Human Papilloma Virus (HPV) DNA Testing Followed by HPV Genotyping Based Cervical Cancer Screening in Magway Region

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Human Papillomavirus (HPV) is the primary cause of cervical cancer, the first leading cause of female cancer in Myanmar. So, screening women for the presence of HPV is a critical aspect for prevention and early treatment of cervical cancer. The objectives were to conduct HPV DNA testing followed by HPV genotyping based Cervical Cancer Screening in Magway Region and to identify the cervical histological abnormalities among women who have HPV infection. It was a prospective cross-sectional descriptive study. Total of asymptotic 264 married women (median age 40 years; range 30-50) residing in Magway Region were screened in 2019. Cervical cells were obtained by sterile disposable *care*Brush and collected in *care*HPV Collection Medium. HPV-DNA-testing was performed using *care*HPV test which detects pooled-14-high-risk-HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68. HPV-positive-cases were followed by colposcopy-directed-biopsy and histology and HPV16/18 genotyping using GeneXpert HPV -Test. HPV was identified in 6.1% of screened women in Magway Region. Among HPV-positive cases, other-HR-HPV-genotypes (HPV -31, -33, -35, -39, -51, -52, -56, -58, -59, -66 and -68) were 75%, HPV -16 (12.5%) and HPV-18/45 (12.5%). Histologically, 43.8% had chronic cervicitis, 37.5% had cervical intraepithelial neoplasia (CIN-1), 12.5% (CIN-2) and 6.2% Carcinoma-in-situ (CIS). Among HPV infected chronic cervicitis cases, HPV-16 was 6.2%, HPV18/45 (12.5%) and other-HR-HPV genotypes (25%). All HPV-positive CIN-1 and CIN-2 cases had other-HR-HPV-genotypes. All HPV-positive Carcinoma-in-situ cases had HPV-16. This study highlighted that using HPV-DNA test in cervical cancer screening, the most-highest risk women who may develop cervical cancer can be determined. Using HPV-DNA test followed by HPV genotyping, early detection and effective management of cervical pre-cancers and cancers can be performed. By using this strategy in cervical cancer screening, unnecessary patient- referral and patient-follow up to hospitals and burden of health care providers can be reduced.

Keywords: Cervical Cancer Screening, HPV DNA testing, HPV genotyping, Histopathology

INTRODUCTION

According to information center on Human Papillomavirus (ICO-HPV) and cancer report (2019), cervical cancer is the second most common female cancer in the women aged 15 to 44 years and the 2nd most common female cancer deaths in the world with an estimated 569,847 new cases and 311,365 deaths in 2018 (GLOBOCAN).[1] In Southeast

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Asia, incidence and mortality of cervical cancer are about 62,456 cases and 35,738 deaths, respectively.[1]

In Myanmar, women at risk for cervical cancer (Female population aged >=15 years) were 20.8 million. Cervical cancer is the first most common female cancer and 1st leading cause of cancer deaths in women aged 15 to 44 years in Myanmar.[1] Age standardized incidence rate of cervical cancer were 13.1% in the world, 17.2% in Southeast Asia, and 21.5% in Myanmar. Age standardized mortality rate of cervical cancer were 6.9% in the world, 10% in Southeast Asia, and 13.1% in Myanmar.[1]

HPV causes virtually 100% of cases of cervical cancer. Worldwide, HPV16 and 18 (the two vaccine-preventable types) contribute to over 70% of all cervical cancer cases, between 41% and 67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions. After HPV16/18, the six most common HPV types are the same in all world regions, namely 31, 33, 35, 45, 52 and 58; these account for an additional 20% of cervical cancers.[1] Up to 80% of sexually active women are infected at some point in their lives and 10-20% develops persistent infection.[2]

