictive value. This was an 8-year study that showed that a single HPV test outperformed cytology tests with no reported cervical cancer deaths. Hence it is strongly proposed that screening with a HR HPV test can be used as an alternative to cytology alone or cotesting in women 25 years and older and at intervals not less than 3 years. Immediate colposcopy is recommended if HPV 16/18 is identified. If other HR HPV types are found, then triage to re cytology is recommended. Colposcopy is performed for any cytologic abnormality.

2.2.2. Cobas HPV test

The cobas human papillomavirus (HPV) test, approved by the FDA, is a fully automated assay for the detection of 14 high risk (hr)HPV genotypes from cervical specimen collected in liquid based cytology medium using real time PCR amplification of the L1 gene and TaqMan probes. Results are simultaneously reported as positive or negative for the pooled 12 oncogenic HPV types (HPV 31, 33, 35, 39, 45, 51,52, 56,58,59, 66 and 68).

2.2.3. Why Cobas HPV test ?

1. Only clinically validated, US FDA approved and CE-IVD marked test for first line primary screening.
2. Only test which provides 3 results in single test, pooled results on known high risk genotypes and individual on two highest risk genotypes HPV 16 and 18.
3. Only test which has internal control for every test to reduce false negativity and external control for reducing false positivity.
4. The cobas HPV test is clinically validated in one of largest and first screening trial- Athena,[2] a multicenter, prospective trial enrolling over 47,000 women >-21

years of age undergoing routine cervical screening.
5. The Cobas HPV test supports a safely extended screening interval (3 years) for women with normal cytology who are HPV negative, similar to other hrHPV DNA tests.
6. The Cobas HPV can be done with primary vial itself and self-collected sampling.
7. The Cobas HPV test demonstrates no cross reactivity with non-high risk HPV genotypes, ensuring that positive results are clinically meaningful.
8. The Cobas HPV test is performed on Cobas 4800 system, which offers true walk away automated extraction, PCR setup and amplification to reduce manual errors and high efficiency. Cobas 4800 system currently have oncology (EGFR, KRAS, BRAF) and CT/ NG parameters and awaited approvals for infectious disease portfolio.
9. -The Cobas HPV test has high reproducibility with no indeterminate/ grey zones, Positive is positive, no need to repeat.

2.2.4. Principles of the procedure

The cobas HPV test is based on two major processes:

1. Automated specimen preparation to simultaneously extract HPV and cellular DNA.
2. PCR amplification of target DNA sequences using both HPV and beta globin specific complementary primer pairs and real – time detection of cleaved fluorescent- labeled HPV and beta globin specific oligonucleotides detection probes.

The concurrent extraction, amplification and detection of beta globin in the cobas HPV test monitors the entire test process. The mastermix reagent for the cobas HPV test contains primer pairs and probes specific for the 14 high risk HPV types and beta globin DNA. The detection of the amplified DNA (amplicon) is performed during thermal cycling using oligonucleotide probes labeled with four different fluorescent dyes. The amplified signal from 12 high risk HPV types (31, 33, 35, 39, 45, 51, 52, 58, 59,66 and 68) is detected using the same fluorescent dye, while HPV 16, HPV 18 and beta globin signals are each detected with their own dedicated fluorescent dyes.

2.2.5. Specimen preparation

Specimen preparation for the cobas HPV test is automated with the use of the cobas instrument. Specimen collected in Surepath preservative fluid must undergo the preanaltyic procedure. On the cobas instrument, pretreated Surepath specimen and PreservCyt specimens are digested under denaturing conditions at elevated temperatures and then lysed in the presence of chaotropic reagent. Released HPV nucleic acids along with the beta globin DNA serving as process control, are purified through adsorption to magnetic

glass particles washed and finally separated from these particles, making them ready for PCR amplification and detection.

3. Materials and Methods

This study was conducted on 100 women with high risk and cervical lesions during the period of 18 months. Women attending the NCD clinic of Government Thoothukudi Medical College hospital were taken for the study. The women underwent VIA/VILI, pap smear study, colposcopy and subjected to cobas HPV test after their consent. VIA/VILI: visual inspection of cervix with hand held magnifying lens. 3% acetic acid and lugol's iodine was used for the procedure. Acetowhite areas were taken positive and areas unstained with lugol's iodine were taken positive. Pap smear: smear from cervix using Ayer's spatula will be fixed in a koplik's jar containing 95% ethanol and 5% ether. Slides were sent for pathological analysis. Cobas HPV test: cervical specimen collected in the PreservCyt solution using an endocervical brush / spatula were recruited in Department of Radiation oncology, Government Thoothukudi medical college for storage at low temperature for a short period, after pooling of samples, these were sent through the department of radiation oncology to Adayar cancer institute, Chennai, with which the department has treated with for cobas HPV test. The results were analysed and proceeded.

3.1. Type of study

Prospective observational study on women of reproductive age group.

3.2. Source of data

Women aged more than 21years attending colposcopic clinic of Government Thoothukudi medical college.

