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Genotyping Human Papillomavirus in Women Attending Cervical Cancer Screening Clinic in Harare, Zimbabwe

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NC, RSDM, JM, EZ and BSP designed and supervised the study. Authors TM, RSDM, NC and GC were responsible for specimen collection, laboratory work and data acquisition. All authors participated in data analysis and writing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the prevalence of human papillomavirus genotypes in women attending a cervical cancer screening VIAC (visual inspection with acetic acid) clinic.
Study Design: Cross-sectional study.
Place and Duration of Study: VIAC clinic at Parirenyatwa Referral Hospital in Harare in Zimbabwe between February and April 2015.
Methodology: Sexually active women were recruited and they provided their socio-demographic data and self-collected vaginal swabs. HIV status of the participants was determined. DNA was

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extracted from the swabs using the standard phenol-chloroform method. HPV DNA was detected using the standard consensus MY09/11-GP5+/GP6+ nested polymerase chain reaction. Amplicons were sequenced and sequences analyzed using bioinformatics tools to identify the HPV genotypes.
Results: Sixty women were recruited. Their age ranged from 21-83 years, with a mean of 40.1 years. Most of the women were married and resided in the urban areas. Of the 60 participants, 50% (30/60) were HIV-positive. The prevalence of HPV genotypes in the study subjects was 56.7% (34/60). HPVs were most prevalent in women aged 30 years and below, and became less prevalent as the age increased. The predominant genotypes detected were HPV-16, -58, -52, -45, -18, -33, -51, -6, -81, -11, -70, -62, -32 and -40.
Conclusion: A number of HPV genotypes were detected in half of women tested. There was no significance association between risk-factors (parity, level of education, residence, history of STI, contraceptive use and sexual debut) and HPV infection. The findings of this study showed that consensus nested PCR and DNA sequencing could be used to detect HPV genotypes in women in cervical cancer screening programs. Although this method is sensitive, it is inefficient at detecting multiple HPV infections.

Keywords: Human papillomavirus; genotypes; consensus nested-PCR; sequencing; Zimbabwe.

ABBREVIATIONS

HPV: Human papillomavirus; HIV: Human immunodeficiency virus; VIAC: Visual inspection with acetic acid; OI: Opportunistic Infection; TRIM5α: Tripartite motif 5 alpha; PCR: Polymerase chain reaction; DNA: Deoxyribonucleic acid; UV: Ultraviolet; OR: Odds ratio; EDTA: Ethylenediaminetetraacetic acid; STI: sexually transmitted Infection.

1. INTRODUCTION

Human papillomavirus (HPV) is a DNA virus that infects mucosal surfaces and has been associated with development of cervical cancer [1]. So far, more than 200 HPV types are known to exist and are broadly grouped as either high- or low-risk types depending on their ability to induce the development of cancer [2]. The high-risk types include HPV-16, -18, -31, -33, -35, -39, -45, -50, -51,-53,-55, -56, -58, -59, -64, -68, and low risk types include HPV -6, -11, -40, -42, -70, -72, -81, -43, -44, -54, -61 and -81 [3]. It is the persistent infection with the high-risk types that normally leads to the development of cervical cancer disease in women [4,5]. In Sub-Saharan Africa, cervical cancer is one of the main causes of mortality and morbidity in women [6]. In Zimbabwe, cervical cancer accounts for about 32% of all cancers in women and is also the main cause of death due to cancer in women [7]. Several studies have already shown a high prevalence of high-risk HPV genotypes in women with cervical cancer in Zimbabwe [8]. However, studies on early detection of cervical cancer in healthy women by screening using approaches such as visual inspection of the cervix after staining with acetic acid are still limited in Zimbabwe. Data on the prevalence of HPV genotypes in healthy women attending such screening programmes are also still lacking in Zimbabwe. The aim of this study was to determine the prevalence of HPV genotypes in women visiting VIAC clinic at Parirenyatwa Hospital in Harare in Zimbabwe.