It is now well-established and widely, if not universally, accepted that virtually all cervical cancer and its immediate pre-cancerous lesions arise from persisting cervical infections by approximately 15 (high-risk) human papilloma virus (HR-HPV) genotypes.[3] A new paradigm of cervical carcinogenesis replaces an older model of stepwise progression from low-grade to high-grade morphological changes and can now be summarized as four reliably measured stages: 1) HPV acquisition, 2) HPV persistence (*vs*. clearance), 3) progression of a persisting infection to cervical pre-cancer (with incidental co-occurrence of both conditions), and 4) invasion.[4] The Society of Gynecologic Oncology (SGO), the American Society for Colposcopy and Cervical Pathology (ASCCP), with input from representatives of five other US national medical organizations (ACOG, ACS, ASCP, ASC, CAP) issued an Interim Guidance Report after the U.S. Food and Drug Administration (FDA) approved the Cobas HPV test as a primary, or first, test performed for cervical cancer screening. This new guidance specifically addresses the implementation of HPV testing in primary screening.[5]

Screening women for the presence of the HPV is a critical aspect for prevention and early treatment of the deadly cancer because HPV is the primary cause of cervical cancer. In several evidence-based guidelines, primary HPV DNA testing is recommended for cervical cancer screening. High-quality molecular HPV tests that are easy to run are critical for expansion of cervical cancer prevention strategies in low-resource settings. The *care*HPV test is one of the cheap molecular diagnostics for high-risk human papillomavirus (HPV) designed to screen women in low-resource settings and it has demonstrated to be a more sensitive alternative to cytology and visual inspection based methods for the detection of pre-cancerous cell abnormalities. The *care*HPV test technology is an *in vitro* nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 14 high risk types of pooled HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 in cervical specimens. It can detect 88 samples per test run within 3 hours and so it is useful in community HPV based cervical cancer screening.[6]

The GeneXpert HPV Test is a rapid, qualitative, real-time Polymerase Chain Reaction (PCR) assay, using disposable cartridges, for the detection of 14 HR-HPVs (14 HR-HPV types i.e, HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68) from a liquid cytology sample. It is an easy-to-use, rapid HPV test with partial genotyping for the most important HPV types (HPV16 and HPV18/45) for risk stratification.[7] Both *care*HPV test and GeneXpert HPV test are World Health Organization (WHO) prequalified tests in vitro diagnostics (IVDs).[6, 7] In Myanmar, various opportunistic screening activities in recent years have covered less

than 1% of the 7.6 million women aged 30-49 who should be screened. This study aimed to conduct HPV DNA testing based Cervical Cancer Screening among married women in Magway Region and to identify the cervical abnormalities among women who had HPV infection.

MATERIALS AND METHODS

Study population and design

This study was a prospective cross-sectional descriptive study. A total of 264 married women (mean age-40 years; range 30-50) residing in Magway Region were screened using HPV DNA testing and genotyping in 2019. After obtaining a written informed consent, a thorough history was taken using structured-proforma. Then, speculum examination was performed under good light source. Cervical cells were obtained by sterile disposable *care*Brush and collected in *care*HPV Collection Medium. The samples were sent to the Technology Development Division, Department of Medical Research (DMR), Yangon at room temperature. Then, those samples were stored in 4°C refrigerator prior to testing.

HPV DNA testing

HPV DNA testing was performed using the *care*HPV test.

Principle

The *care*HPV test was an *in vitro* nucleic acid hybridization assay with signal amplification using microplate chemiluminescence technology. The *care*HPV test system detected the presence of 14 high-risk carcinogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) using full genome RNA probes complementary to the HPV DNA, specific antibodies and chemiluminescent detection. The target DNA combined with specific RNA probes, creating RNA:DNA hybrids. Then, the RNA:DNA hybrids were captured onto a solid phase coated with universal capture antibodies specific for RNA:DNA hybrids. The specimen matrix was washed from the captured hybrids to remove inhibitors. During the signal amplification, captured RNA:DNA hybrids were detected with multiple antibodies conjugated to alkaline phosphatase. The signal resulting from the chemiluminescent reaction was read by the *care*HPV test Luminometer. The results were automatically interpreted by the *care*HPV test System and were displayed graphically on the *care*HPV test Controller screen.[6]