3.3. Period

18 months.

3.4. Inclusion criteria

Sexually active women more than 21 years

1. Non pregnant women.
2. Nulliparous
3. Multiparous women

3.5. Exclusion criteria

1. Women with severe ill health
2. Women allergic to acetic acid and lugol's iodine
3. Women on hormonal therapy
4. Women with previous surgery on cervix

3.6. Design of study

Prospective observational study.

3.7. Sampling method

Simple random sampling. Informed consent for the study was obtained from patients attending the gynecology OPD and enrolled for the study. History and detailed physical and gynecological examination was done. VIA/VILI screening was done in all patients. Lesions were subjected to pap smear. Cobas hrHPV test was done. Colposcopy and subsequent biopsy was done.

3.8. Methodology of study

1. Patient lies in lithotomy position
2. Cusco speculum introduced
3. Nature of discharge noted
4. Smear taken for pap study. Fixation done.
5. Cobas hrHPV test taken. Sample collected in PreservCyt
6. Unstained cervix inspected
7. Transformation soon inspected after application of acetic acid
8. Inspection done after applying lugol's iodine
9. Fornices, vaginal wall examined
10. Findings noted.
11. Colposcopy done
12. Vascular pattern noted
13. Acetic acid and lugol's iodine applied
14. After recording the findings, cervical biopsy done by punch forceps
15. Specimen sent for histopathological examination.

4. Results and Analysis

Table 1: VIA/VILI

VIA/VILI	Frequency	Percent
Negative	42	42
Positive	58	58
Total	100	100.0

Table 2: Colposcopy

Colposcopy	Frequency	Percent
abnormal	34	34
normal	66	66
Total	100	100.0

5. Discussion

This study compares the effectiveness of cobas HPV test with VIA/VILI, pap smear and colposcopy with biopsy as the gold standard for early detection of cervical lesions,

Table 3: PAP smear

PAP Smear	Frequency	Percent
Normal	85	85.0
Abnormal	15	15.0
Total	100	100.0

Table 4: Cobas HPV test

Cobas HPV Result	Frequency	Percent
HPV 16 +VE	5	5.0
HPV 16, 31,35+	2	2.0
HPV 16, 35+	4	4.0
HPV 16,35,31,39 +VE	1	1.0
HPV 16,58+	1	1.0
HPV 35, 31+VE	1	1.0
HPV 35+VE	1	1.0
HPV 56, 58, 59 +VE	1	1.0
NON 16,18 HR HPV	4	4.0
NEG	80	80.0
Total	100	100.0

Table 5:

Biopsy	Frequency	Percent
Chronic Cervicitis	28	45.1
CIN 1	5	8.06
CIN II	4	6.4
CIN III	2	3.2
Infiltrating Adenocarcinoma	1	1.61
Invasive Squamous Cell Carcinoma	2	3.2
Normal	20	32.2
Total	62	100.0

Table 6:

	Frequency	Percent
HPV 16/18	5	25.0
HPV 16+ other HR HPV	8	40.0
NON 16/18 HR HPV	7	35.0
Total	20	100.0

hence, aiming at reduction in frank cervical cancer rates and effective treatment of early cervical changes. In this study, women aged between 30 to 50 years had the highest incidence of premalignant cervical changes (71 percentage) while women less than 30 years had only 9% prevalence. In those aged 50 and above, the prevalence is around 20 percentage. This goes in hand with a study by Fernnandes and Kushtagi, who showed that the incidence of premalignant lesions of cervix is more in women aged 30 years and above. The age of marriage and duration of married life correlates with the occurrence of cervical cancer. In this study, around 43 percentage of women were married before 20 years of age and 53 percentage were married between 20 and 30 years of age. The study by Fernnandes and Kushtagi showed that an early age at marriage and long duration of marriage is associated with an increased risk of cervical malignancies. The most common complaint of presentation was leucorrhea (49%), followed by bleeding/ spotting pv (14%) and abdominal pain (12%) Higher incidence of premalignant cervical lesions is found in multiparous women >3 (51 percentage), while it is only 2% for nulliparous and women with single parity. The incidence of cervical premalignant lesions was more among people belonging to socioeconomic status 4 or less (57 percent), when compared to class 2, where it is only 2 percentage. Among the biopsy positive cases, 11.6% had CIN I, 9.3% had CIN II, 4.7% had CIN III and 7% had invasive cancers (4.7%-invasive squamous cell carcinoma, 2.3%infiltrative adenocarcinoma). Pap smear results: Among 100 samples, 15 pap results were found to be positive and 7 were both pap and biopsy positive. 2% LSIL, 2% HSIL, 9% ASCUS and 2% invasive cancer. Compared with biopsy results, pap smear is 7% true positive, 8% false positive, 40% true negative and 8% false negative. Pap smear had a sensitivity of 50% and a specificity of 43.75% from this study. The diagnostic accuracy is 45.16%. Colposcopy results : Among 100 samples, 34 colposcopy results were found to be positive and 7 were both colposcopy and biopsy positive. Sensitivity was found to be 50% and specificity 83.33 percent. Diagnostic accuracy is 75.81%. Divya Hedge et al.[4] in a study conducted in 2011, showed the sensitivity was 70.8%, specificity was 95%, positive predictive value was 62.9% and NPV was 96.5%. Olaniyan B et al[5] also showed the correlation between colposcopy and biopsy, and the accuracy was 89%. The present study also showed the highest specificity for colposcopy. VIA/VILI results: Among 100 samples, 58 were found to be positive and 10 were both pap and biopsy positive. Compared with biopsy results, VIA/VILI had a sensitivity of 71.43% and very low specificity. Cobas HPV test: 5% had HPV 16 infection, 8 percentage had HPV 16 and other high risk HPV infection, while another 7 percentage had hon 16/ 18 HR HPV infection. This finding goes well with Athena Trial[2] which supports higher occurrence of HPV 16 infection in premalignant lesions. Among 100 samples, 20 HPV results were found to be positive and 11 were both pap and biopsy positive Compared with biopsy results, cobas HPV test had a sensitivity of 78.57% and a specificity of 81.25% from this study. The diagnostic accuracy is 80.65%. Cobas HPV test has the highest sensitivity, colposcopy has highest specificity. HPV testing is the best screening method to detect early cervical lesions, with a diagnostic accuracy par comparable to viavili, colposcopy and pap smear. Though VIAVILI is routinely used in low resource settings, it has a very low specificity.

