

# The Prevalence of Human Papillomavirus Among the Indigenous Population in Serian, Sarawak, Malaysian Borneo

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## Research

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# Abstract

## Introduction:

Cervical cancer remains a significant problem worldwide, particularly in resource-limited countries, and having persistent HRHPV infection is a necessary risk factor. HPV16/18 are generally regarded as the cause of 70% cervical cancer incidences worldwide, and effective vaccines have been developed against these two HPVs. Although Malaysia has officially adopted an HPV vaccination strategy into the National Vaccination Program, the comprehensive prevalence data on each endemic HPV genotypes was absent in East Malaysia and most parts of the country except for major cities in Peninsular Malaysia.

## Objective:

The objective of this study is to elucidate the endemic HPV genotypes that are circulating in the suburban population in Serian Division, Sarawak, Malaysia.

## Methods:

Non-virgin women who were attending the Women's health campaign on the 5<sup>th</sup> and 23<sup>rd</sup> October 2018 were recruited. Informed consent was obtained, and a cervical swab was clinician-collected. The presence of HPV in the cervical swab was detected using MY09/MY11 followed by GP5+/GP6+ nested-PCR and its corresponding genotypes identified via sequencing. HRHPV positive women were screened using

## Results:

We have recruited 43 sexually active women with median age of 51-year-old. HPV18, 39, 52, 56, and 84 were detected at an equal ratio. The overall prevalence rate of HPV, HRHPV and URHPV were 11.62% (5/43), 9.3% (4/43) and 2.3% respectively. Only 20% (n=1/5) of the HRHPV positive women were positive by VIA.

## Conclusion:

HPV18, 39, 52, 56, and 84 were detected in Serian at an equal ratio. HPV16 was absent, and HPV18 was the only vaccine-genotype detected. HPV 39, 52, 56, and 84 were not covered by the bivalent and tetravalent HPV vaccines.

# Introduction

Cervical cancer is ranked as the 3<sup>rd</sup> most common cancer among women in Malaysia and is the second cause of mortality in Malaysian women. On average, about 1682 women are diagnosed with invasive cervical cancer, and 944 die from the disease annually (1). Sarawak has the highest cervical cancer incidence rate in Malaysia despite the 4-decade long free Pap smear program by the Ministry of Health with the national coverage of <22% with the non-adherence rate of 90.5%(2).

Cervical cancer is a preventable cancer linked to the persistent infection by oncogenic human papillomavirus (HPV). HPV belongs to the family of *Papillomaviridae*, and has a double-stranded circular DNA genome of about 8000bp long within a non-enveloped viral capsid. The L1 gene encodes for the capsid protein, and its gene sequence is universally used for the construction of the phylogenetic tree and genotyping. There are currently 198 HPVs identified to date, with about 40 genotypes being sexually transmitted. These HPVs are epidemiologically classified into four risk groups, namely: high-risk (HR), probably high-risk (PHR), undetermined risk (UR) and low risk (LR) (3,4).

There are at least 14 HRHPV genotypes that are highly oncogenic. Two HRHPV, HPV16 and HPV18 were thought to be responsible for approximately 70% of all cervical cancer incidences worldwide (5,6) and have been the priority target genotypes for both vaccine and diagnostic tool development.

The bivalent (Cervarix, GSK; HPV16,18) and quadrivalent (Gardasil; Merck; HPV6,11,16,18) HPV vaccines were launched in Malaysia since 2006 and have been formally adopted into the National Immunisation Programme (NIP) in 2010 and administered to schooling female students aged-13, projecting to reduce the cervical cancer rate by 70% in the long run (7). However, there is a paucity of published epidemiological surveillance data in Malaysia when the decision was made with most epidemiological data came from large cities in Peninsular Malaysia and none from East Malaysia. A nonavalent HPV vaccine (Gardasil9, Merck; (HPV6,11,16,18,31,33,45,52,58) was launched in 2019, offering additional protection against 5 HRHPVs but has yet to be adopted into the NIP. Although HPV vaccines are proven to be effective, protection is near genotype-specific, and any mismatch between the vaccine candidates and the endemic HPV genotypes may provide a false sense of protection and alter the behaviour of seeking early cervical cancer screening. To this, we seek to elucidate the endemic human papillomavirus (HPV) in the suburban population in Serian, Sarawak.

