International STD Research & Reviews
5(1): 1-8, 2017; Article no.ISRR.31094
ISSN: 2347-5196, NLM ID: 101666147

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Molecular Detection and Typing of Genital Human Papillomavirus among Female Medical and Nursing Students in Harare, Zimbabwe

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Authors' contributions

This work was carried out in collaboration between both authors. Authors NLS and NC designed the whole study and wrote protocols. Author NLS recruited study subjects, processed samples and did the laboratory bench work. Authors NLS and NC analyzed data and wrote the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ISRR/2017/31094
Editor(s):
(1) Kailash Gupta, Division of AIDS, NIAID, NIH, USA.
Reviewers:
(1) Luigi Tagliaferro, Laboratory "Pignatelli" – Lecce, Italy.
(2) Jonatas R. de Oliveira, UNESP, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/17565

Original Research Article

Received 19th December 2016
Accepted 13th January 2017
Published 19th January 2017

ABSTRACT

Aims: to detect and type genital human papillomavirus (HPV) in young female medical and nursing students.
Study Design: Cross sectional study.
Place of Study: The study was conducted in Harare, Zimbabwe at the College of Health Sciences, University of Zimbabwe and Harare Central Hospital's School of Nursing in 2014.
Methodology: Self-collected cervico-vaginal swab specimens from female college students were processed and genomic DNA extracted. HPV-DNA was detected by consensus polymerase chain reaction (PCR) using two primer sets, MY09/MY11 and GP5+/GP6+. Positive PCR samples were typed by DNA sequencing and bioinformatics analysis.
Results: Cervico-vaginal swabs were self-collected from 125 students. The age range of the participants was 20-25 years with a mean age of first sexual activity of 19.22 years. One hundred and fourteen (114) out of the 125 swabs from the students had genomic DNA successfully extracted. Of these, 36 tested positive for HPV-DNA, giving a prevalence of 31.58%. Both high-risk

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(HPV 16, 18, 35, 45, 58, 53 and 56) and low-risk (HPV 6, 11, 40, 53, 54, 72, 81 and 86) genotypes were detected among the students.

Conclusion: A high prevalence of HPV infection in medical and nursing students in Harare was observed. Further studies are necessary to establish the true prevalence of HPV types in young and healthy women in Zimbabwe and the data generated will be useful in informing reproductive public health policies.

Keywords: Genital; human papillomavirus; female; students; infection; Zimbabwe.

1. INTRODUCTION

Genital human papillomavirus (HPV) is one of the most common sexually transmitted viruses worldwide [1]. It is the persistent infection with specific genital HPV genotypes that results in the development of genital and cervical cancers as well as warts [2]. Of the many HPV genotypes known to date, more than 40 of them infect the anogenital tract [3]. These genotypes that affect the anogenital mucosa are classified as either low- or high-risk genotypes depending on their ability to cause cancer and warts [4]. The high-risk HPV genotypes that may cause development of cervical cancer include HPV 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, 64, 68, 73 and 82 [5]. The majority of cervical cancer cases are however caused by HPV 16 and 18 [6]. The low-risk genotypes include HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and the majority of anogenital warts are caused by HPV 6 and 11 [7]. In Zimbabwe, cervical cancer is the most common malignancy in black women and accounts for 35.5% of all cancers [8]. Community-based studies on the genital human papillomavirus incidence and persistence in a cohort of HIV-negative women in Zimbabwe have shown HPV 16, 18, 31, 33 and 58 to be the most prevalent in women [9]. In cervical cancer patients, the high-risk genotypes, HPV 16, 18, 31, 33, 52, 58 and 70 have been found to be prevalent in a number of studies in Zimbabwe [10]. Most studies on genital HPV in Zimbabwe have been done on adult women. Epidemiological data on the prevalence of genital HPV genotypes in adolescents and young healthy women such as university or college students are not available. The aims of the study were to detect and to genotype genital HPVs in young female medical and nursing students at the University of Zimbabwe's College of Health Sciences and Harare Hospital's School of Nursing in Harare, Zimbabwe. We targeted students because genital HPV is the most common sexually transmitted virus on campuses and infection by the virus is the best biological marker of their sexual behaviours and activities [11]. Medical and nursing students were also targeted in this study because they were easy to recruit and self-sampling of genital specimens was likely to be easy for them.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Genital Swabs from Students

