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Comparison of Cervical and Vaginal Specimens for HPV/DNA Test for Screening Cervical Carcinoma among 35 Year Age Cohort Ever Married Women in a District of Sri Lanka: A Cross Sectional Study

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Keywords: *cervical cancer screening, HPV/DNA test, cervical specimens, vaginal specimens, coverage.*

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Comparison of Cervical and Vaginal Specimens for HPV/DNA Test for Screening Cervical Carcinoma among 35 Year Age Cohort Ever Married Women in a District of Sri Lanka: A Cross Sectional Study

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Abstract- Background: Currently, pap smears are used to screen cervical cancers in Sri Lanka. Two major drawbacks of pap smear screening method are the suboptimal sensitivity and the low coverage. HPV/DNA screening method can be used as a cervical cancer screening method and high sensitivity (92.9%) is the major advantage of it. Specimens can be collected for HPV/DNA test from two sampling site a) from the cervix b) from the vagina. Vaginal specimen collection is more feasible as it does not require speculum examination, therefore primary healthcare worker can be collected a specimen. The objective of the study is to compare HPV/DNA test results of specimens from two sampling sites as a measure to improve the quality and coverage of the National Cervical Cancer Screening programme in Sri Lanka.

Method: A descriptive cross-sectional study was conducted among 35 year old ever married women in a district of Sri Lanka. Total number of 682 women were recruited randomly from the field. Total number of 621 women were first subjected to vaginal HPV/DNA specimen collection by primary healthcare workers followed by cervical HPV/DNA specimen collection by Medical officers (MOO) or Public Health Nursing Sisters (PHNSS). Specimen screening was carried out at a laboratory using Polymerase Chain Reaction (PCR) technique by cobas 4800 HPV/DNA screening machine. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the vaginal specimens against cervical specimens were computed with their 95% confidence intervals (CI).

Results: The sensitivity, specificity, PPV and NPV of the vaginal HPV/DNA specimen were 100% (95%CI; 90.7%-100%), 98.9% (95% CI; 97.8%-99.6%), 86.4% (95% CI; 74.1%-93.3%) and 100% respectively. Kappa coefficient between vaginal vs cervical HPV/DNA specimen screening method was 0.92 (95%CI: 0.86%-0.98%).

Conclusions: There is a good concordance between cervical vs vaginal HPV/DNA specimen screening method. Vaginal specimen collection method can be used to improve the detection of cervical lesions.

Keywords: cervical cancer screening, HPV/DNA test, cervical specimens, vaginal specimens, coverage.

I. Introduction

Cervical cancer is the 2nd leading cause of female cancer in Sri Lanka (1). Hence in 1998, Sri Lanka took an initiative to include screening for cervical cancer with conventional papaniculaou (pap) smear in the Well Woman Clinics (WWCs) (2). However, even after 20 years of cervical cancer screening (with pap smears), there is no marked reduction in incidence, morbidity and mortality of cervical cancer in Sri Lanka. Two major drawbacks of the present programme are, the suboptimal sensitivity of the pap smear (53%) (3) to detect Cervical Intraepithelial Neoplasia (CIN) II and the low coverage of the cervical cancer screening programme.

Cervical cancers are virtually associated with human papillomavirus (HPV) infection. HPV/DNA screening test screens for high risk carcinogenic HPV antigens. Cobas 4800 HPV/DNA screening test detects fourteen high risk cervico-vaginal carcinogenic HPV genotypes such as; 16, 18 and 12 pooled high risk (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) (4).There are two methods of specimen collection for HPV/DNA screening test such as; vaginal and cervical. The sensitivity of cobas 4800 HPV/DNA screening test for cervical specimen to detect CIN II (92.9%) (4) is high, therefore the detection rate of cervical lesions are very high.

The major problem lies behind the lower coverage of pap smear screening programme in a country is the requirement of a vaginal speculum examination by field public health Medical Officers called Medical Officers of Health (MOOH), Medical Officers attached to health care institutions or Public Health Nursing Sisters (PHNSS) as well as qualified staff categories for cyto-screening (Consultant Histopathologists and cytoscreeners). Non-cytological

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screening method, which doesn't requires vaginal speculum examination (i.e. HPV/DNA vaginal specimen) may improve the coverage of National Cervical Cancer Screening Programme in Sri Lanka. The objective of the study is to compare HPV/DNA test results of specimens from two sampling sites a) from the cervix and b) from the vagina among 35 year age cohort ever married women in Kalutara district as a measure to improve the quality and coverage of the National Cervical Cancer Screening programme in Sri Lanka.