2. METHODOLOGY

2.1 Study Subjects and Sample Collection

The study was done on sexually-active women who visited the VIAC clinic situated at Parirenyatwa Referral Hospital in Harare, Zimbabwe. All women visiting the clinic were invited to participate in the study and issued with an enrolment screening tool. Women were eligible for participation if they were sexually active and at least 18 years old. Women who had undergone total abdominal hysterectomy were excluded from the study. A nurse explained the study and written informed consent was obtained from each woman who agreed to participate. Each participant completed a self-administered questionnaire which included information on socio-demographic data and sexual behaviour. HIV testing was offered to all women, however some of them had valid documentations from the OI (Opportunistic Infection) clinic and preferred to provide their HIV results. After enrolment,

participants were given written instructions and a diagram on how to obtain vaginal samples. The self-collection of vaginal swabs was then conducted. Dacron swabs with plastic applicators were used with 500 µl Biomeriuex/Nuclisens-NASBA lysis buffer (containing guanidine thiocyanate) and were stored at -80°C until DNA extraction.

2.2 DNA Extraction from Swabs

DNA was extracted by adding 15 µl of 10 mg/ml Proteinase K to 300 µl of sample, followed by vortex and incubation at 50°C in a water-bath with shaking overnight. After incubation at 95°C for 15 minutes to inactivate the proteinase K, 1 ml of 1:4 ammonium-acetate/acetate solution was added. The DNA was precipitated with 1 volume of absolute ethanol. The DNA/ethanol samples were mixed by gentle inversion. The DNA precipitates were incubated at -80°C for 1 hour and then centrifuged at 14000 g for 30 minutes to pellet the DNA. The supernatant was discarded leaving the DNA pellet. The DNA pellets were washed once with 70% ethanol and allowed to dry overnight and were suspended in 25 µl TE (10 mM Tris pH 8.3/ 1 mM EDTA pH 8.0). The DNA was stored at -80°C until further use. The integrity of the extracted DNA was determined by amplifying the TRIM5α (Tripartite motif 5 alpha) gene, using the R136Q-R/R136Q-F primers which amplify a 526 bp sequence. Each amplification reaction contained 31.75 µl nuclease free water, 10 µl of 5X Taq standard buffer (Zymo Research Corp, U.S.A), 1 µl dNTPs (10 mM), 1 µl Primer 1 - 136F (10 µM), 1 µl Primer 2 - 136R (10 µM) and 0.25 Taq Polymerase (1U; New England Biolabs Inc, U.S.A). A thermocycler (Perkin Elmer Gene Amp PCR System 2400, USA) was used for amplification with the following cycling program: initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94 °C for 30s, annealing at 50°C for 30s, extension at 68°C for 30s and a final extension at 68°C for 5 minutes. The PCR products were analysed by running them on a 3% agarose gel at 100 volts for 1 hour.

2.3 HPV Amplification by Consensus Polymerase Chain Reaction

HPV detection from purified DNA from vaginal swabs was carried out using consensus MY09/11-GP5+/6+ nested-PCR, performed in two rounds. The first round PCR was carried out using the MY09/11 primer sets (MY09: CGT CCM ARR GGA WAC TGA TC; MY11: GCM CAG GGW CAT AAY AAT GG) which amplify the 450 bp sequence within the L1 region of HPV genome. Each amplification reaction contained: 31.75 µl nuclease-free water, 10 µl of 5X Taq standard buffer (New England Biolabs, UK), 1 µl of 10 mM dNTPs, 1 µl of MY09 primer (10 µM), 1 µl of MY11 primer (10 µM) and 0.25 Taq polymerase (1U) (New England Biolabs, UK) and 5 µl of DNA template. The first round MY09/11 PCR cycling conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s, extension at 68°C for 30s, and a final extension for 5 min at 68°C. For the second round PCR, GP5+/GP6+ primers (GP5+: TTT GTT ACT GTG GTA GAT ACT AC; GP6+: GAA AAA TAA ACT GTA AAT CAT ATT C) were used and amplified an approximately 150 bp DNA band. Each amplification reaction contained: 31.75 µl nuclease-free water, 10 µl of 5X Taq standard buffer (New England Biolabs, UK), 1 µl of 10 mM dNTPs, 1 µl of GP5+ primer (10 µM), 1 µl of GP6+ primer (10 µM) and 0.25 Taq polymerase (1U) (New England Biolabs, UK) and 5 µl of first-round PCR as DNA template. PCR cycling conditions were as follows: Initial denaturation for 5 mins at 94°C, 35 cycles of denaturation at 94°C for 30s, annealing at 44°C for 30s, extension at 72°C for 30s and a final extension for 5 min at 72°C. The PCR products were analysed by 3% agarose gel electrophoresis.