Procedure

The experimental operation was performed according to the *care*HPV instruction manual provided. Briefly, to detect HPV with the *care*HPV system, supplied lysate was added to the specimens to dissolve the cells. The exposed double-stranded HPV DNA was denatured to become single-strand DNA by heating the lysate and this single-stranded DNA then hybridizes with the full-length complementary RNA to form HPV DNA/RNA hybrids. Magnetic beads coated with monoclonal antibody for HPV DNA/RNA hybrid mixtures were then added. Alkaline phosphatase was also added to combine with the monoclonal antibody and a chromogenic substrate is acted upon by the alkaline phosphatase. The light intensity generated by the reaction of the chromogenic substrate reflects the amount of HPV DNA contained in the specimen. The ratio of relative light unit (RLU) to the mean RLU of the minimum positive control (RLU/CO) was used for diagnosis. The standard definition for a sample to be positive for HPV DNA was a reading ≥0.5 pg/ml in one specimen. RLU/CO=1.0 is used as a cut-off point.[6] After performing *care*HPV test, the cervical specimens were stored in -20 °C refrigerator.

HPV Genotyping

HPV positive cases were followed by partial HPV16/18 genotyping using GeneXpert HPV test.

Principle

The GeneXpert HPV assay was a rapid, qualitative, and an automated real-time PCR assay. Sample processing, cell lysis, purification, NAA, and detection of the target sequences in clinical samples were performed

automatically using disposable cartridges. In this assay, the E6 and E7genes of the 14 targeted HR-HPV types (Genotypes-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68) were amplified simultaneously in five fluorescent channels: (1) HPV16; (2) HPV18/45; (3) HPV31/33/35/52/58; (4) HPV 51/59; and (5) HPV39/56/66/68. For quality control of each test, sample adequacy control (SAC) and probe check control (PCC) were included. Each fluorescent channel had its own cutoff parameters for target detection/ validity. The cut-off for positivity with the GeneXpert HPV assay was <40 Ct for HPV16 and HPV18/45 and <38 Ct for HPV31/35/33/52/58, HPV51/59, and HPV39/ 68/56/66.[7]

Procedure

Cervical specimens were collected with a sterile disposable careBrush and stored in 1ml *care*HPV Collection Medium and stored at 4°C. It is important to note that *care*HPV Collection Medium is not a manufacturer's approved collection medium for the Xpert HPV. The ThinPrep transport medium is one of the approved collection medium and is 20 ml while *care*HPV Collection Medium is 1 ml. For each Xpert HPV test, 200 µl of cervical sample from *care*HPV Collection Medium was transferred to 800 µl of ThinPrep transport medium. One millilitre of that diluted specimen was used to run Cepheid Xpert HPV according to manufacturer's instructions. Xpert HPV gives results from 6 separate channels: (a) sample adequacy control (SAC), (b) P1-HPV16, (c) P2-HPV18 /45, (d) P3-HPV31/33/35/52/58, (e) P4-HPV51 /59 and (f) P5-HPV39/68/56/66. Then, colposcopy-directed-biopsy and histopathological diagnosis were performed in all HPV positive cases.

Data analysis

All study data were double-entered using Microsoft Excel. Statistical analysis of the data was performed using Statistical Package for Social Sciences (SPSS-16.0).

Funding

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Ethical consideration

This study was approved by Institutional Review Board, Department of Medical Research, Yangon (IRB No-2019-74, Approval No- Ethics/DMR/2019/081).

RESULTS

A total of asymptotic 264 married women residing in Magway Region were screened in 2019. Regarding the baseline characteristics of women who participated in this cervical cancer screening, mean age was 40 years (±SD 6.3 years, range: 30-50). The mean age of first marriage was 23 years (±SD, 5.4 years, range: 15-42) and of menarche was 14 years (±SD, 1.6 years range: 11-19). Education of those women was graduated (21.3%), high school level (26.8%), middle school level (32.3%), primary school level (18.6%) and illiterate (0.5%).

HPV DNA testing

HPV was identified in 6.1% (16/264) of screened women in Magway Region (Fig. 1a).