II. Methods

A descriptive cross-sectional study was conducted in public health administrative areas called MOH areas of Kalutara district since September/2018 to January/2019 to compare screening results between cervical and vaginal methods of HPV/DNA specimen collection. The study population comprised of ever married women in 35 year of age in Kalutara district. Women with diagnosed invasive cervical cancer, women with vaginal bleeding and active infection at the time of examination with evidence of medical records or by visual inspection, women currently on treatment for HPV infection, pregnant women and women ≤ 3 months in the post partum period, women who had undergone hysterectomy, women with diagnosed physical or mental retardation or disease status and women who are not resident within the district continuously for ≥ three months prior to the date of the survey were excluded from the study.

Sample size calculation for diagnostic test accuracy was done (5). Sensitivity of vaginal specimen vs cervical specimen for CIN II or worse in Polymerase Chain Reaction (PCR) based screening method was 99% (6). Prevalence (P) of the disease in the target population was 3.3% (7). In a case of preliminary studies, if there is a resource limitation setting, investigators may use a lower precision of >10% (8), (9). Therefore, we needed 386 women. Further adjustment to the sample size was made by considering the previous year WWC non-response rate (42.4%) in Kalutara district (10) and the final required sample size was 671.

A MOH area is divided in to several Public Health Midwife (PHM) areas. Total number of PHM areas in Kalutara district was 413. Public Health Midwife area eligible families register/s was/were the sampling frame. Two women from each PHM area eligible families register/s were randomly selected to the study. Total number of 682 ever married women of 35 year old aged were recruited to the study after applying exclusion criteria at field setting and invited to field WWCs in Kalutara district (89). Age was calculated using the date of birth by recall or by using an National Identity Card and was approximated to the last completed year.

Staff trainings for PHMM to collect upper vaginal HPV/DNA specimen and MOOH or Public Health Nursing Sisters (PHNSS) to collect cervical HPV/DNA specimen was done by the first author at each MOH office level in Kalutara district. Fifteen such staff trainings were conducted on monthly PHMM in-service training day at each MOH office. Videos created for staff trainings by the cobas 4800 HPV/DNA screening machine manufactures (Hologic company for women's health) was used in staff trainings. Instruction regarding accurate numbering of specimens, completion of specimen request forms and preparation for transport were also included in the training sessions. Cyto-screeners were uniformly trained for specimen barcoding, handling the machine and report writing to ensure the quality of performance by team of experts. Colposcopists were uniformly trained to ensure the quality of performance.

Information regarding socio-demographic characteristics were gathered by using an interviewer administered questionnaire. First HPV/DNA vaginal specimen collection was carried out by well-trained PHM and then cervical specimen collection from the same client by MOH/PHNS at the same clinic session in a separate place. Cusco's speculum was inserted to visualize cervix before obtaining HPV/DNA cervical specimen. HPV/DNA specimen obtained from the cervix and vagina using a special broom-like devices were separately placed into HPV/DNA specimen collection containers. Cervical and vaginal specimen from the same client were separately packed with the same identification number. In vaginal specimens, the letter "V" was written after the identification number for identification purpose.

Prepared guidelines were strictly adhered during data collection, barcoding and preparation for transport. Monitoring and supervision of the ongoing field activities and specimen collection were carried out by the first author. Specimen identification numbers were closely supervised by the first author at community clinic level for vaginal and cervical specimens including in request forms. Barcoding of specimens at the laboratory before entering to the cobas 4800 machine were done under first author's close monitoring and supervision. Result report writing by cyto-screeners at the laboratory were randomly checked.

Cervical specimens were screened at the laboratory by well trained cyto-screeners with cobas 4800 HPV/DNA automated PCR machine, which consists of cobas 4800x instrument and cobas analyzer. Cobas 4800 HPV/DNA screening machine was included several quality control mechanisms such as internal quality control, external quality control and contamination control.

The test sensitivity and specificity to detect ≥ CIN II is 92.9% and 71% respectively (4). It detects 14 high risk carcinogenic HPV genotypes, such as; 16,18

and 12 pooled high risk (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

Data were analysed by using SPSS version 20. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of vaginal specimen vs cervical specimen (gold standard) and 95% Confidence Intervals (CI) were calculated. Overall cervico-vaginal HPV infection and subgroup analysis (genotype 16 & 18) by cervical and vaginal specimen and 95% CI were calculated.