This was a cross-sectional study conducted on young and female students based at the University of Zimbabwe's College of Health Sciences and the Harare Central Hospital's School of Nursing. The inclusion criteria were: being a female medical and nursing student at the above mentioned institutions; aged between 17-25 years and being willing to voluntarily participate in the study. The exclusion criteria were: being a non-female medical and nursing student at the above mentioned institutions; being female and aged low 17 years or above 25 years and being unwilling to voluntarily participate in the study. A total of 125 female participants aged between 17-25 years were therefore recruited from the University of Zimbabwe College of Health Sciences and Harare Central School of Nursing employing the random sampling technique. A 10-minute recruitment Powerpoint presentation was given to the potential participants and those consenting were recruited into the study. The presentation was not to give participants information about the virus, its infection or prophylaxis, but to convince the students to join the study and to advise them on how to complete the questionnaire and how to self-collect swabs. Each student answered a questionnaire that provided demographic information on aspects such as age, sexual behaviour, age of first sexual encounter, number of sexual partners. The self-collected genital/vaginal swabs were collected using sterile cotton swabs with transport media. Participants were instructed by the researcher on the correct procedure of self-collection of cervico-vaginal/ genital swabs. The self-collection was done at their place of residence or home in the morning

before bathing. They were instructed to open the swab and insert the cotton tip about 4 cm into the vagina, turning it left and right on the vaginal wall to aid collection of vaginal excretions. They were then instructed to immediately place the swab in the tube containing the transport media avoiding putting it down or touching the surface of the skin. Participants were issued with a self-sampling instructional sheet for reference when carrying out the procedure. Specimens where then collected by the researchers within 24 hours, and transported in biohazard labeled specimen bags at room temperature to the Department of Medical Microbiology laboratory. They were stored at 4℃ until extraction of DNA within 24-48 hours.

2.2 Genomic DNA Extraction from the Swabs

Genomic DNA from the swabs was extracted using the Quick-gDNA™ MicroPrep Kit (Zymo Research, USA) according to manufacturer's recommendations. Briefly, vaginal swabs were processed in batches of 20. The swabs were individually washed in 500 µl of lysis buffer in 1.5 ml Eppendorf tubes to dislodge cells from the cotton tip surface, followed by vortexing for 5 seconds. The vortexed mixtures were allowed to stand at room temperature for 10 minutes after which they were pipetted into Zymo-Spin IC Columns inserted into collection tubes. The columns were spun at 10000 g for one minute and the collection tubes and flow through discarded. The columns were then placed in clean collection tubes followed by pipetting 200 µl of DNA pre-wash buffer into each column and spun at 10 000 g for one minute. To the spin columns, 500 µl of genomic DNA wash buffer was added and the columns spun at 10 000 g for one minute. DNA was eluted in a total elution buffer volume of 50 µl after incubation at 25-30℃ for 2 minutes, and high speed spinning for 30 seconds. The samples were then stored at -20℃ until PCR amplification.