## Materials And Methods

### Medical Ethics Approval

The study was approved by Universiti Malaysia Sarawak Medical Ethics Committee UNIMAS/NC-21. 02/03-02 Jld. 3 (17).

### Study design

This is a cross-sectional study involving non-virgin women from Kampung Balai Ringin and Kampung Tarat (*kampung means village in the Malay language*) within the Division of Serian. The two villages are located about 30km apart by road.

We designed the algorithm based on the WHO Guidelines for screening and treatment of precancerous lesions for cervical cancer prevention (Figure 1). HPV DNA test was used as the primary cervical cancer screening tool, and women who were HPV positive were triaged for visual inspection using acetic acid (VIA). Women who are HPV-positive and VIA-positive will be referred for colposcopy at the nearest tertiary

healthcare facilities. For the feasibility reason, VIA was offered immediately after the cervical swab was clinician-collected.

## **Informed consent**

All women who have had at least one sexual encounter and presented to the cervical cancer screening program were recruited. They were briefed regarding the program, and informed consent was obtained before proceeding to obtain a clinician-collected cervical swab. A cervical swab was collected using BD Rover Cervical Broom (Becton-Dickinson, Dun Laoghaire, Ireland) and preserved in BD Surepath medium (Becton-Dickinson, Dun Laoghaire, Ireland). Specimens were maintained at 4°C and transported immediately to the Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak.

## **DNA Extraction**

The BD Surepath medium was pulse-vortexed trice, and 2 mL of the medium were pelleted at 10 000 x g for 10 minutes at 4 °C. The supernatant was decanted and the pellet resuspended in 200 µL of phosphate-buffered saline (PBS) pH 7.6. The viral DNA was extracted using High Pure Total Viral Nucleic Acid Kit (Roche, USA) and eluted in 50 µL of elution buffer. HeLa cells (HPV18 transformed cells) and PBS were included in the extraction process and used as the positive and negative (template-free) controls respectively for the nested-PCR.

## **HPV genotyping assay**

Nested PCR was performed using a combination of MY09/MY11 as outer primers and GP5+/GP6+ as inner primers that amplify the L1 gene of the viral genome. The primers used for the first round of PCR were MY09 (5'-CGTCCMARRGGAWACTGATC-3') and MY11 (5'-GCMCAGGGWCATAAYAATGG-3') while the primers for the second round were GP5+ (5'- TTTGTTACTGTGGTAGATACTAC-3') and GP6+ (5'-GAAAATAAACTGTAAATCATATTC-3') (8). An internal control targeting the human beta-globin gene amplification using PC03 forward (5'-ACACAACCTGTGTTCACTAGC-3') and PC05 reverse (5'-GCTAGTGAACACAGTTGTGT-3') primers. All oligonucleotides were synthesised by Integrated DNA Technologies (IDT) (Kuala Lumpur, Malaysia).

PCR mixtures with total volumes of 50µl contained 10µl of template DNA, 3.0 mM of MgCl<sub>2</sub> (Thermo Scientific, USA), 2mM of dNTP Mix((Thermo Scientific, USA), ten pmol of each primer (IDT, Malaysia) and 10X PCR buffer (Thermo Scientific, USA). The amplicon for the MY09/MY11 amplification was 10-fold diluted in deionised water, and 10µl was used as the template for the nested-PCR using the GP5+/GP6+ primers set. PCR amplification was performed using Verity<sup>®</sup> 96-well thermocycler (Applied Biosystems, Massachusetts, USA) with initial denaturation at 95°C for 2 min, denaturation at 95°C for 45 sec, annealing at 55°C 45 sec, extension at 72°C for 45sec and a final extension at 72°C for 5 min. The PCR reaction was carried out for 40 cycles. The PCR products were electrophoresed through a 2% (w/v) agarose gel prepared in 1X Tris-boric acid-EDTA (TBE) buffer, stained in ethidium bromide and viewed under ultraviolet light. The nested-PCR positive bands of the correct size were excised using sterile scalpel

extracted from the gel and subjected to BIG DYE™ Terminator v3.1 cycle automated DNA sequencing (Apical Biotechnology, Malaysia). Nucleotide sequences were analysed using BLAST software (<http://www.ncbi.nlm.nih.gov/blast/html>).