III. Results

Six hundred eighty two women were recruited to the study and only 631 of them were attended to community WWCs, therefore the response rate was 92.5%. Of them only 621 were subjected to vaginal and cervical specimen collection after applying exclusion criteria at clinic setting.

One recruit was excluded at the clinic setting from the study, as she was pregnant (n=1), while others were excluded due to cervical erosion (n=3), vaginal discharge (n=3), cervicitis (n=2) and fungal infection (1).

Majority of respondents were Sinhala (94.5%) and Buddhist (94.4%). Out of the total subjects 9% had not completed years of school education beyond the 5thgrade and another 12.9% of the subjects were remained at 6-11th grade of level education. Majority were educated only up to O/L passed level of education (58.9%). (Table 1)

Table 1: Distribution of participants according to ethnicity, religion, educational level and occupational status

Characteristics	Number of women (n)	Percentage %
1. Nationality		
Sinhala	587	94.5
Tamil	13	2.1
Muslim	21	3.4
2. Religion		
Buddhism	586	94.4
Catholic	3	0.4
Hindu	11	1.8
Islam	21	3.4
3. Education level		
No schooling	2	0.3
Grade 1-5th	54	8.7
Grade 6-11th	80	12.9
O/L passed	230	37.0
A/L passed	164	26.4
Degree& above	91	14.7
4. Occupational status		
Working women	184	29.6
Non-working women	437	70.4
Total	**621**	**100.0**

Sensitivity, specificity, PPV and NPV of the vaginal vs cervical HPV/DNA specimen collection screening method were 100% (95%CI: 90.7%-100%), 98.9% (95% CI: 97.8%-99.6%) 86.4% (95% CI: 74.1%-93.3%) and 100% (95%CI: 90.7%-100%) respectively (Table 2).

Table 2: HPV/DNA vaginal specimen collection screening method vs cervical HPV/DNA specimen collection screening method (as a gold standard) by using cobas 4800 HPV/DNA screening test

Screening test +ve/-ve	Cervical HPV/DNA specimen +vefor HR-HPV	Cervical HPV/DNA specimen –vefor HR-HPV
Vaginal HPV/DNA* specimen +ve for HPV	38	06
Vaginal HPV/DNA specimen – ve for HPV	0	577
Total	**38**	**583**

**HPV/DNA- Human papillomavirus/DNA*

Diagnostic agreement between vaginal vs cervical HPV/DNA specimen collection screening method was 99.0% (95% CI; 97.9%-99.6%). Positive Likelihood Ratio (LR +), Negative likelihood ratio (LR-) for vaginal specimen screening were 97.2 (95% CI; 43.8-215.4), and 0.0 respectively. Kappa coefficent between vaginal vs cervical HPV/DNA specimen screening method by cobas 4800 HPV/DNA test was 0.92(95% CI; 0.86-0.98%).

Prevalence of cervico-vaginal HPV infection among 35 year age cohort ever married women in Kalutara district, according to the cervical specimen screening method by cobas 4800 was 6.12% (95% CI; 4.26%-8.3%), while the percentage of HPV infection positives among 35 year age cohort ever married women in Kalutara district, according to the vaginal specimen screening method by cobas 4800 test was 7.08% (95% CI; 5.2%-9.4%).

Prevalence of HR-HPV 16 & 18 genotype infection among 35 year age cohort ever married women in Kalutara district according to the cervical specimen collection method by cobas 4800 was 1.9% (95% CI; 1.89%-1.91%) (Table 3). Percentage of HPV 16 and 18 genotypes infection positives among 35 year age cohort ever married women in Kalutara district according to the vaginal specimen collection screening method was 2.1% (95% CI; 2.09%-2.11%) (Table 4).

Table 3: Distribution of participants according to cervical HPV/DNA specimen screening result for HR-HPV genotypes

Cervical HPV/DNA specimen results for HR-HPV genotype	Number of women	Percentage %	95% CI for percentages %
Negative	583	93.9	
12 pooled positive	26	4.2	4.18-4.22
16positive	10	1.6	1.59-1.61
18positive	02	0.3	0.29-0.31
Total	**621**	**100.0**	

Table 4: Distribution of participants according to vaginal specimen screening result for HR-HPV genotypes

Vaginal HPV/DNA specimen results for HR-HPV genotypes	Number of women	Percentage %	95% CI for percentage%
Negative	577	92.92	92.88-92.92
12 pooled positive	31	5.0	5.28-5.32
16positive	11	1.78	1.79-1.81
18positive	02	0.3	0.29-0.31
Total	621	100.0	

IV. Discussion

According to 2012 estimates, annually 1721 new cervical cancer cases are diagnosed in Sri Lanka and 690 are died due to the disease (1). Total number of 111,798 of 35 year age cohort ever married population were screened under the National Cervical Cancer Screening programme in 2016 and the coverage was 52.8% (10). Cervical smears reported as malignant were 44 (0.03%) and cervical smears reported as high and low grade lesions were 665 (0.5%), which shows the underreporting due to low coverage of the programme.