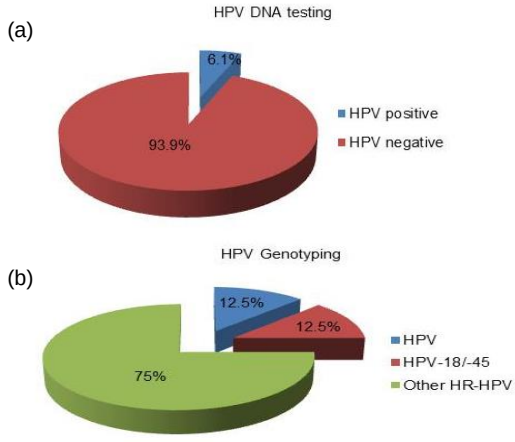


Fig. 1. Proportion of (a) HPV infection in women residing in Magway Region (b) HPV genotypes in HPV infected women residing in Magway Region

HPV Genotyping

Among HPV-positive-cases, HPV-16 genotype was 12.5% (2/16), HPV-18/45 genotype was 12.5% (2/16) and other-HR-HPV-genotypes (HPV-31, -33, -35, -39, -51, -52, -56, -58, -59,-66 and -68) was 75% (12/16) (Fig. 1b). HPV DNA testing analyzed by *care*HPV test and Partial HPV genotyping analyzed by GeneXpert HPV test were shown in Fig. 2 & 3, respectively.

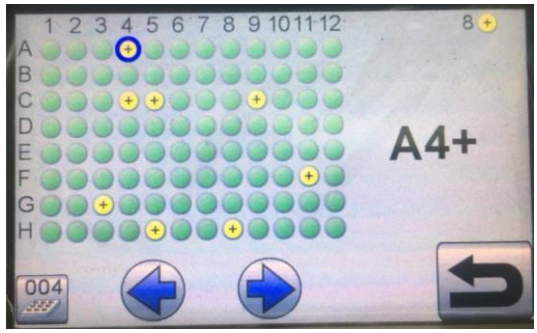
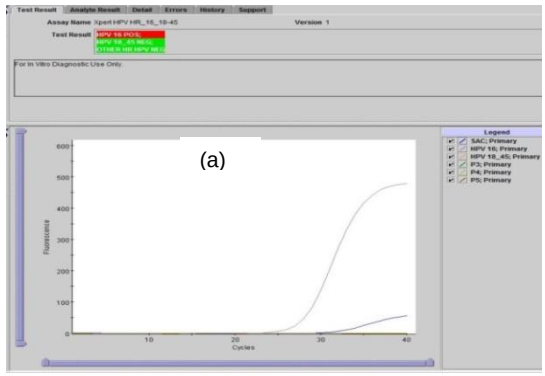
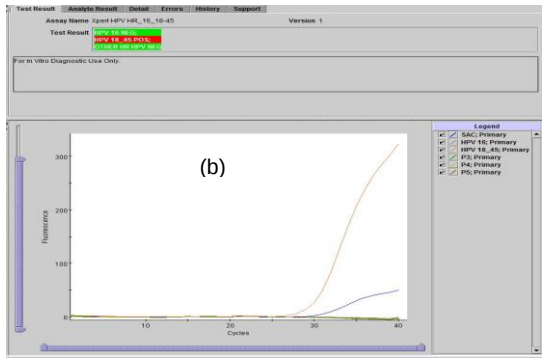


Fig. 2. HPV DNA testing using *care*HPV test showing (1) A1, B1, C1- positive calibrator (2) D1, E1, F1- negative calibrator (3) A4 -positive control (4) H12-negative control (5) C4, C5, C9, F11, G3, H5, H8- positive HPV





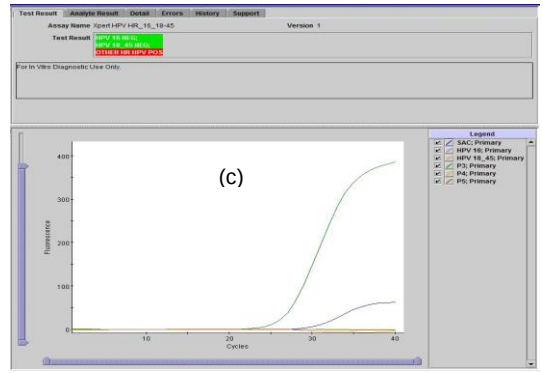


Fig. 3. Partial HPV Genotyping using GeneXpert HPV test showing (a) HPV-16 (b) HPV-18/58 (c) Pooled HR-HPV -31, -33, -35, -39, -51, -52, -56, -58, -59, -66, -68

Histopathology diagnosis

In histopathology diagnosis of HPV positive women, 43.8% (7/16) had chronic cervicitis, 37.5% (6/16) had cervical intraepithelial neoplasia (CIN-1), 12.5% (2/16) (CIN-2) and 6.2% (1/16) (Carcinoma-in-situ) (CIS) (Fig. 4).