2.4 Evaluation of the Consensus Polymerase Chain Reaction Method

To evaluate the consensus nested PCR method, we also amplified DNA samples that had been spiked with known HPV genotypes. The panel HPV DNA samples were derived from material procured in the Global HPV DNA Proficiency Study in which our laboratory had participated. The DNA samples contained purified plasmids carrying the complete genomes of known HPV types and these samples consisted of different HPV types, either single or in pools with multiple HPV types in different dilutions. Each sample was composed of 100 µl purified HPV plasmid DNA in TE buffer with 1 mM EDTA and 10 ng /µl of human placenta DNA. DNA extraction was not required prior to PCR testing. First round PCR was performed as outlined above with 5 µl of the samples used as DNA template. The second round PCR was also performed as outlined above with 5 µl of the first-round PCR being used

as DNA template. The PCR products were also analysed by 3% agarose gel electrophoresis.

2.5 Sequencing and Bioinformatics Analysis

A total of 30 positive PCR amplicons from the participant samples were sent for sequencing at Inqaba Biotechnical Industries (Pretoria, South Africa). As positive controls, a total of 7 out of 8 amplicons from the Global HPV Proficiency Study were also sent for sequencing. DNA sequence data (chromatographs and sequences) for all the samples were sent back by email for analysis. DNA sequences were analysed using the Geneious Basic program version 8.1 (Biomatters, USA) (http://www.geneious.com) and BLAST tools (www.ncbi.nlm.nih.gov).

2.6 Statistical Analysis of Data

Socio-demographic data were compared among the HPV positive and HPV negative women to correlate any association between the socio-demographic variables and HPV acquisition. Statistical analysis of data was done using the Stata Version 11 (Inc., Chicago, IL, USA). A p-value below 0.05 was considered statistically significant. HPV results were also stratified by HIV status using the Pearson chi-square method.

2.7 Ethical Approval and Considerations

The study was approved by the Joint Research Ethics committee of Parirenyatwa Hospital and the University of Zimbabwe College Of Health Sciences (JREC210/14) and the Medical Research Council of Zimbabwe (MRCZ/B/809). The experiments were performed in accordance with the ethical standards laid down by the Declaration of Helsinki of 1964. All participants signed informed consent forms according to the recommendations of the ethics committees.

3. RESULTS

3.1 Demographic Characteristics of the Study Participants

The study participants consisted of a total of 60 women. The mean age of the study participants was 40.1 years, range 21-83 years and standard deviation (SD) of ±11.7 years. Most of the participants lived in the urban area (80%), while the remaining (17%) resided in the rural area. The majority (68%) were married, 2% were single, while the divorced and widowed accounted for 10% and 20% respectively. More than half (53%) of the participants were using contraception, while 45% were not using any contraception. The mean number of pregnancies or children (parity) in participants was 3.3 (± 2.3). The average age at which women engaged in the first sexual activity (sexual debut) was 19.8 (± 3.3) years. The majority (58%) of the participants had no history of any STI infection besides HIV, while a significant number (40%) of the participants confirmed to have previously suffered from an STI. The level of education of most women in the study population was secondary (ordinary) level (38%), followed by primary level (37%), advanced secondary level (12%) and tertiary level (13%) of education.

3.2 Amplification of HPV DNA

The PCR products of the house-keeping gene TRIM5α were visualized under UV radiation (Fig. 1). Amplification of a housekeeping gene (TRIM5α) signified that there was sufficient DNA in a sample suitable for PCR amplification and detection of the PCR product by gel electrophoresis. Most samples were positive for the TRIM5α gene. However, 8 out of 60 samples (13%) were negative for the TRIM5α.

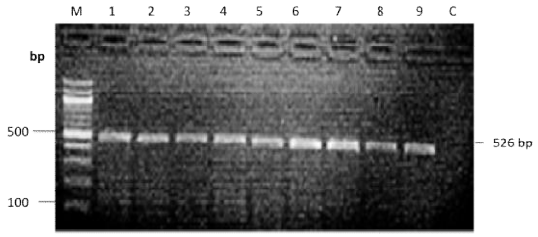

Fig. 1. Representative gel showing amplification of TRIM5α gene
Lane M: DNA marker, Lanes 1-9: amplified Trim5α gene, Lane C: PCR negative control

Nested PCR was performed on 60 samples and a 150 bp band visualized under UV radiation was scored as a positive result for a sample with HPV DNA. The absence of a band showed that there was no HPV DNA in a sample (Fig. 2). HPV DNA was detected in 34 samples (56.7%) and 26 samples (43.3%) were negative. Of the 34 samples that were positive, 30 were selected for DNA sequencing. From the Global HPV Proficiency Study samples investigated, 7 out of 8 were positive by nested PCR (Fig. 3) and these were also sent for DNA sequencing. Sample 1 was known not to have been spiked with HPV DNA and that was why it turned out to be PCR-negative.