Sensitivity, specificity, positive predictive value and negative predictive value of the vaginal HPV/DNA specimen screening vs cervical HPV/DNA specimen screening were 100%, 98.9%, 86.4% and 100% respectively, while the false positive rate was high in vaginal HPV/DNA specimen screening (1.1%).

As a screening method most précised value of the vaginal HPV/DNA specimen screening was zero reporting of the false negatives vs gold standard screening which would not let missing and under reporting cases. Similar pattern of results were observed in the world by using different PCR tests in comparison studies of vaginal HPV/DNA specimen screening vs cervical HPV/DNA specimen screening (5, 10, 11).

Prevalence of overall cervico-vaginal HPV infection among 35 years old ever married women was 6.12%, while the percentage of cervico-vaginal infection by vaginal HPV/DNA specimen screening method was 7.08%. Prevalence of HPV infection by genotypes 16 & 18 was 1.9%, while the percentage of cervico-vaginal infection positive by vaginal HPV/DNA specimen screening method was 2.1%.

Slight over reporting rate of vaginal HPV/DNA specimen screening method was observed due to the lower specificity rate, which might lead to over treatment and unnecessary anxiety due to the fear of disease.

Agreement between the vaginal HPV/DNA specimen screening method vs cervical HPV/DNA screening was 0.99, while the kappa coefficient between the two test was 0.92. Similar agreement was shown in the world by using different PCR tests in comparison studies of vaginal HPV/DNA specimen screening vs cervical HPV/DNA specimen screening (5, 10, 12).

Specimens were transferred to the laboratory in a special regiform box with a cool pack to maintain the temperature regime (2c°-30c°), while during the

transport. Vaginal HPV/DNA specimen can be obtained with a cotton swab combined with glass slide and successfully attempted in some other countries (13), which can be a suitable measure to overcome the associated challenge with specimen storage and transportation in "thinprep cell collection media".

The major advantage of the vaginal HPV/DNA specimen collection was it doesn't require speculum examination and PHMM can be collected specimens. Therefore, vaginal specimen collection method can be used to improve the quality and coverage of the National Cervical Cancer Screening programme in Sri Lanka. This study was restricted to one district out of 25 districts in Sri Lanka due to logistic constrains. Population characteristics and the public health infrastructure of the district favored generalizability of the research findings to the whole country.

V. Conclusion

The concordance between cervical vs vaginal specimen screening method by cobas 4800 PCR based HPV/DNA screening test was very high. Vaginal specimen collection method is more feasible and can be used to improve the detection of cervical lesions, therefore improve the quality and coverage of the National Cervical Cancer Screening programme.

Abbreviations

WWC: Well Woman Clinic,

CI: Confidence Interval,

MOOH: Medical Officers of Health,

PHNSS: Public Health Nursing Sisters,

PHMM: Public Health Midwives,

PCR: Polymerase Chain Reaction

PPV: Positive Predictive value

NPV: Negative Predictive Value

Ethical approval and consent to participate

Ethical clearance was obtained from the Ethics Review Committee (ERC), National Institute of Health Science, Kalutara. Informed written consent was obtained from each of the selected participants at the field during the study. Confidentiality was highly maintained, While handing over individual HPV/DNA result reports. Administrative clearance to conduct the study was obtained from Provincial Director of Health Services Western Province, Regional Director of Health Services Kalutara district, Director District General Hospital Kalutara and Director National Institute of Health Science Kalutara.

Consent for publication

Not applicable

Availability of data and materials

The datasets used to analyse in this study is available at corresponding author on reasonable request.

Competing interests

Authors were declared that they have no competing interests.

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Authors contribution

KCMP was participated in the design of the study, coordinated data collection performed the statistical analysis and drafted the version of the manuscript. HTCSA and NM were participated in the design of the study. HTCSA was performed the statistical analysis and interpreted data. Both HTCSA and NM were helped to draft the manuscript. All three authors were read and approved the final manuscript.

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