### **Visual inspection using Acetic Acid (VIA)**

VIA was performed by direct application of 4% acetic acid to the cervix and the development of acetowhite lesions were recorded as Negative (Neg) or Positive (Pos) (9)

## **Results**

### **Sampling**

A total of 43 women from Kampung Balai Ringin (n=29) and Kampung Tarat (n=14) had responded to the study. The mean age was 50-year-old, and the median age was 51-year-old. The respondents were mostly from the older age group, probably due to the rural-to-urban migration of the younger generations (10) (see Table 1).

### **HPV screening and genotyping**

All 43 samples were  $\beta$ -globin positive. The amplification results for the HeLa cell genomic extract (positive control, HPV18 transformed cancer cell line), and PBS extract (negative control, template-free control) were as expected. The nested-PCR yielded 5/43 positive amplification, indicating the prevalence of human papillomavirus in 11.62% of the women (see Figure 1). The sequencing of the resolved bands yielded clean electropherograms without mixed-peaks, and the partial sequences matched HPV18, 39, 52, 56, and 84 in the GenBank (see Figure 2). Each of the HPVs was equally distributed and had an individual prevalence of 2.3% in the population.

HPV Negative women were advised to seek to rescreen after five years. HPV positive women were offered VIA as per the standard operating procedure.

### **VIA**

Only 20% (1/4) of the HPV positive woman was VIA positive (see Table 2). The HPV genotype corresponding to the VIA positive woman was HPV56. She was referred for colposcopy at the participating tertiary healthcare facility as per study standard operating procedure. The outcome of the histologic and colposcopic findings is beyond the scope of this manuscript. 80% (4/5) of the HPV positive women were advised to seek to rescreen after one year.

## **Discussion**

In this study, we have found that 11.62% of the women in Serian were infected with HPV. This rate is slightly lower than the prevalence rate of 9.6% in neighbouring Sabah (12) and 14% as estimated for

Southeast Asia (13). The prevalence of HRHPV in Serian was determined to be 9.3% (4/43), which is similar to the prevalence of 8% as determined using the careHPV System in Bario, northern Sarawak (14). The overall prevalence of HRHPV in Serian is lower compared to the studies conducted in West Malaysia [25.6-46.7%] (15,16).

All HRHPV positive women were from the Iban ethnicity, but our sample size is skewed towards this ethnic group, which predominates in both of the study sites. HRHPV genotype 18, 39, 52, and 56 and UDRHPV genotype 84 were identified in the population. The median age of the HRHPV positive women was 58, with two women aged 60 and above. Other studies have also reported a higher prevalence of HRHPV in the older age cohorts (17,18) correlating to the 'second HRHPV peak' observed among the premenopausal and menopausal. The second peak may be due to the reactivation of latent HPV infection resulted from hormonal changes and decreased immunity (19). However, Monsonego and colleagues had reported a single peak in HRHPV prevalence, which decreases with age (20).

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One sole HPV18 infected women in our sample size has old woman has exceeded the eligible screening age of 65-year, both under the National Cervical Cancer Screening Programme and the *Guidelines for Primary HPV Testing for Cervical Cancer Screening in Malaysia (2020)* which may predispose her to the risk of developing invasive cervical cancer within her lifetime. A retrospective study in Michigan, US has revealed that half of the patients with invasive cervical cancer above 65-years were adherent to the cervical cancer screening programme before ceasing screening at the age of 65. Other researchers have also reported increasing incidences of cervical cancer among women above the recommended screening age. Their prognosis is often worst than those within the recommended screening age (21–23). Therefore, we strongly believe that it is crucial to extend the cervical cancer screening age beyond 65-year (24) while waiting for more studies to be carried out on the older age cohorts to understand the natural progression of HRHPV to pre-cancer and cancer (23).

