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Brief Communication

A pilot study on detection and genotyping of *Human Papilloma* virus isolated from clinically diagnosed Ethiopian women having cervical intraepithelial neoplasia

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ABSTRACT

Background: *Human Papilloma virus associated cervical cancers are more prevalent in developing countries compared to developed countries. Cervical cancer is reported as the most frequent malignancy among women visiting hospitals in Ethiopia. This study is a pilot study designed to examine the prevalence and genotypes of HPV in twenty Ethiopian women, clinically diagnosed to have cervical neoplasia, while visiting gynecology unit of a tertiary level referral hospital in Addis Ababa. The objective of this study was to detect the presence of HPV L1 gene and respective genotypes among women clinically diagnosed with different grades of cervical neoplasia.*

Methodology: *A total of 20 fresh biopsy samples were collected from clinically diagnosed cases, DNA extracted and further amplified using PCR for HPV L1 and beta globin genes. The PCR amplicons were denatured and allowed for hybridization onto a nitrocellulose strip containing the type-specific probes for 27 HPV genotypes representing both high and low risk groups as well as beta globin genes. Socio-demographic characteristics and clinical findings of the participants were recorded on structured questionnaires.*

Results: *Amplification of HPV L1 gene by PCR detected 17 cases out of 20. Based on reverse line blot hybridization assay, the most frequent genotype identified was HPV16 (13/20). Mixed infection of HPV 16 with HPV 33, HPV 35, HPV 45 and HPV 58 was detected from other four study participants.*

Conclusion: *Human papilloma virus type 16 was the most prevalent genotype identified from the subjects screened. Further investigation with statistically sound sample size would help to clearly visualize the existing trend in Ethiopia regarding factors for high risk HPV positivity and multiple gravidity, young age at first coitus and cervical neoplasia.*

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INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide and Ethiopia is a country with a huge burden affected by the disease (1). Possibility of development of cervical intraepithelial neoplasia (CIN) due to infection with oncogenic *human papilloma virus* (HPV) has already been reported earlier, which is one of the most common sexually transmitted viruses (5). This virus is amongst the most common sexually transmitted agents. *Human papilloma virus* types 16 and 18 are incriminated to be responsible for about 70% of all cervical cancer cases worldwide (9). A recent survey has ranked cervical cancer as second most common cancer in women living in Ethiopia (8). Early coitus and multiple gravidity have been reported to be the major risk factors associated with cervical neoplasia (2). A pilot study focusing on detection and genotyping of the circulating HPV genotype in Ethiopia would therefore be an important step for planning a subsequent broader study which in turn would be helpful for designing a recombinant vaccine and better management of the disease. The objective of this pilot study was to detect the presence of high risk HPVs in 20 Ethiopian women who were clinically diagnosed with different grades of cervical neoplasia.

MATERIAL AND METHODS

Study participants were recruited from gynecology unit of Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, who were clinically diagnosed to have gynecology related problems. Biopsy specimens were collected from clinically diagnosed patients who were suspected to have cervical neoplasia after visual inspection. Section of the biopsy specimen was sliced with a sterile scalpel, immersed into 0.9% normal saline and transported at room temperature to the laboratory at the Armauer Hansen Research Institute (AHRI) and kept at -20°C until further HPV genotyping. DNA was extracted from cryopreseved biopsy specimens using phenol-chloroform DNA extraction method, followed by alcohol precipitation and later amplified by PCR using primer sets specific for HPV L1 gene (MYO9: 5'-CGTCCMAARGGAWACTGATC-3' and MY11:5'-GCMCAGGGWCATAAYAATGG-3') as described earlier (6). In addition, beta globin gene (GH20: 5'-GAAGAGCCAAGGACAGGTAC-3' and PCO4: 5'-CAACTTCATCCACGTTCACC-3') was used as a housekeeping gene. Along with experimental samples, know HPV L1 gene positive sample and a non-template control (PCR grade dH_2O) were used as positive and negative controls, respectively in each reaction series. The PCR condition was set up in 25µl of total reaction volume containing 2.5µl of 10 x buffer II, 4µl of 25Mm $MgCl_2$, 0.5µl of DNTP blend, 0.375µl of 5U/µl AmpliTaq Gold, 0.5µl of 50µM each PGMY 09/11, 0.0125µl of B_PCO4/B_GH20, 15.35µl of PCR grade H_2O and 1.76µl of 100ng/µl of the extracted DNA. The PCR was run in a T3000 Thermocycler (biometra) with the following condition (Initial activation at 95°C for 1 min, followed by denaturation 95°C for 30 s, annealing at 55°C for 1 min and polymerization at 72°C for 1 min for 40 cycles, further followed by final extension step at 72°C for 5 min). Reverse line blot hybridization assay was run using

the PCR amplicons with line blot strips impregnated with 29 probes lines; detecting 27 individual HPV genotypes and two beta globin control probes with low and high intensities as described earlier (3, 4). The line blot assay makes use of L1 consensus primer-based PCR product amplified with PGMY09/11 primers. The PCR product was denatured and allowed to undergo hybridization to the strips. This was followed by visual detection of bands on the strips, where binding of the impregnated probe and the corresponding denatured DNA has occurred. Bands were detected on the strips fluorescing due to luminescent reaction where there was hybridization.

