IJIMM, Volume 3, Issue 7, 2025 ISSN: 2995-5319
http://medicaljournals.eu/index.php/IJIMM/issue/view/3

INTERNATIONAL JOURNAL OF INTEGRATIVE AND MODERN MEDICINE

Prevalence of Human Papillomavirus and Herpes Simplex Virus-2 Infections among Young Female Students in Oyo State

Oduola Aderonke Aminat
Department of Virology, College of Medicine, University of Ibadan

Alimi Grace O. Annabelle
University of Ibadan

Ayinde Abayomi. O
Public Health Epidemiology

Annotation: Introduction: Human papillomavirus (HPV) and herpes simplex virus type 2 (HSV-2) infections among young female students have become a significant public health concern primarily due to their association with the development of certain cancers, such as cervical, anal, and oropharyngeal cancers.

Objective: This study aimed to determine the prevalence of HPV and HSV-2 infection and explore potential risk factors among this population.

Method of Analysis: A cross-sectional study was conducted among a sample of young female students aged 16 to 25 years residing at Queen Idia Hall, University of Ibadan. The study was approved by the University of Ibadan/University College Hospital Institutional Review Committee (UI/UCH IRC). Data were collected through structured interviews. Vaginal swab sample was collected from 232 study participants. The study was carried out at the department of Virology, UCH, Ibadan. HPV and HSV-2 DNA were detected using polymerase chain reaction (PCR).

Results: HPV DNA was not detected while a low prevalence (6%) of HSV-2 infection was observed among the young female students. Several risk factors were associated with the acquisition of HPV and HSV-2 infections. These included early sexual debut, multiple sexual partners and unprotected sexual intercourse.

Conclusion: The findings of this study indicate absence of HPV infection among the young female students. These results underscore the importance of implementing preventive measures, specifically HPV vaccination, among this particular age group. Given the limited exposure to the virus observed in this study, extending the age range for HPV vaccination is warranted to provide protection against future HPV infection. Efforts should also be directed towards increasing access to screening and early detection of HPV and HSV-2 infections among this population. There is need for comprehensive sexual health education programs targeting young female students, promoting safe sexual practices, and increasing awareness about HPV vaccination.

Keywords: HPV, HSV-2, Infection, Cervical cancer, Vaccination, young female students, University of Ibadan.

Introduction

Human papillomavirus (HPV) is a non-enveloped double-stranded DNA virus that infects squamous epithelia of the skin and mucosae, with over 200 types identified [1]. Certain high-risk types of genital HPV, such as HPV-16 and HPV-18, have been found to promote the disruption of normal cell-cycle control, targeting the retinoblastoma (Rb) family of proteins, and p53 and inducing telomerase, leading to the development of malignancy [2]. High-risk HPV types such as HPV-16, -18, -31, -33, and -35 have been linked to various precancerous lesions and malignant tumors, especially cervical cancers,

with one or more of these types found in over 90% of women diagnosed with cervical cancer [3]. Genital warts with low malignant potential are associated with HPV-6 and HPV-11.