The HPV genotyping results did not correlate well with the VIA findings. Only 25% (1/4) of HRHPV infection correlates with the formation of acetowhite cervical lesions in VIA. This observation may be attributed to the high sensitivity of the nested-PCR assay but low specificity in clinical correlation to cervical intraepithelial neoplasia (25). However, HPV testing on a cohort of 1.2 million women had identified more women who subsequently diagnosed with pre-cancer and cancer (26). It is worth to mention that VIA has a reduced sensitivity (59.4%) and specificity (76.2%) in premenopausal and menopausal women due to the contraction of the transformation zone into the cervix. (23,27). Despite the poor HPV-VIA correlation, the prevalence rate of HPV by nested-PCR may represent the true prevalence of HPV within the population as compared to HPV DNA tests that have been calibrated to correlate to CIN2 or worse (25).

Interestingly, we did not detect HPV16 ( $\alpha$ -9) in this population. This observation is of public health importance as HPV16 is a vaccine-genotype included in the NIP. All women in this study have never had any HPV vaccine before, and this observation cannot be a benefit from the HPV vaccination programme.

However, HPV16 is present in Sabah at lower prevalence (12), but in high prevalence in Peninsular Malaysia and Singapore (16,28,29). The only vaccine-genotype detected was HPV18 ( $\alpha$ -7). The prevalence of each HPV genotype is geographically unique. For instance, HPV18 was also absent among the sex-workers in Tunisia (30).

HPV52 ( $\alpha$ -9), HPV56 ( $\alpha$ -6), and HPV39 ( $\alpha$ -7) detected here are of public health interests as they are the non-vaccine-genotypes and are ranked as the 3<sup>rd</sup>, 7<sup>th</sup>, and 8<sup>th</sup> aetiological agent in cervical cancer in Malaysia (1). Even with the inclusion of the more comprehensive Gardasil9, HPV39 and 56 remained not covered. HPV52 is also prevalent among the ethnic Kelabits in the Highland of Bario (Northern Sarawak) (14) but not detected in Sabah (12). Studies in West Malaysia have shown the presence of HPV52 in the northern and southern regions but absent in the midland. (28,29). Recent studies have shown the presence of HPV56 in Sabah but not documented in West Malaysia, suggesting its endemicity in East Malaysia.

HPV84 ( $\alpha$ -3) is the only UDRHPV detected in this study. HPV84 was initially thought to cause asymptomatic infection in both healthy and HIV-infected women (31) and have higher tropism to the vagina rather than the cervix (32). However, HPV84 was later detected in genital warts (33) and cervical specimens with abnormal cytology ranging from cervical intraepithelial neoplasia 1-3, to cervical adenocarcinoma (34,35). Thus, HPV84 is not a low-risk HPV as conveniently regarded by many authors (36,37) but is epidemiologically classified as undetermined-risk (3,4) due to the lack of clinical evidence to associate it with cervical cancer and abnormal lesions at the time of classification. HPV84 is probably present in Peninsular Malaysia too, but the method used by the authors failed to discriminate HPV84 with HPV26 (28). The general omission of HPV with undetermined-risk such as HPV84 in commercial HPV diagnostic panels may cause the missed opportunity in the early diagnosis of cervical cancer.

## Conclusion

The prevalence of HPV in suburban villages in Serian was determined to be 11.62% (n=42). HPV18, 39, 52, 56 and 84 were detected at an equal ratio of 2.3%. HPV16 was not detected in this study and HPV18 is the only vaccine genotype present while HPV52, 56, and 39 are not covered by the vaccines used in the National Immunisation Programme.

## Abbreviations

HPV human papillomavirus

HR high-risk

UD undetermined-risk

PHR probably high-risk

LR low-risk

VIA visual inspection using acetic acid

PBS phosphate-buffered saline

PCR polymerase chain reaction

POS positive

NEG negative

## **Declarations**

### **Ethical Approval and Consent to participate**

The study was approved by Universiti Malaysia Sarawak Medical Ethics Committee UNIMAS/NC-21. 02/03-02 Jld. 3 (17). Standard procedures were followed to obtain informed consent from each participant before undergoing the procedures.

### **Consent for publication**

The results obtained from those who have provided their consent for publication were included in this manuscript.