This study has received ethical approval from the institutional and national ethical clearance committees and a written informed consent was obtained from all participants before inclusion to the study.

RESULTS

Of the 20 study participants, we were able to retrieve the socio-demographic information from only 13 study participants. The median age of the study participants was 49 years (range: 23-60) and the median gravidity score was 6 (range: 4-14). The median age at which the participants had first coitus was 15 years, with a higher age at 20 years and the least age at 12 years. The median parity score was 5, ranging between 3 and 11. Of the 13 participants whose socio-demography was complete, 8 were married, 3 separated and 2 were widowed. Six of the 13 (46.2%) were rural dwellers whereas 7 (53.8%) were from urban areas. In addition, 9/13 had a history of at least one abortion.

The beta globin gene was amplified at 268 bp and HPV L1 gene at 452 bp (Figure 1) and a total of 17 cases were detected by PCR for HPV L1 gene, of which genotypes 33, 35, 45 and 58 were detected as mixed infection with HPV16 in 4 study participants and the rest 13 detected as single infection (Figure 2). Based on reverse line blot hybridization assay (RLBH) assay, HPV16 was the most frequent 65% (13/20) genotype identified. In 13 of the women whose isolates were typed as HPV 16, the number of gravidity ranged between 5 and 14. The highest age in years reported at first coitus was 20 while the least was 12. Histo-pathological analyses indicated moderately differentiated squamous cell carcinoma (MDSCC) in the cervix with stages varying from IIA to IIIB in 10/13 (77%) biopsy specimens, of which 6 (60%) were further typed as HPV 16 by RLBH assay and 3 (30%) showing mixed infection of HPV 16 with 33, 45 and 58. In addition, 2/13 (15.4%) were histologically diagnosed as poorly differentiated squamous cell carcinoma (PDSCC) of the cervix with stage IIB and IIIB, which were further typed as HPV16

and 16 + 35 by RLBH assay. Only 1/13 (7.7%) of the biopsy was histologically diagnosed to be adeno carcinoma (AC) stage IIB with further typing as HPV 16 by RLBH assay.

Figure 1. L=Lane; Lane 1 represents 100 bp DNA ladder; L2 – L7 represent biopsy samples from patients; L8 and L9 are positive and negative controls, respectively

Figure 1.PCR amplifications of HPV L1 and beta globin genes at 452 and 268 base pairs, respectively