2.3 HPV DNA Amplification by Consensus Polymerase Chain Reaction

The HPV DNA amplification was performed by polymerase chain reaction (PCR) using consensus primers, MY09/MY011 set (MY09: 5'-CGT CCM ARR GGA WAC TGA TC-3'; MY11: 5'-GCM CAG GGW CAT AAY AAT GG-3') which amplified a 450 bp sequence within the L1 region of the HPV-DNA as well as the GP5+/GP6+ primer set (GP5+: 5'-TTT GTT ACT GTG GTA GAT ACT AC-3'; GP6+: 5'-GAA AAA TAA ACT GTA AAT CAT ATT C-3') which amplified a 150 bp sequence in the same region. The final reaction volume for each sample was 50 µl. A master mix for each PCR run was made while working on ice all the time. Each reaction contained 36.8 µl nuclease free water; 5 µl Taq buffer with Magnesium chloride; 1 µl dNTPs (10 mM); 1 µl Primer 1 (either MY09 or GP5+); 1 µl Primer 2 (either MY11 or GP6+); 0.2 µl Taq Polymerase (1U). To PCR tubes, 45 µl of the master mix was added and 5 µl of DNA template from each specimen to make the 50 µl reactions. The samples were run on the PTC-100™ Programmable Thermal Controller, MJ Research, Inc. The cycler was programmed according to the primers being used. For the MY09/11 primer set, the program used was: initial denaturation 5 minutes at 95°, then 40 cycles with denaturation at 95℃ for 30 seconds; annealing at 50℃ for 30 seconds, extension for 45 seconds at 72℃, and a final 7 minute extension at 72℃. For the GP5+/6+ primer set: initial denaturation was 5 minutes at 95°, 40 cycles with denaturation at 95℃ at 30 seconds; annealing at 44℃ for 30 seconds, extension for 45 seconds at 72℃, and a final 7 minute extension at 72℃. A house-keeping gene, the Trim5α (Trim5alpha) gene was also amplified for all samples. The final reaction volume was 25 µl with the pipetting scheme for the master mixes containing: 15.8 µl nuclease free water; 2.5 µl Taq buffer with MgCl2; 0.5 µl dNTPs (10mM); 0.5 µl Primer 1 (R136Q-F, sequence: 5'-ATG GCT TCT GGA ATC CTG GTT-3'); 0.5 µl Primer 2 (R136Q-R, sequence: 5'-CCC GGG TCT CAG GTC TAT CAT-3'); 0.2 µl Taq Polymerase (1U). Negative controls with master mix and nuclease free water in place of the template were used in every PCR run. The PCR program was an initial denaturation 5 minutes at 95°, 40 cycles of denaturation at 95℃ for 30 seconds; annealing at 50℃ for 30 seconds, extension for 45 seconds at 72℃, and a final 7 minute extension at 72℃. All PCR products were checked by 2.0% agarose gel electrophoresis.

2.4 DNA Sequencing, HPV Genotype Identification and Data Analysis

DNA sequencing of the HPV DNA-positive PCR products was performed at Inqaba Biotech (Pretoria, South Africa) by the Sanger sequencing method on an automated ABI-prism 3100 Genetic Analyser (Applied Biosystems, Califonia, USA). DNA sequences were analyzed using the Geneious Basic program

(Biomatters, USA). Nucleotide sequence searches in public genbanks were done using the NCBI online BLAST tool program(http://www.ncbi.gov/BLAST). Nucleotide sequences were assigned HPV genotypes if the sequence corresponded 100% with a known HPV genotype available in the genbanks. Published literature was used to separate the genotypes into low- and high- risk genotypes upon data analysis. The data were captured, sorted and cleaned in Microsoft Excel 2011. Data were exported to Stata 11 software for both descriptive analysis of the demographic data and bivariate analysis on the diagnostic performance the PCR primers in detecting HPV infection, prevalence and genotyping.

3. RESULTS

3.1 Demographic Data of Student Participants

The study population consisted of a total of 125 young female students who were recruited into the study. Of the 125, 78 (62%) were from the University of Zimbabwe's College of Health Sciences and were MBChB Part III students and the other 47 (38%) were nursing students from the Harare Central Hospital's School of Nursing. All the participants reported being of African descent. The mean age of the students was 22.4 years with age range of 20-25 years. The majority (68.8%) of the students were single, while only 24.8% were married and the remaining ones were either engaged or separated. Of the 125 students, 80 (64.0%) were sexually active and the other 45 (36.0%) reported not having had sex before. Of the 91 respondents on a question concerning number of sexual partners, 35 (38.5%) reported to have zero or one sexual partner and 56 (61.5%) reported to have more than one sexual partners since being sexually active. Thirty-four students did not respond to the question concerning the number of sexual partners they had. Only a few (8 out 91 respondents) of the students reported to have a positive STI infection history. Only 11.3% had previously gone for PAP smear examination for cervical cancer screening.

3.2 HPV DNA Amplification from Swabs

Total genomic DNA was successfully isolated from all the 125 swab samples from the students. To check the quality of DNA preparations, a house-keeping gene, TRIM5α gene was amplified (Fig. 1). The majority of the samples (114 out of 125) showed amplification of the gene. Gel electrophoresis showed the presence of the expected PCR band of 526 bp (Fig. 1). There was no amplification in only 11 (9%) of the 125 DNA preparations. These were excluded from further analysis for the presence of HPV DNA.