When HPV infects a cell, its DNA integrates into the host cell genome, producing proteins necessary to commandeer the DNA synthesis machinery of the host cell. Two viral genes, E6 and E7, act as oncogenes by binding to the protein products of important tumor suppressor genes, p53 and RB, respectively, blocking their actions, and allowing the cell to grow and divide [4]. HPV infection is widespread, with an estimated incidence of 1% in sexually active adults and 3% in sexually active adolescents in the US [5]. Globally, the prevalence of cervical HPV infection in women with non-cancerous cytology was estimated to be 11.7% in 2016, with HPV-16, HPV-18, HPV-31, and HPV-58 being the most frequent types [6]. Cervical cancer is the fourth most common cancer in women worldwide, with HPV type 16 being responsible for 50% of cervical cancer cases and types 16 and 18 accounting for 70% of cases [6]. In addition to cervical cancer, HPV has also been linked to other malignancies, including anal, vaginal, vulvar, and oropharyngeal cancers, with HPV being the cause of 99% of cervical cancers, 90% of anal cancer, 65% of vaginal cancers, 50% of vulvar cancers, and 45-90% of oropharyngeal cancers [7] [8] [9]. According to the Center for Disease Control and Prevention, approximately 80% of women and 90% of sexually active men will have been infected with at least one HPV type at some point in their lives [10]. Anogenital verruca is the most common sexually transmitted disease in the United States, affecting 1% of sexually active adults and 3% of sexually active teenagers, with both genders being susceptible, with 67% of patients being women [5]. HPV infection is highly prevalent in Africa, with Nigeria being one of the countries burdened by a high HPV prevalence rate. Studies have shown a high prevalence of HPV infection among women in Nigeria, ranging from 20% to 47% depending on the region and population studied [11] [12]. Nigeria has a population of approximately 60.9 million women aged 15 years and older who are at risk of cervical cancer, the burden of the disease is substantial. Annual estimates indicate that 12,075 women receive a cervical cancer diagnosis, and 7,968 lose their lives to this condition. Cervical cancer stands as the second most prevalent cancer among Nigerian women and the second most common cancer among females aged 15 to 44 years. Within the general population, an estimated 3.5% of women harbor cervical HPV-16/18 infection at any given time, and an overwhelming 66.9% of invasive cervical cancers are attributed to HPVs 16 or 18 [13].

The Herpesviridae family is a large group of DNA viruses that can cause infections and various diseases in animals, including humans [14]. It is estimated that around 491 million individuals are infected with HSV-2 globally, with variations in prevalence rates across different populations and geographic regions [15]. HSV-2 is primarily transmitted through sexual contact, including vaginal, anal, and oral sex. The virus can be transmitted even in the absence of visible symptoms or lesions, known as asymptomatic shedding, which contributes to its high transmission potential. Direct contact with the mucosal surfaces or skin areas that are shedding the virus facilitates its transmission [16]. HSV-2 infection commonly leads to the development of genital herpes, characterized by painful blisters or ulcers in the genital area. While genital herpes can cause significant discomfort and distress, it is important to note that many individuals infected with HSV-2 may experience mild or no symptoms at all [17].

In recent years, research has highlighted the interplay between HSV-2 and human papillomavirus (HPV) infection, particularly in the context of cervical cancer. HPV is a highly prevalent STI and a major cause of cervical cancer, which is one of the leading cancers affecting women worldwide [18]. When HSV-2 co-infection occurs with HPV, it can potentially exacerbate the progression of HPV infection and increase the risk of cervical cancer [19]. The synergistic effect of HSV-2 on HPV infection and cervical cancer progression is thought to be multifactorial. HSV-2 infection can compromise the immune response, leading to a reduced ability to control HPV infection and increasing the likelihood of persistent HPV infection. Persistent infection with high-risk HPV types is a necessary factor for the development of cervical cancer [20].

Furthermore, HSV-2 infection can induce local inflammation and create a microenvironment that facilitates HPV replication and persistence. This inflammatory response may also lead to the disruption

of the cervical epithelial barrier, allowing HPV to enter deeper into the cervical tissue and promoting the development of cervical cancer [21]. The co-infection of HSV-2 and HPV has been associated with an increased risk of high-grade cervical intraepithelial neoplasia (CIN), a precancerous condition, and cervical cancer. It is crucial to consider the potential synergistic effects of these two infections in designing preventive strategies, early detection, and management of cervical cancer [22]. In this study, we investigated the prevalence of Human Papillomavirus and Herpes Simplex Virus-2 infections among young female students in Oyo State.

Materials and methods

Study Location

This is a cross sectional study aimed at the molecular detection and prevalence of Human Papillomavirus and Herpes Simplex Virus-2 Infection among young female students attending University of Ibadan. This study was approved by the University of Ibadan/University College Hospital Institutional Review Committee (UI/UCH IRC) with research approval number UI/EC/21/0589. The study was carried out at the Department of Virology, University College Hospital, Ibadan. Socio-demographic data and samples were collected from 232 consenting young female students attending University of Ibadan, Oyo State. Only consenting young female students in Oyo State within the age range of 16-25years were recruited in the study. Vagina swab sample was collected from consented individuals using self-sampling method into 500ul of virus transport medium (VTM) and transported into the lab while maintaining cold chain. The transport medium containing the swabs were vortexed rigorously in the lab, the medium was then aliquoted and stored at -20^{0}C until analysis.