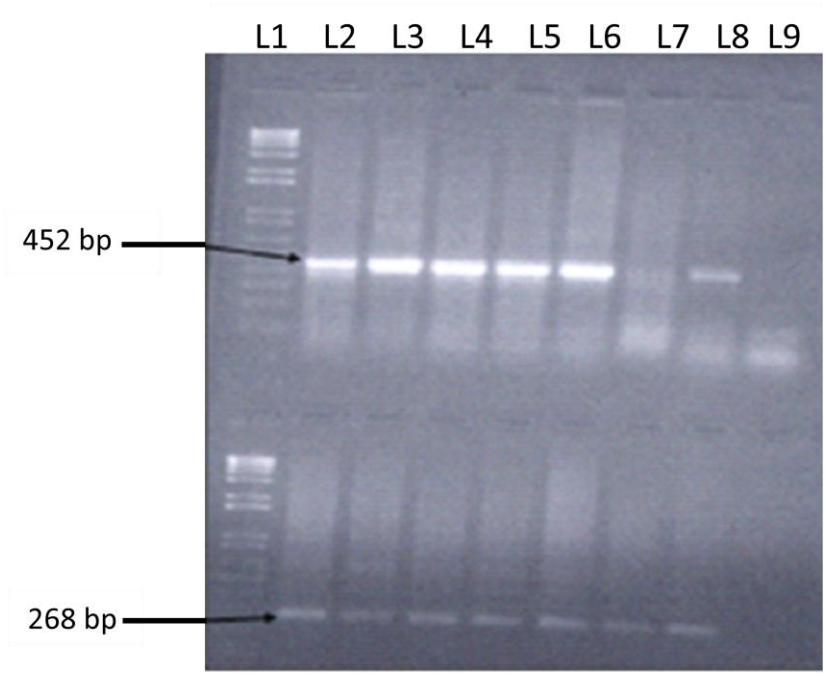
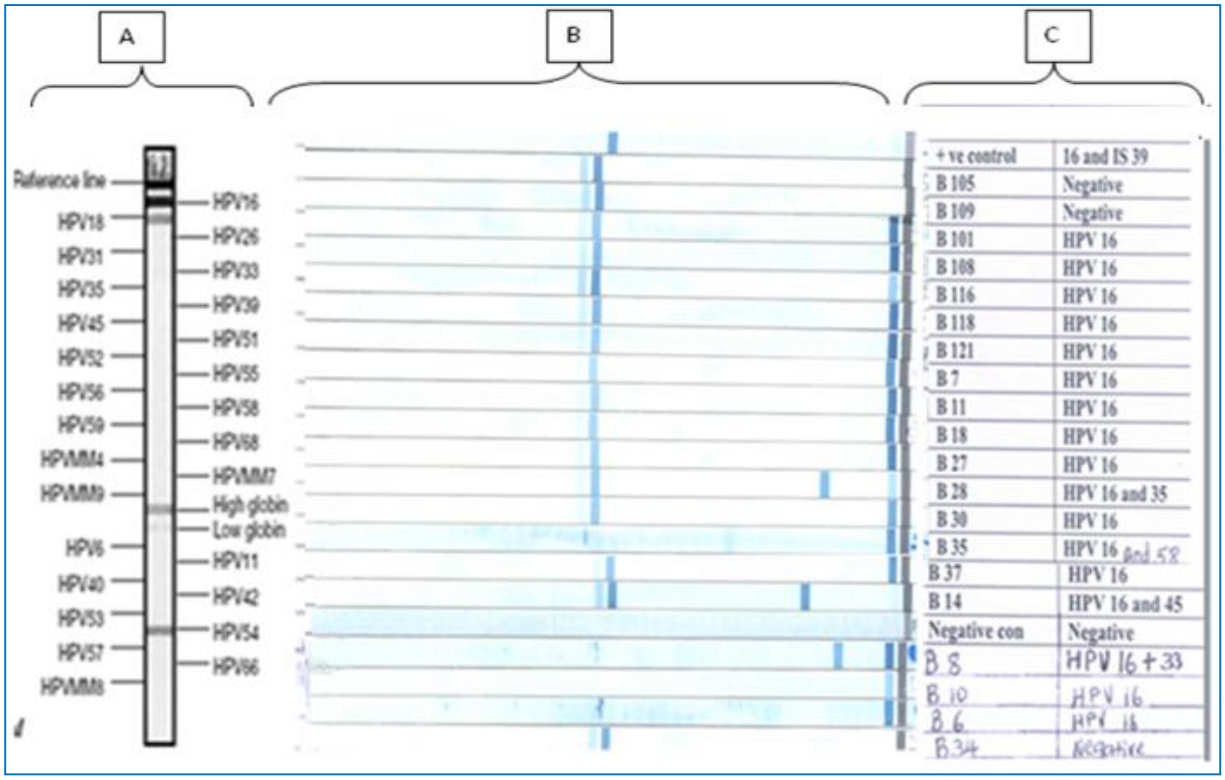


Figure 2. "**A**" represents standard strip displaying HPV genotype probes; "**B**" represents the reverse line blot hybridized strips, where the thick bands indicate high globin and thin bands indicate low globin and "**C**" indicates HPV genotypes interpretation reading from the middle genotyped strips based on the standard

Figure 2. Reverse line blot hybridization strips displaying lines of hybridized HPV probes with denatured HPV DNA



DISCUSSION

HPV16 which is the leading cause of cervical neoplasia, is the most frequent genotype identified in this study. Amplification of beta globin gene assured the quality of specimens and confirmed presence of suitable DNA. Like other earlier studies (9, 10), a trend of association is reflected between HPV 16 positivity with different grades of CIN, socio-demographic characteristics like early age coitus, multiple gravidity, multiple parity and low socio-economic status. In addition, HPV 16 has been described as one of the high risk types of HPV leading to a higher chance of cervical lesions when compared to the infection of other high-risk HPV types (11, 12). The occurrence of HPV16, as single and mixed infection, and its association with invasive CIN like MDSCC, PDSCC and AC is a remarkable finding of this study.

This pilot study highlights the most abundant circulating genotypes of HPV, i.e., HPV16. Furthermore it indicates the need for in-depth investigation of such cases with possible risk factors using a robust sample size to better identify cases, improve patient management and for future vaccine intervention program.

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