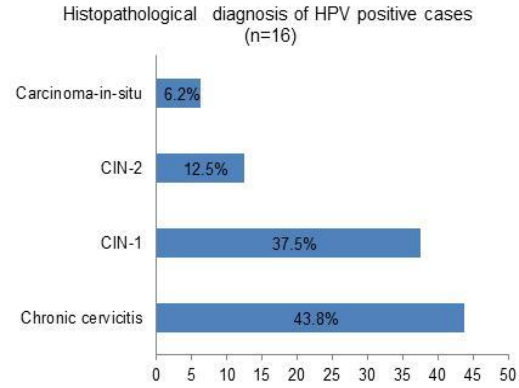


Fig. 4. Prevalence of cervical epithelial cell abnormalities using histopathology in HPV infected women residing in Magway Region

Association of HPV genotypes with histopathology diagnosis

Among HPV infected chronic cervicitis cases, HPV-16 was 6.2%, HPV18/45 (12.5%) and other-HR-HPV-genotypes (25.0%). All HPV-positive CIN-1 and CIN-2 cases had other-HR-HPV-genotypes. All HPV-positive Carcinoma-in-situ cases had HPV-16 (Table 1). Among HPV-16 positive women,

50% of women had Carcinoma-in-situ and 50% had chronic cervicitis. All HPV-18/45 positive women had chronic cervicitis. Among women infected with other HR-HPV, 50% of women had CIN-1, 33.3% had chronic cervicitis and 16.7% had CIN-2 (Table 1).

Table 1. Association of HPV genotypes with histopathology diagnosis of HPV infected women residing in Magway Region

Histopathology		HPV genotypes			Total
		HPV-16	HPV-18/45	Other HR-HPV	
Chronic cervicitis	Count	1	2	4	7
	% within histology	14.3	28.6	57.1	100
	% within HPV genotypes	50	100	33.3	43.8
	% of Total	6.2	12.5	25	43.8
CIN-1	Count	0	0	6	6
	% within histology	0	0	100	100
	% within HPV genotypes	0	0	50	37.5
	% of Total	0	0	37.5	37.5
CIN-2	Count	0	0	2	2
	% within histology	0	0	100	100
	% within HPV genotypes	0	0	16.7	12.5
	% of Total	0	0	12.5	12.5
Carcinoma-in-situ	Count	1	0	0	1
	% within histology	100	0	0	100
	% within HPV genotypes	50	0	0	6.2
	% of Total	6.2	0	0	6.2
Count		2	2	12	16
% within histology		12.5	12.5	75	100
% within HPV genotypes		100.	100	100	100
% of Total		12.5	12.5	75	100

DISCUSSION

ICO/IARC Information Centre on HPV and Cancer (2019) reported: Asia has an estimated population of 1673.2 million women aged 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 315,346 women are diagnosed with cervical cancer and 168,411 die from the disease. Cervical cancer ranks as the third most frequent cancer among women in Asia.

In Southeast Asia, HPV were determined in 3% of women with normal cytology, 27.4% of women with low grade lesion, 33.4% of women with high grade lesion, and 70.4% of women with cervical cancer.[1] Recently, American Society of Clinical Oncology (ASCO)'s Resource-Stratified Clinical Practice Guideline, HPV DNA testing is recommended in all resource settings; recommended age ranges and frequencies by setting are as follows: maximal setting: ages 25 to 65, every 5 years; enhanced setting: ages 30 to 65, if two consecutive negative tests at 5-year intervals, then every 10 years; limited setting: ages 30 to 49, every 10 years; and basic setting: limited setting: ages 30 to 49, every 10 years; and basic setting: ages 30 to 49, one to three times per lifetime.[8]