The 114 valid samples which tested positive for the Trim5α gene were further tested for the presence of HPV DNA using MY09/11 and GP5+/GP6+ primer sets. Samples that were positive for HPV DNA using the MY09/11 primers showed the presence of an expected 450 bp band on gel electrophoresis (Fig. 2). Samples that were positive for HPV DNA using the GP5+/GP6+ primers showed the presence of an expected 150 bp band on gel electrophoresis (Fig. 3). A summary of the results of HPV DNA amplifications are in Table 1. Briefly, the MY09/11 primer set gave an HPV prevalence of 31.58% (36 out of 114) while GP5+/6+ primer set gave a prevalence of 22.81% (26 out of 114). Overall, a total of 36/114 samples (31.58%) tested positive for HPV-DNA with both MY09/11 and GP5+/6+ primer sets.

Table 1. Detection of genital HPV by two PCR primer sets (MY09/11 and GP5/6) in students (n=114)

Outcome	Frequency (%)
MY09/11 primer set	
Positive	36 (31.58)
Negative	78 (68.42)
Total	114 (100)
GP5+/6+ primer set	
Positive	26 (22.81)
Negative	88 (77.19)
Total	114 (100)
Overall HPV status	
Positive	36 (31.58)
Negative	78 (68.42)
Total	114 (100)

3.3 Genital HPV Genotypes Detected in Medical and Nursing Students

The 36 PCR samples that were positive for HPV DNA by either MY09/11 or GP5+/6+ primer sets were sequenced. Out of these samples, 29 gave sequence data that could be used for identification of HPV genotypes. Analysis of the sequence data showed that 10 out of 29 (34.48%) had infection with a high-risk HPV genotypes; 1 out of 29 (3.45%) with a probable high risk HPV genotypes; 8 out of 29 (27.59%)

were infected by a low-risk HPV genotypes; 2 out of 29 (6.90%) were infected by HPVs of unknown or not yet well-characterized genotypes. Mixed infections with either a high-risk or low-risk genotype were found in 13 samples. The 17 known HPV genotypes detected in the students were HPV 81, 53, 6, 90, 72, 40, 11, 86, 54, 16, 68, 45, 18, 58, 35, 56 and 67. Three samples had HPV sequences that could not align 100% with any genotype sequences in the Genbanks. Of the total (17 known and 3 unknown), 7 out of 20 (35%) were high-risk (HPV genotypes 16, 18, 35, 45, 58, 53, 56), 8 out of 20 (40%) were low risk (HPV genotypes 6, 11, 40, 53, 54, 72, 81, 86) 1 out 20 (5%) was probable high-risk (HPV genotype 67), 1 out of 20 (5%) was probable low-risk (HPV genotype 90), and 3 out of 20 (15%) were of unknown HPV genotypes. Probable high- or low-risk genotypes are those that have not been classified into either category but assumed to be either while more research is being carried out around them.

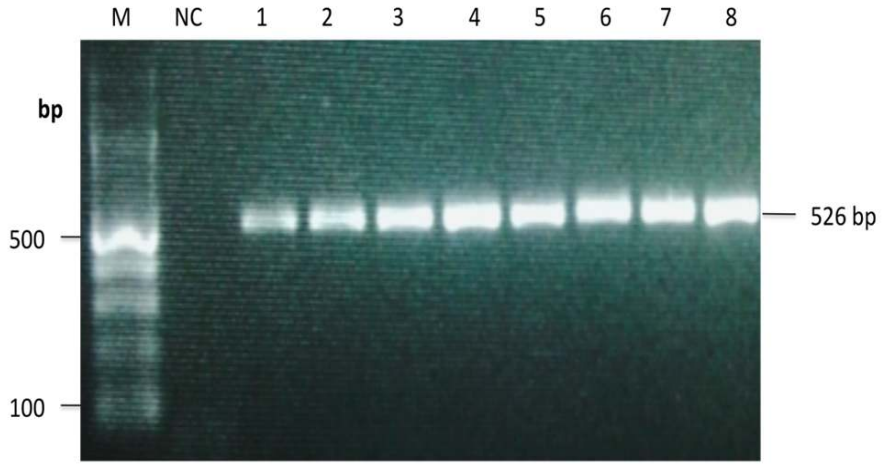

Fig. 1. A representative gel photograph showing amplification of a house-keeping gene, TRIM5α gene from swabs