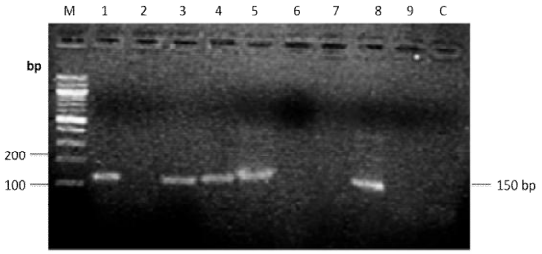

Fig. 2. Representative gel showing amplification of HPV L1 gene from swab DNA using nested PCR

Lane M: DNA marker, Lanes 1, 3-5, 8: amplified HPV L1 gene, Lanes 2, 6, 7: no amplification of HPV L1 gene, Lane C: PCR negative control

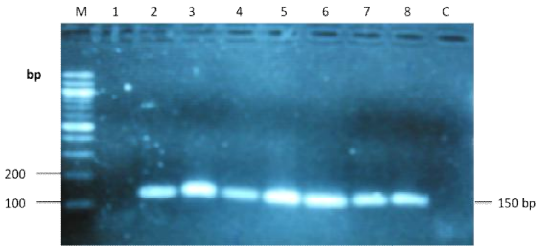

Fig. 3. Gel showing amplification of HPV L1 gene from 8 samples from the global HPV proficiency study L1 using nested PCR

Lane M: DNA marker, Lane 1: sample 1 which was PCR negative, Lanes 2 -8: amplified HPV L1 gene from the 7 samples, Lane C: PCR negative control

3.3 Prevalence of HPV in the Subjects

Out of the 34 samples that showed HPV DNA amplification, 30 (88%) samples with strong PCR bands were selected for DNA sequencing of the HPV L1 fragment. The 4 samples (12%) with faint bands, though of the expected size, did not qualify for DNA sequencing. The DNA sequences of 30 samples were analysed using Geneious Basic program in order to identify the different HPV genotypes. This was done by searching the gene banks available for DNA sequence similarities.

Of the 30 samples sequenced, only one (3%) of the sequences did not match with any HPV genotypes from the gene banks. The presence of multiple HPV genotypes was observed in some of the samples. The most prevalent HPV types were 16 and 6, present in 8 and 9 of the HPV infection cases respectively (Table 1). HPV 58, 52 and HPV 18 had a significant high contribution to the HPV infection cases. The least common genotypes were HPVs 33, 51, 70, 62, 32 and 40. HPV 16 and 18 were identified in 8/30 and 2/30 cases respectively, which gives a prevalence of 33% (10/30) for the HPV subtypes 16 and 18.

Table 1. Frequency of HPV types detected in 30 subjects

HPV genotype	Frequency (n=30)
6	9
16	8
58	4
52	4
11	3
45	3
81	3
18	2
32	1
33	1
40	1
51	1
62	1
70	1

We also used the Geneious Basic program to identify HPV genotypes from the sequences from the Global HPV Study samples. The findings are given in Table 2. The spiked HPV genotypes were detected in samples 2, 4, 5 and 7. These samples were spiked with single HPV types. Where samples were spiked with multiple HPV types (samples 3, 6 and 8), PCR-sequencing was not able to detect all genotypes (Table 2). In samples 3 and 6, only HPV genotypes 66 and 11 were detected respectively. In sample 8 which was spiked with 4 genotypes, only 2 types (HPV 6 and 58) were detected.

3.4 Risk Factors for HPV Infection

The socio-demographic characteristics were compared between HPV positive and HPV negative women (Table 2). The mean age for HPV positive women was 37.2 (±10.2) years compared to 43.7 (±12.7) years among those with no HPV infection. There was a statistically significant difference in age between women infected with HPV (HPV positive) and those not infected with the HPV (HPV negative) (95% CI, 0.90-0.99, p=0.042*). For a unit increase in age the odds of HPV are 0.95 times less. Other risk factors that were compared between HPV positive and negative include parity, level of education, residence, history of STI, contraception use and sexual debut. There was

no significance association between these risk-factors and HPV infection.