In Myanmar (2016), Mu Mu Shwe, *et al* reported that HR-HPV was identified in 85.8% of cervical cancer patients attending Central Women's Hospital, Yangon using GeneXpert HPV test. The most prevalent HPV genotype was HPV-16 (63.7%), followed by HPV-18/45 (17.6%), HPV-31/ 33/35/52/58 (9.9%), HPV-51/59 (4.4%), and HPV-39/56/66/68 (1.1%). Mixed HPV-16 with other HR-HPV genotypes was 3.3%. [9, 10] Meta-analysis of previous studies done between 2012-2014[11] shown that HR-HPV was identified in CINI (39.3%), CINII (58.6%), CINIII (66.7%), and squamous cell carcinoma (SCC) of the cervix (77.1%) of women attending Thingangyun General Hospital, Yangon[12] and Central Women's

Hospital, Mandalay.[13] The most common genotypes were HPV-16(68.1%), followed by HPV-31(16.5%), HPV-18 (7.7%), HPV-58 (6.6%), and HPV-35 (1.1%). Among squamous cell carcinoma (SCC) of the cervix, HPV-16 was the most common genotype (66.7%) followed by HPV-18 (14.8%), HPV-31 (11.1%), HPV-35 (3.7%), and HPV-58 (3.7%).[11]

Vaccine preventable genotype, HPV-16 was the most common genotype among cervical cancer patients in Myanmar.[9-15] In 2003, HR-HPV was detected in 81.2% of cervical tissue with SCC, 5.56% with CINI and 8.33% with normal cytology.[16] In worldwide as well as in Myanmar, among cervical cancer cases, HPV-16 was the most common genotype followed by HPV-18.

In Asia, the prevalence of HPV among women with normal cervical cytology varies as follows: 1.7-45.6% in China, 2.3-36.9% in India, 3.1-46.7% in Malaysia, 3.3-40.6% in Thailand, 9.3% in the Philippines, and 1.5-10.2% in Vietnam.[1] In Myanmar (2017), Mu Mu Shwe, *et al* published the prevalence of HPV in married women residing in general population of North Okkalapa Township, Yangon: in which HPV was detected in 5.5% of those screened women.

Among positive HPV cases, pooled-12-HR-HPV types were 66.7%, HPV-16 (25.0%) and HPV-18 (8.3%).[17] Another study in 2018 reported that HPV was determined in 10% of women who had no previous cervical cancer screening in North Okkalapa General and Teaching Hospital and Thingangyun General Hospital, Yangon comprising pooled-12-HR-HPV genotypes (5%), HPV-16 (3.2%) and HPV-18 (1.8%).[18]

In the ATHENA HPV study (USA), a total of 47,208 women were enrolled. The prevalence of cytological abnormalities was 7.1%. Overall prevalence of ASCUS, LSIL and HSIL were 4.1%, 2.3% and 0.3%, respectively. Pooled 12 HR-HPV, HPV-16 and HPV-18 were detected in 12.6%, 2.8% and 1.0% of women, respectively. HPV-16 was identified in 12.8% of CIN1, 29.7% of CIN2, and 51.2% of CIN3.[19] Van, *et al* reported that HR-HPV were found out in 9.5% of women residing in two districts of Vietnam. The proportion of high-risk genotypes other than 16 and 18 was relatively high.[20]

In this study, HPV was identified in 6.1% of screened women (mean aged 40-years, ±SD 6.3 years, range: 30-50) in Magway Region. Among HPV-positive-cases, other-HR-HPV-genotypes (HPV-31, -33, -35, -39, -51, -52, -56, -58, -59, -66 and -68) were (75%), HPV-16 (12.5%) and HPV-18/45 (12.5%). The proportion of high-risk genotypes other than 16 and 18 was relatively high in the general population which was similar with previous Myanmar studies, ATHENA HPV study and Van study.[20]

In meta-analysis of Southeast Asia studies (2019), prevalence of HPV-16/18 among women with normal cytology, low grade lesion, high grade lesion and cervical cancer were 1.0%, 30.4%, 49.3%, 88.7%, respectively in Malaysia and 3.4%, 25.5%, 29.6%, 67.6%, respectively in Thailand. The prevalence of HPV-16/18 among women with normal cytology was 4% in Indonesia, 2.9% in the Philippine and 2.1% in Vietnam.[1]

In this study, cervical cancer screening was performed from asymptotic women residing in general population of Magway Region. Histologically, among HPV-positive cases, 43.8% had chronic cervicitis, 37.5% had cervical intraepithelial neoplasia (CIN-1), 12.5% (CIN-2) and 6.2% (Carcinoma-in-situ) (CIS).