Lane M: DNA marker, NC: polymerase chain reaction negative contro, Lanes 1-8: successfully amplified Trim5α gene

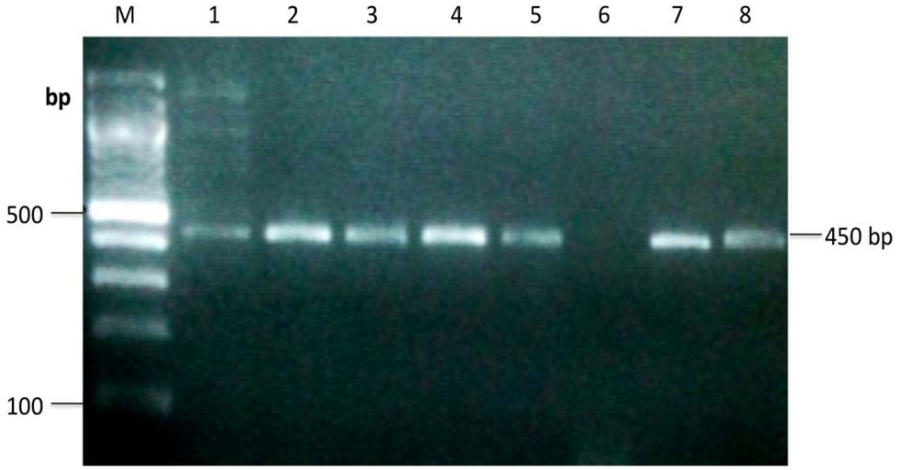

Fig. 2. A representative gel showing amplification of HPV DNA from swabs using MY09/11 primer sets

Lane M: DNA marker, Lanes 1-5, 7 and 8: amplified HPV DNA, Lane 6: no amplification of HPV DNA

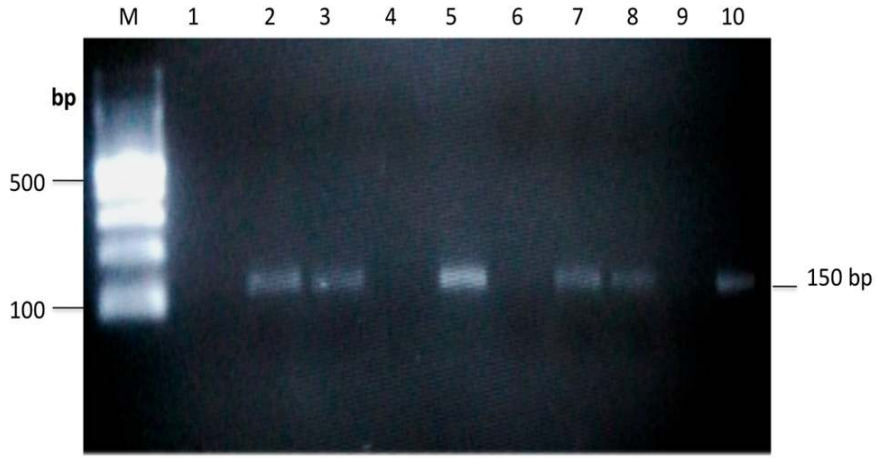

Fig. 3. A representative gel showing amplification of HPV DNA from swabs using GP5+/GP6+ primer sets

Lane M: DNA marker, Lanes 2,3,5,6,7 and 10: amplified HPV DNA, Lanes 1,4,6 and 9: no amplification of HPV DNA

4. DISCUSSION

Genital HPV infections are very common in sexually active individuals [12]. Most epidemiological studies on the prevalence of these viral infections have only been done in old women especially those with cervical cancer. Data on genital HPVs in adolescents and young women are still very scarce. Data on the prevalence of genital HPVs in young and health college students are also limited. In this study, we therefore set out to investigate the prevalence of genital HPV in young female medical and nursing students in Harare, Zimbabwe. Analysis of data from the administered questionnaire showed that more than half of the participants reported being sexually active with some of the subjects having more than one sexual partner. Overall, sexual activity was high in the students and hence the risk of HPV infection was also high. This aspect was investigated in our study subjects because exposure to multiple partners is a well-known risk factor for genital HPV infection [13]. HPV is known to be one of the most common sexually transmitted viruses on university or college campuses, hence its presence can be used as a biological marker of sexual behaviour among students [11,14]. It has also been established that unprotected penetrative intercourse, especially with multiple partners is a behavioral risk for HPV infection [15]. There was also a low level of HPV screening with the majority of the participants never having had a Pap smear examination. This was expected since most of participants were still young and healthy and did not expect to have cervical cancer which takes a long time to develop after persistent infection with HPV.