For the 60 women in this group, who participated in this study, 50% (30) of them were HIV positive and 50% (30) were HIV negative (Table 3). Amongst the 34 women that were HPV positive, only 22 (37%) were HIV sero-positive while the remaining 12 (20%) were HIV negative. There was no statistical significance association between HPV infection and HIV status.

4. DISCUSSION

Cervical cancer remains one of the major causes of morbidity and mortality in women in most developing countries including Zimbabwe. Since the disease can be treated if detected early, women have to regularly go for screening for precancer. In this study, we set out to investigate the prevalence of HPV genotypes in women attending a cervical cancer screening programme in Harare, Zimbabwe. Self-collected

Table 2. Identification of HPV genotypes from the global proficiency study

Panel sample	Content (IU or GE per 5 μl)	Expected HPV type(s) in the panel	Findings by nested-PCR- sequencing
1	0	Negative	negative (not sequenced)
2	500	58	58
3	500	35, 39, 59, 66, 68b	66
4	5	16	16
5	500	6	6
6	50	11, 18, 31, 52	11
7	50	31	31
8	500	6, 56, 58, 68a	6, 58

Table 3. Socio-demographic data of the study population: Bivariate analysis

Variable	HPV DNA status		OR (95% CI)	p-value
HPV DNA status, n (%)	Case (n=34)	Control (n=26)		
Age, mean (SD)	37.2 (10.2)	43.7 (12.7)	0.95 (0.90-0.99)	0.042*
Parity, mean (SD)	2.9 (2.2)	3.9 (2.4)	0.81 (0.63-1.05)	0.107
Education				
Primary	12 (35%)	10 (39%)	-	
Secondary	12 (35%)	11 (42%)	0.91 (0.28-2.93)	0.873
Advanced level	5 (15%)	2 (8%)	2.08 (0.33-13.1)	0.435
Tertiary	5 (15%)	3 (12%)	1.39 (0.26-7.30)	0.698
Residence				
Rural	5 (15%)	5 (20%)	-	
Urban	29 (85%)	18 (72%)	1.61 (0.41-6.35)	0.496
Sexual debut, mean (SD)	20.0 (3.5)	19.6 (3.1)	1.03 (0.88-1.21)	0.709
Marital status				
Single	1 (3%)	-	-	
Married	23 (68%)	18 (69%)	1.28 (0.35-4.64)	0.709
Divorced	4 (12%)	2 (8%)	2.00 (0.26-15.4)	0.505
Widowed	6 (18%)	6 (23%)	1.33 (0.76-1.45)	0.828
History of STI				
No	19 (56%)	16 (62%)	-	
Yes	14 (41%)	10 (38%)	1.18 (0.41-3.37)	0.758
No information	1 (3%)	-	NA	NA
Contraception use				
No	15 (44%)	12 (46%)	-	
Yes	18 (53%)	14 (54%)	1.03 (0.37-2.89)	0.957
No information	1 (3%)	-	-	-

* *P < 0.05 significant difference, OR odds ratio*

vaginal swabs were used for the isolation of HPV DNA from the women. The successful amplification of a house-keeping gene from most swabs indicated that the swabs contained enough cells for HPV DNA analysis from the study subjects. An HPV prevalence of 56.7% was recorded by consensus nested PCR using MY9/11 and GP5/6 primer sets. The prevalence value observed in this study was much higher than that reported in previous studies in Zimbabwe. Previous studies have indicated HPV prevalence values of 24.5-48.7% [9-11]. The prevalence disparities between the previously reported studies and the current study could be due to differences in study population and HPV detection strategies employed. The high HPV prevalence in this study can be attributed to the high sensitivity of the consensus nested PCR detection assay used. Several studies have revealed that nested PCR using MY9/11 and GP5/6 primer sets is a highly sensitive HPV detection assay compared to conventional PCR which was used in previous studies [12]. Further evaluation of the nested -PCR method showed that the method was very sensitive and all 7 proficiency-test samples known to contain HPV DNA had 150 bp bands for positive HPV L1 gene amplification. The nested PCR method was efficient since it picked low levels of HPV DNA. This justifies the use of consensus primers targeting the L1 region for HPV-DNA amplification in many laboratories [13]. DNA sequencing results for HPV proficiency-study amplicons were generally consistent with expected outcomes. However in three of the samples that were spiked with several genotypes, some of the genotypes could not be identified. This is due to nested PCR method which preferentially amplifies abundant genotypes leaving out minority genotypes. This could imply that the actual prevalence of HPV genotypes detected in the study population could be even greater than those we detected. One of the samples showed the presence of the PCR positive but confirmed to be a non-HPV sample and this underscored the need for confirmation by DNA sequencing after PCR amplification in order to eliminate false positives. It should however be noted that the rate of false positives was quite low (3%). The false positive result usually arises due to non-specific amplification of some non-HPV DNA sequences or amplification of novel HPV types.