### **Availability of supporting data**

The data generated during this study are not publicly available, but a transcribed file is available from the corresponding author Cheng-Siang Tan ([cstan@unimas.my](mailto:cstan@unimas.my)) on reasonable request.

### **Competing interests**

The authors declare no competing interests.

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### **Authors' contributions.**

MSY, SL, ARJ and MK obtained the informed consent, performed the cervical examination, obtained the cervical swabs, and critically reviewed and provided clinical input to the manuscript.

ARJ organised the medical outreach programme.

CST performed the HPV genotyping and wrote the manuscript.

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## Tables

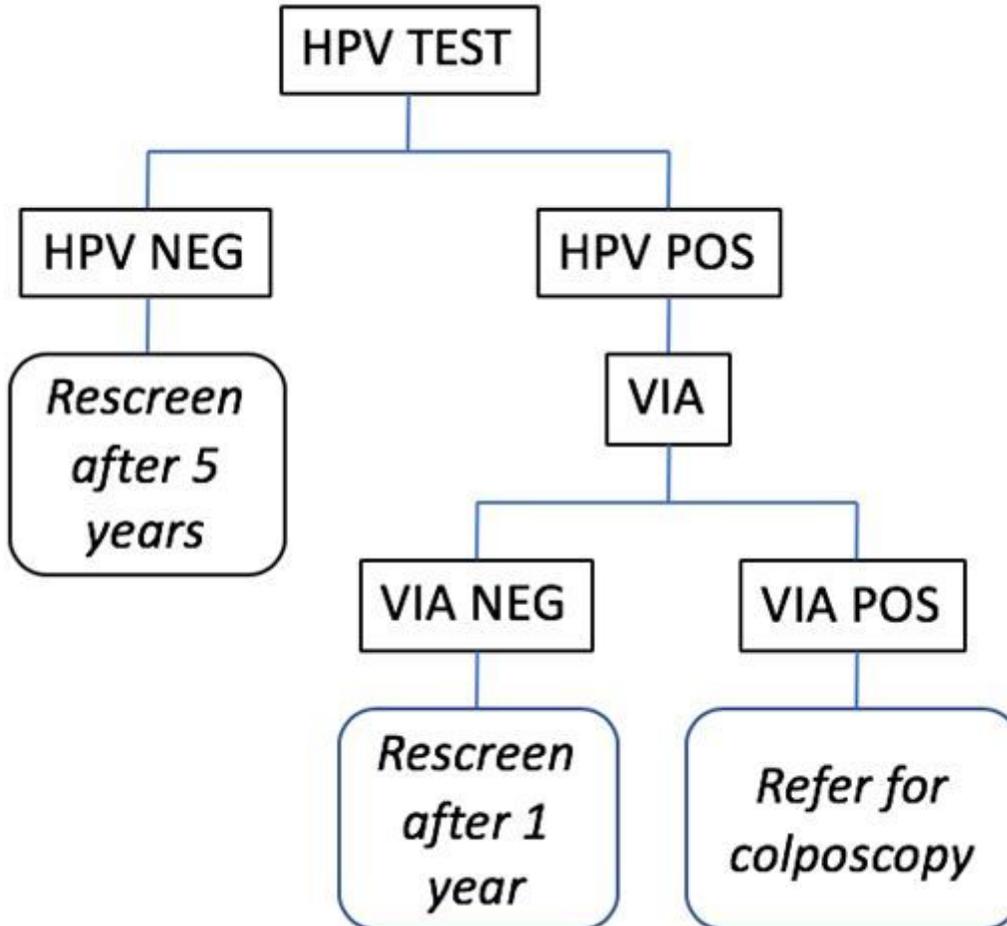
**Table 1:** HPV genotypes according to age cohorts.