Polymerase chain reaction (PCR) is a molecular method which is increasingly being used to detect HPV. In this study, we first checked the quality of genital samples collected from students by amplifying a house-keeping human gene. The fact that the house-keeping gene amplified in most samples (Fig. 1) showed that there were sufficient cells self-collected by the students for HPV DNA analysis [16]. PCR of the L1 region of the HPVs was targeted using the consensus HPV-specific primers (MY09/11 and GP5+/6+ sets. In this study, a greater number of positive samples were observed using MY09/11 primers when compared to GP5+/6+ primers. The MY09/11 primers are degenerate and can therefore pick more HPV genotypes than the GP5+/GP6+ primers which are not degenerate and therefore miss some genotypes. The current study showed an overall HPV-DNA prevalence of 31.58% which was considered to be high. The students were infected with both high-risk and low-risk HPV genotypes, some of which are known to cause cervical and vaginal cancers as well as genital warts. Other studies conducted elsewhere have also shown a high prevalence of HPVs in young women such as female college students. A cross-sectional study in Honduras in female university students aged 18-35 years showed HPV DNA prevalence of 45% and 73% of those infected had high-risk HPV types [17]. In the study, 36 HPV genotypes were detected in the students and the most prevalent ones were HPV types 16, 51, 84, 66, and 39 [17]. A study in

South Korea in sexually active students showed a higher prevalence of genital HPV in females (38.8%) than in males (10.6%) [18]. In the study, having multiple sexual partners and smoking were risk factors of HPV infection in female students [18]. A cross-sectional study of female students (18-35 years of age) in Brazil showed a prevalence of HPV DNA in self-collected vaginal swabs to be 28.9% and the most prevalent genotypes were HPV types 6, 16, 31, 33, 45 and 83 [19]. A study in Finland showed an HPV DNA prevalence of 33.0% in self-collected vaginal swabs in female university students [20]. Of the positive samples, 84.3% had high-risk HPV genotypes [20]. A study in young women who were not students in Kwazulu-Natal in South Africa (mean age 21 years) showed a high HPV DNA prevalence of 76.3% and most of them had high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58) [21]. The variations in the prevalence of HPV genotypes in different parts of the world could be due to methods used collecting samples and in detecting the HPV DNA in the laboratory as well as difference in sexual behavioural and biological factors of different human populations. Although both high-risk and low-risk HPV genotypes are found in sexually active female college students, most infections are likely to be transient and will not cause disease. It is only the persistent infection with certain HPV genotypes that causes genital cancers and warts [22].

Our study was the first to evaluate the prevalence of genital HPV, a biological marker of sexual activity, in young female college students in Zimbabwe. More studies that look at the biological markers of sexual activities in students and young people may also need to be conducted at other colleges and universities in Zimbabwe. The data generated could be used by various stakeholders in designing sexual and reproductive public health policies for adolescents and young women at colleges and universities in Zimbabwe.

5. CONCLUSION

Prevalence of high-risk human papillomavirus (HPV) was found to be high in young sexually active female university and college students in Harare, Zimbabwe. Further epidemiological studies on HPV prevalence, with increased sample sizes, are required in Zimbabwe. Data generated will be useful in designing public health policies especially on prevention of sexually transmitted diseases and anogenital cancers.

CONSENT AND ETHICAL APPROVAL

The study was approved by the Joint Research Ethics committee of Parirenyatwa Hospital and the University of Zimbabwe College of Health Sciences (JREC) (Number JREC/41/14) and the Medical Research Council of Zimbabwe (MRCZ) (Number MRCZ/B/649). The experiments were performed in accordance with the ethical standards laid down by the committee and council. All students signed informed consent forms. As per international or JREC/MRCZ standards, patient's written consent has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17565