Table 7: Comparison of VIA/VILI, PAP smear, colposcopy and HPV PAP smear and biopsy report

		Biopsy Report		Total
		Negative	Positive	
PAP SMEAR	Negative	40	7	47
	Positive	8	7	15
Total		48	14	62

VIA/VILI and Biopsy Report

		Biopsy Report		Total
		Negative	Positive	
VIA/VILI	Negative	0	4	4
	Positive	48	10	58
Total		48	14	62

Colposcopy and Biopsy Report

		Biopsy Report		Total
		Negative	Positive	
Colposcopy	Negative	21	7	28
	Positive	27	7	34
Total		48	14	62

Cobas HPV Result and Biopsy Report

		Biopsy Report		Total
		Negative	Positive	
Cobas HPV result	Negative	39	3	42
	Positive	9	11	20
Total		48	14	62

6. Conclusion

The burden of cancer cervix has increased in developing countries like India, where the lack of awareness among people make them succumb to the disease. Through the use of VIAVILI in massive screening programmes in low resource settings and in areas with inadequate infrastructure and man power, the detection of premalignant lesions of cervix has improved. Though the sensitivity of VIA/VILI is good, it is highly non-specific thus, has become questionable in cervical cancer screening. Colposcopy is referred by studies as the best screening tool owing to its higher sensitivity and specificity. Pap smear has very low sensitivity as derived from this study. This study has proven that screening for high risk HPV test in cervical specimen can easily pickup the premalignant lesion with high sensitivity, specificity. This test also can reduce the time factor that is involved with VIA/VILI followed by pap/colposcopy. It also helps in detecting the prevalence of HPV serotypes in population groups and counselling regarding HPV vaccines at the earliest even before reproductive age. Hence, this study emphasizes the importance of implementing HPV tests that can provide a 3-year screening free interval, compared to yearly pap screening. The other advantage is that the results are available immediately, reducing the frequency of visits to hospital for further followup on colposcopy/biopsy. Hence for good resource settings, high risk HPV testing is the most effective screening tool for early detection of premalignant lesions of cervix.

7. Conflict of Interest

The authors declare no conflict of interest.

References

1. Hoffman BL, Schorge JO, Bradshaw KD, Halvorson LM, Schaffer JI, Corton MM. Precancerous lesion of cervix. In: Williams Gynecology. McGraw Hill; 2016. p. 625–52.
2. Rao A, Young S, Erlich H, Boyle S, Krevolin M, Sun R, et al. Development and Characterization of the cobas Human Papillomavirus Test. *J Clin Microbiol*. 2013;51(5):1478–84.
3. Shankaranarayanan R, Wesley R, Somanathan T, Dhakad N, Chandralekha B, Sebastian P, et al. Test charecteristics of visual inspection with acetic acid and lugol's iodine(VILI) in cervical cancer screening in Kerala, India. *Int J Cancer*. 2003;106(3):404–8.
4. Hegde D, Shetty H, Shetty PK, Rai S. Diagnostic value of acetic acid comparing with conventional Pap smear in the detection of colposcopic biopsy-proved CIN. *J Cancer Res Ther*. 2011;7(4):454–8.
5. Olaniyan OB. Validity of colposcopy in the diagnosis of early cervical neoplasia–a review. *Afr J Reprod Health*. 2002;6(3):59–69.

Author biography

T V Indirani, Associate Professor ⓘD https://orcid.org/0000-0003-0733-1569

Lalitha Subramanian, Associate Professor

Igena Sharo Suji I, Postgraduate