Age cohort (year)	HPV genotype	Species (11)	Phylogenetic classification (3)
20-29	-	-	-
30-39	84	α-3	Undetermined risk
40-49	56	α-6	High-risk
50-59	52	α-9	High-risk
60-69	18, 39	α-7	High-risk

**Table 2:** HPV positive women with their corresponding VIA results.

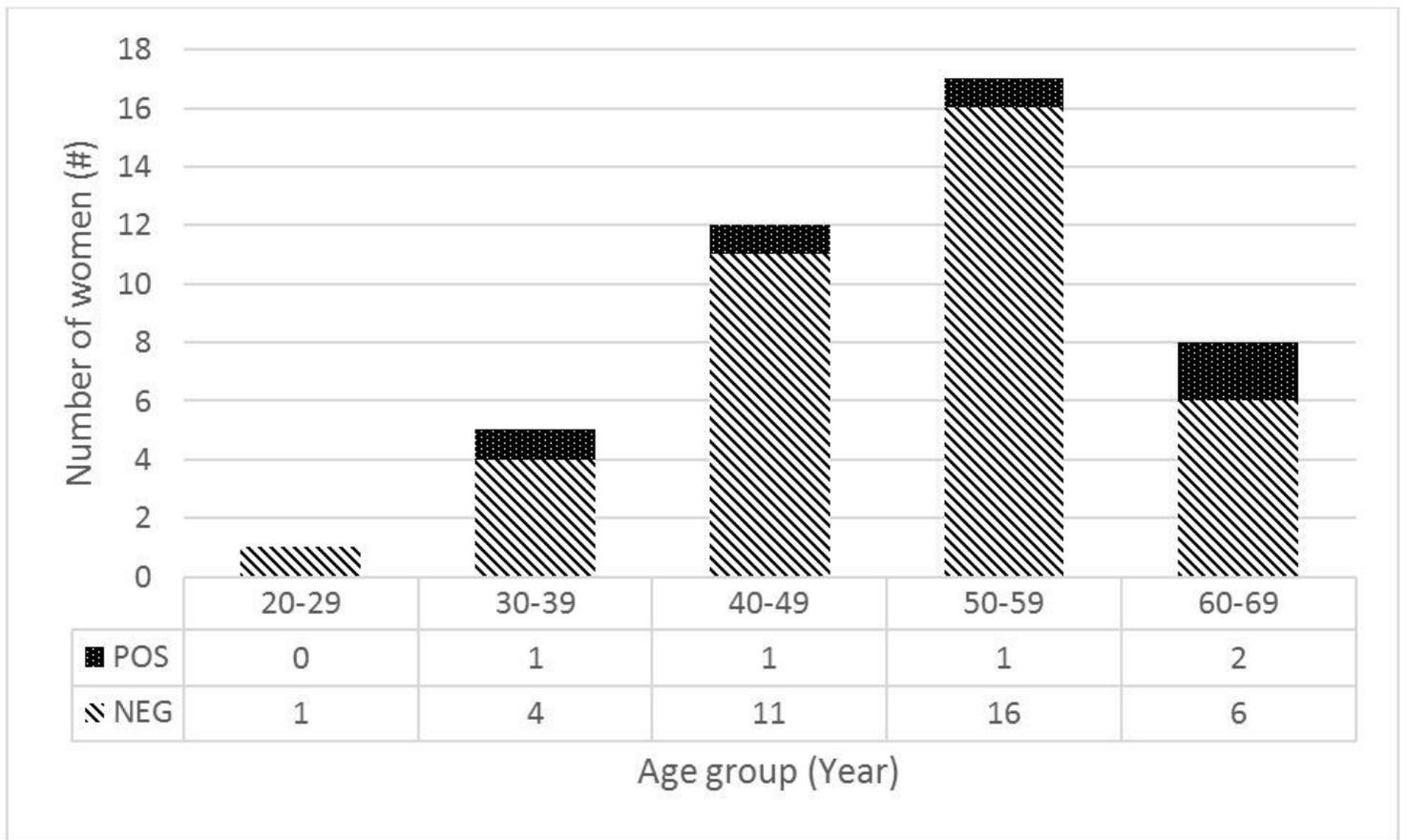
Age (year)	Ethnicity	HPV genotype	VIA
36	Iban	84	NEG
47	Iban	56	POS
56	Iban	52	NEG
60	Iban	39	NEG
67	Iban	18	NEG

## Figures



**Figure 1**

Screen with HPV DNA Test and followed by VIA. (adapted from the WHO guidelines for screening and treatment of precancerous lesions for cervical cancer prevention.



**Figure 2**

Distribution of human papillomavirus detected according to age group.