A total of 14 genotypes identified (Table 1) showed a high HPV genetic variation in positive samples. Of these, 7 (HPV16, 18, 33, 35, 45, 51, 52, 58) were classified as high risk in terms of their oncogenic potential for cervical intraepithelial neoplasia while 7 were low risk types (HPV 6, 11, 32, 40, 62, 70, 81). The findings of this study are consistent with previous reports that HPV 16, 18, 35, 51, 52, 58 and 70 are the most prevalent genotypes among Zimbabwean women [11,9]. These HPVs (16, 18, 45, 33 and 35) were reported to be most frequent among women of Sub-Saharan Africa and HPV type 16, 18, 35, 45 and 31 noted to be most frequent worldwide [14]. In this study, all commonly previously reported types were identified with the exception of HPV 31 and 35. A total of 11 out of the 30 (36.6%) sequenced samples consisted of the HPV types 16, 18 and 33. The most prevalent LR-HPV types detected in this study were HPV6 and 11 which made up 12 of the 30 samples (Table 1). The types are also usually associated with recurrent respiratory papillomatosis and condyloma acuminata (genital warts).

It is crucial to note that this study identified the HPV 16/18/6/11 amongst the most prevalent HPV types. These HPV types are being covered in the current vaccines and this justifies their introduction in our society as a great effort in reducing the burden of HPV related infections. Although the vaccines do not cover all the most prevalent HPVs, they will prevent the majority of cases since HPV 16/18 have been found in 79.6% of ICC cases in the country [14]. In sub-Saharan Africa, the prevalence of HPV 16/18 in ICC was estimated to 69.2% [14]. Basing on genotypes detected in this study, the HPV nonavalent vaccine which covers HPV genotypes 6/11/16/18/31/33/45/52/58 will be more relevant for Zimbabwean women. If we are to combat HPV infections in our society there is need to incorporate subunits of the detected HPVs in the production of new vaccines. Prophylactic mass HPV vaccination will be crucial in the control of the high risk and associated disease burden in young women in the country. Moreover, a high HPV prevalence detected in this study highlight that we should put our main thrust on developing therapeutic vaccines for use against HPV. Interestingly, a statistically significant association between age and HPV infection ($p= 0.042$, Table 1) was observed. As the age increased, the risk of being infected with HPV decreased. There was a significant decrease in age specific HPV prevalence with increasing age. The same trend was reported in previous studies done in Zimbabwe and other countries [10,15]. The trend observed could have been due to the majority of

HPV infections that resolve spontaneously, mostly within a 24 month period in young women [16], as well as differences in sexual behavior with age increase. However, there was no significant association between other factors such as parity, level of education, residence, sexual debut, history of STI and contraception use with HPV infection ($p>0.05$, Table 1). Therefore, with a high prevalence of HPV found in this Zimbabwean group, there is a serious and urgent need for introducing HPV vaccination for young boys and girls before their sexual debut.

A total of 50% of the women who participated in this study were HIV positive. The majority of women who were HPV positive were co-infected with HIV (22/34). The findings are consistent with previous studies that reported a 2-4 fold increased risk of HIV acquisition in cervico-vaginal HPV infections [11]. Furthermore, studies revealed that women infected with HIV were likely to develop persistent infection with multiple HPV types and this was notably observed in this study. Other studies showed that women with HIV develop cervical cancer at an earlier age than women who are HIV-negative [17]. A rapid progression to invasive cervical cancer is expected to occur in HIV positive women in compared to HIV negative women. HIV infection suppresses the immune system and therefore the outcome of an HPV infection in an HIV positive patient is worsened [18].

5. CONCLUSION

HPV genotypes were found in 50% of the women tested. Other risk factors compared between HPV positive and negative included parity, level of education, residence, history of STI, contraception use and sexual debut. There was no significance association between these risk-factors and HPV infection. Although 50% were also HIV-positive, there was no significance association between this risk-factor and HPV infection. The findings of this study showed that consensus nested PCR using MY9/11 and GP5/6 primer sets and sequencing could be used to detect HPV genotypes in women in cervical cancer screening programs. Although this method is sensitive, it is inefficient at detecting multiple HPV infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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