From this study, early detection of cervical neoplasia cases (CIN) as well as CIS could be done. Therefore, early management could be given to those cervical abnormalities cases. All HPV-positive CIN-1 and CIN-2 cases had other-HR-HPV-genotypes. All HPV-positive Carcinoma-in-situ cases had HPV-16. Among HPV infected chronic cervicitis cases, HPV-16 was 6.2%, HPV18/45 (12.5%) and other HR-HPV genotypes (25.0%). From this study, high risk cases

necessary to follow-up could be identified. Therefore, primary HPV DNA testing followed by HPV genotyping takes very crucial role for the early detection of cervical cancer.

Currently, HPV testing alone or with cytology triage is increasingly being used as a primary screening approach for cervical neoplasia. Whilst HPV tests are less likely to miss cases of cervical pre-cancer and cancer, primary HPV screening test is very useful which don't lead to more unnecessary referrals. Negative HPV test is more reassuring than a negative cytological test, as the cytological test has a greater chance of being falsely negative, which could lead to delays in receiving the appropriate treatment.

Conclusions

This study highlighted that using HPV-DNA test in cervical cancer screening, the most-highest risk women who may develop cervical cancer can be determined. Using HPV-DNA test followed by HPV genotyping, early detection and effective management of cervical pre-cancers and cancers can be performed. By using this strategy in cervical cancer screening, unnecessary patient-referral and patient-follow up to hospitals can be reduced. In addition, burden of health care providers can also be reduced.

Competing interests

The authors declare that they have no conflict of interest.

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REFERENCES

1. Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, *et al.* ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papilloma virus and related diseases in the World. *Summary Report* 17 June 2019.
2. Einstein MH, Schiller JT, Viscidi RP, Strickler HD, Coursaget P, Tan T, *et al.* Clinician's guide to human papillomavirus immunology: Knowns and unknowns. *Lancet Infectious Diseases* 2009; 9:347-56.
3. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, *et al.* International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *New England Journal of Medicine* 2003; 348:518-527.
4. Snijders PJ, Steenbergen RD, Heideman DA & Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. *Journal of Pathology* 2006; 208:152-164.
5. The American College of Obstetricians and Gynecologists (ACOG), The Society of Gynecologic Oncology (SGO), the American Society for Colposcopy and Cervical Pathology (ASCCP) guideline (2016) (www. hpv 16 and 18.com/ hcp /cervical cancer screening guidelines/ new-us-primary-screening-guidance)
6. WHO Prequalification of *in vitro* Diagnostics PUBLIC REPORT 2018 July [Internet]. Care HPV Test (PQDx 0085-028-00) Available from: https://extranet. who.int/pqweb/sites/ default/files/180713_pqpr_pqdx_0085_028_ 00_carehpv_with_labelling.pdf
7. WHO Prequalification of *in vitro* Diagnostics PUBLIC REPORT 2017 December [Internet]. Xpert HPV Test (PQDx0268-070-00. Available from: https:// extranet. who.int/ pqweb/ sites/ default/files/ PQDx0268-070-00-XpertHPV_4.0.pdf
8. Castle PE, Jeronimo J, Temin S & Shastri SS. Screening to prevent invasive cervical cancer: ASCO Resource-stratified Clinical Practice Guideline. *Journal of Clinical Oncology* 2017; 35(11): 1250-1252.
9. Mu Mu Shwe, San San Myint, Yin Yin Soe, Sandar Win, Lynn Pa Pa Aye, Htwe Htwe

Nyunt, *et al*. Detection of high risk HPV genotypes using GeneXpert HPV assay among cervical Cancer patients in Myanmar. 6th Annual Symposium on Global Cancer Research, New York, USA 15th March 2018. (Poster)