Laboratory Analysis

Nucleic acid extraction

Genomic DNA was extracted from each of the samples using Da An Gene extraction kit according to the manufacturer's instructions.

Polymerase Chain Reaction (Amplification of Human Papillomavirus DNA)

The extracted DNA was subsequently used as a template for the detection of Human Papillomavirus DNA by PCR. The consensus region of the HPV DNA was amplified by PCR using primers targeting the conserved sequence in the HPV L1 gene [one forward primer MY09 (5'-CGTCCMARRGGAWACTGATC-3') and one reversed primer MY11 (5'-GCMCAGGGWCATAAYAATGG-3')] as described by [23]. The cycling conditions for PCRs with MY-09/11 consensus primers were preceded by an initial denaturation step at 95°C for 10 min, followed by 40 amplification cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min. The last cycle was followed by a final elongation step at 72°C for 7 min. All PCRs were performed in a final volume of 15µL reaction mix containing 3µL of the extracted DNA, 12µL of a premix of PCR buffer, dNTPs, Magnesium chloride and Taq Polymerase enzyme in optimized concentration (Jena Bioscience, Germany), and 1 µL of each primer. The amplified HPV DNA was detected by electrophoresis on 2% agarose gel and visualised using Bio-Rad Gel Doc™ XR+System. The size of the PCR products that should be generated with MY-09/11 consensus primers used is 450bp.

Another set of primers (GP-E6/E7) were used on the same DNA extracted product for further investigation. The consensus region of the HPV DNA was amplified by PCR using primers targeting the E6/E7 gene region [one forward primer GP-E6-3F (5'-GGGWGKKACTGAAATCGGT-3') and two back primers GP-E6-5B (5'-CTGAGCTGTCARNTAATTGCTCA-3')and GP-E6-6B (5'-TCCTCTGAGTYGYCTAATTGCTC -3')] as previously described by [24]. The cycling conditions for PCRs with GP-E6/E7 consensus primers were preceded by an initial denaturation step at 95°C for 5 min, followed by 45 amplification cycles of 95°C for 30 s, 45°C for 30 s, and 68°C for 45 s. The last cycle was followed by a final elongation step at 72°C for 7 min. All PCRs were performed in a final volume of 15µL reaction mix containing 3µL of the extracted DNA, 12µL of a premix of PCR buffer,

dNTPs, Magnesium chloride and Taq Polymerase enzyme in optimized concentration (Jena Bioscience, Germany), and 0.5 µL of each primer. The amplified HPV DNA was detected by electrophoresis on 2% agarose gel and visualised using Bio-Rad Gel Doc™ XR+System. The size of the PCR products that should be generated with GP-E6/E7 consensus primers used is 602-666bp.

Visualization of the amplified DNA using gel electrophoresis

The gel tray was placed accordingly into the gel tank and filled up with TBE running buffer. 4µl of amplicon was loaded into each well alongside the appropriate molecular ladder, positive and negative controls. The agarose gel was subjected to electrophoresis following manufacturer's instruction for the electrophoresis apparatus. The tank was covered up and the gel was allowed to run at 120volts for 30mins to allow the migration of the nucleic acids across the matrix of the agarose from the negative to the positive and this forms distinct bands that can be read under the biorad transilluminator. Samples were run until the pink dye (~25 bp) had migrated at least 2/3 of the way through the gel. A band at 450bp and 660bp was considered a positive depending on the primers used for amplification. The negative processing control and no template control lacked amplification. Positive and negative results were confirmed with repeated assays.

Polymerase Chain Reaction (Amplification of Herpes Simplex Virus-2 DNA)

The extracted DNA was subsequently used as a template for the detection of Herpes simplex virus-2 DNA by PCR. Detection of HSV-2 was performed using a sensitive PCR protocol targeting the UL28 gene region of the viral genome. The consensus region of the HSV-2 DNA was amplified by PCR using primers targeting the conserved sequence in the HSV-2 UL28 gene [one forward primer H2M40 (5'-GTACAGACCTTCGGAGG-3') and one reversed primer H2P4 (5'-CGCTTCATCATGGGC-3')] as described by [25]. The cycling conditions for PCRs with the consensus primers were preceded by an initial denaturation step at 95°C for 5 min, followed by 45 amplification cycles of 95°C for 30 s, 52°C for 40 s, and 72°C for 50 s. The last cycle was followed by a final elongation step at 78°C for 15 min. All PCRs were performed in a final volume of 25µL reaction mix containing 5µL of the extracted DNA, 18µL of a premix of PCR buffer, dNTPs, Magnesium chloride and Taq Polymerase enzyme in optimized concentration (Jena Bioscience, Germany), and 1 µL of each primer. The amplified HSV-2 DNA was detected by electrophoresis on 2% agarose gel and visualised using Bio-Rad Gel Doc™ XR+System. The size of the PCR products that should be generated with H2M40 and H2P4 consensus primers used is 227bp.

Statistical analysis

Data obtained were analyzed using the Statistical Package for Social Sciences (SPSS) program Version 20 (Chicago, IL, USA). Analyses were carried out using descriptive statistics with mean and standard deviation (SD).

Results

Table 1. Sociodemographic characteristics of study group

Variables	Number of participants tested	Percentage(%)
Gender		
Female	232	100.0
***Age-group (years)**		
16-20	147	63.4
21-25	85	36.6
Marital status		
Single	232	100
Married	0	0
Educational level		
Primary	0	0

Secondary	0	0
Tertiary	232	100
Are you sexually active		
Yes	44	19
No	188	81
Number of sexual life partners	40	91
One	4	9
More than one		
Use of condom during sexual intercourse		
Yes	33	75
No	11	25
Have you ever had STI?		
Yes	0	0
No	232	100
Do you smoke		
Yes	0	0
No	232	100
Result of the Analysis		
Positive	0	0
Negative	232	100

*Mean±SD for age:

20.17±2.32

A total of 232 female students aged 16-25 were recruited in this study. This study investigated the prevalence of HPV and HSV-2 infections among young female students in Oyo State. The demographic characteristics of the study participants are presented in the table 4.1 below. The age range of the enrolled population ranges from 16-25 years, with a mean age (± SD) of 20.17 ± 2.32years. Majority (63.4%) of the study participant were within age group 16-20 years. The study participants were all females 232(100%). The table 1 shows the age group distribution of the participant. Two hundred and thirty-two samples were tested for HPV, 232(100%) females. Of the 232 tested samples, 0(0%) were positive for Human Papillomavirus, giving low prevalence. The marital status of all the study participants were single 232(100%) with educational level been tertiary level 232(100%). 44(19%) of the study population were sexually active while 188(81%) were not sexually active. The table shows that among the sexually active group, 40(91%) has one sexual partner while 4(9%) has more than one sexual partner. The table also illustrates the responses on the use of condom among the sexually active group of the study population, 33(75%) responded “Yes” to the use of condom while 11(25%) responded “No” to the use of condom.

Table 2. Prevalence of HSV-2 among study groups

	HSV-2 DNA			
Variables (n=232)	**Positive (%)**	**Negative (%)**	**χ^2**	**p-value**
Age-group (years)				
16-20	2 (14.0)	145 (67.0)	32.479	<0.01
21-25	12 (86.0)	73 (33.0)		
Are you sexually active				
Yes	14 (100.0)	30 (14.0)	63.385	<0.01
No	0 (0)	188 (86.0)		
No of partner				

1	14(100.0)	26(87.0)	71.516	<0.01
>1	0(0)	4(13.0)		
Use of condom				
Yes	5(36.0)	28(93.0)	70.423	<0.01
No	9(64.0)	2(7.0)		