10. Mu Mu Shwe, San San Myint, Yin Yin Soe, Khin Lay Kywe, Sandar Win, Lin Pa Pa Aye, *et al.* Rapid Diagnosis of High-Risk Human Papillomavirus (HR-HPV) Genotypes using New Point of Care (POC) GeneXpert HPV Assay in Cervical Cancer Patients. Abstract of *45th Myanmar Health Research Congress*; 2017; Jan 9-13; Yangon, Myanmar. p. 81.
11. Mu Mu Shwe, Kyi Kyi Nyunt, Khin Saw Aye, Hlaing Myat Thu, Hla Myat Mo Mo, Zin Mon Kay Khine Win, *et al*. High-Risk Human Papillomavirus (HR- HPV) genotypes among women with cervical precancer and cancer in Myanmar. *Journal of Global Oncology* 2016; 2:3_suppl, 18s-19s.
12. Mu Mu Shwe, Kyi Kyi Nyunt, Shigeru Okada, Teruo Harano, Hlaing Myat Thu, Hla Myat Mo Mo, *et al.* Trend of human papillomavirus genotypes in cervical neo-plasia observed in the newly developing township in Yangon, Myanmar. *Acta Medica Okayama* 2015; 69(1): 51-59.
13. Mu Mu Shwe, Kyi Kyi Nyunt, Zin Mon Kay Khine Win, Ohnmar Kyaw, Khin Than Maw, Thazin Myint, *et al*. Genotypes of Human Papillomavirus (HPV) among women with squamous cell carcinoma of the cervix and those with different cervical cytology in Central Women's Hospital, Mandalay. *Abstract of 43rdMyanmar Health Research Congress* 2015; Jan 5-9; Yangon, Myanmar. p. 35.
14. Mu Mu Shwe, Teruo Harano, Shigeru Okada, Aye Aye Win, Khin Saw Aye, Hlaing Myat Thu, *et al.* Prevalence of high risk human papillomavirus (HR-HPV) infection among women with normal and abnormal cervical cytology in Myanmar. *Acta Medica Okayama* 2014; 68(2): 79-87.
15. Mu Mu Shwe, Hlaing Myat Thu, Mo Mo Win, Khin Saw Aye, Khin Khin Oo, Ko Ko Zaw, *et al.* Genotyping of high risk type human papillomavirus (HR-HPV) in women with cervical cytological abnormalities. *Myanmar Health Sciences Research Journal* 2013; 25(1): 1-7.
16. Win Win Mya. Association of HPV-DNA cervical tissues with cervical intraepithelial neoplasia I and carcinoma in Myanmar women attending Gynaecological Clinic of Teaching Hospitals in Yangon. [DrMedSc *thesis*]. University of Medicine 1: Yangon; 2003.
17. Mu Mu Shwe, Kyi Kyi Nyunt, Lin Pa Pa Aye, Kham Mo Aung, Myat Noe Swe, Ni Ni Aung, *et al.* Community-based cervical cancer screening using cobas Human Papillomavirus (HPV) test and Liquid Based Cytology (LBC) in married women living in North Okkalapa Township, Yangon. *Abstract of* the *46th Myanmar Health Research Congress* 2018; Jan 8-12; Yangon, Myanmar. p. 57.
18. Mu Mu Shwe, Kyi Kyi Nyunt, Saw Kler Ku, Khin May Thin, Aye Aye Tint, Lynn Pa Pa Aye, *et al.* Human Papillomavirus (HPV) DNA testing in community-based and hospital-based cervical cancer screening. *Abstract of the 47th Myanmar Health Research Congress* 2019; Jan 7-11; Yangon, Myanmar. p. 179.
19. Wright TC, Stoler MH, Behrens CM, Apple R, Derion T, Wright TL. The ATHENA human papillomavirus study: design, methods, and baseline results. *American Journal of Obstetrics & Gynecology* 2012; 206: 46.e1-11.
20. Van SN, Khac MN, Dimberg J, Matussek A & Henningsson AJ. Prevalence of cervical infection and genotype distribution of human papilloma virus among females in Da Nang, Vietnam. *Anticancer Research* 2017; 37(3): 1243-1247.