*Statistically significant at p<0.05

Table 2 presents the prevalence of Herpes Simplex Virus Type 2 (HSV-2) DNA among the study participants (n = 232) based on selected socio-demographic and behavioural variables. The distribution of HSV-2 infection differed significantly across age groups, with a higher proportion of positive cases observed among individuals aged 21–25 years (86.0%) compared to those aged 16–20 years (14.0%) ($\chi^2 = 32.479$, $p < 0.01$). Sexual activity was strongly associated with HSV-2 positivity; all HSV-2 positive individuals (100.0%) reported being sexually active, while none of the sexually inactive participants tested positive ($\chi^2 = 63.385$, $p < 0.01$). Regarding the number of sexual partners, HSV-2 prevalence was higher among individuals with multiple partners (64.0%) than those with a single partner (36.0%), and this association was statistically significant ($\chi^2 = 71.516$, $p < 0.01$). Similarly, condom use showed a significant relationship with HSV-2 infection; all individuals who tested positive for HSV-2 (100.0%) reported not using condoms during sexual intercourse, whereas no positive cases were recorded among condom users ($\chi^2 = 70.423$, $p < 0.01$). These findings suggest that younger age, sexual activity, multiple sexual partners, and non-use of condoms are significantly associated with HSV-2 infection among the study population.

Fig. 1. Agarose Gel Electrophoresis Image Showing Amplified Products of the Human Papillomavirus DNA

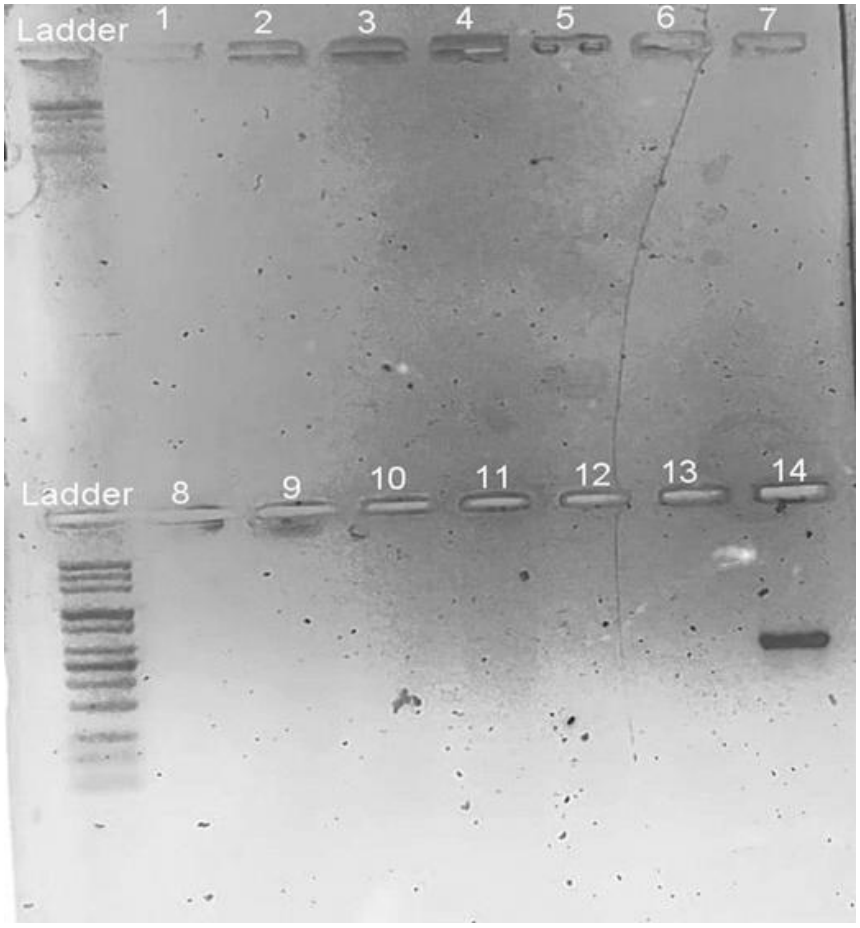


Figure 1 shows the PCR amplification results of DNA extracted from the samples collected from study participants, alongside appropriate controls. The expected amplicon size was 450 base pairs (bp). Lanes 1–12 represent negative samples, Lane 13 contains the negative control, while Lane 14 contains

the positive control, which shows a distinct band at the expected 450 bp. A mid-range DNA ladder was used as the molecular weight marker. All 232 samples tested were negative for Human Papillomavirus (HPV) DNA by PCR, as indicated by the absence of bands at the target size, resulting in an overall HPV prevalence of 0% (0/232).

Fig. 2. Agarose Gel Electrophoresis Image Showing Amplified Products using beta micro globin to confirm DNA extraction Internal Primer

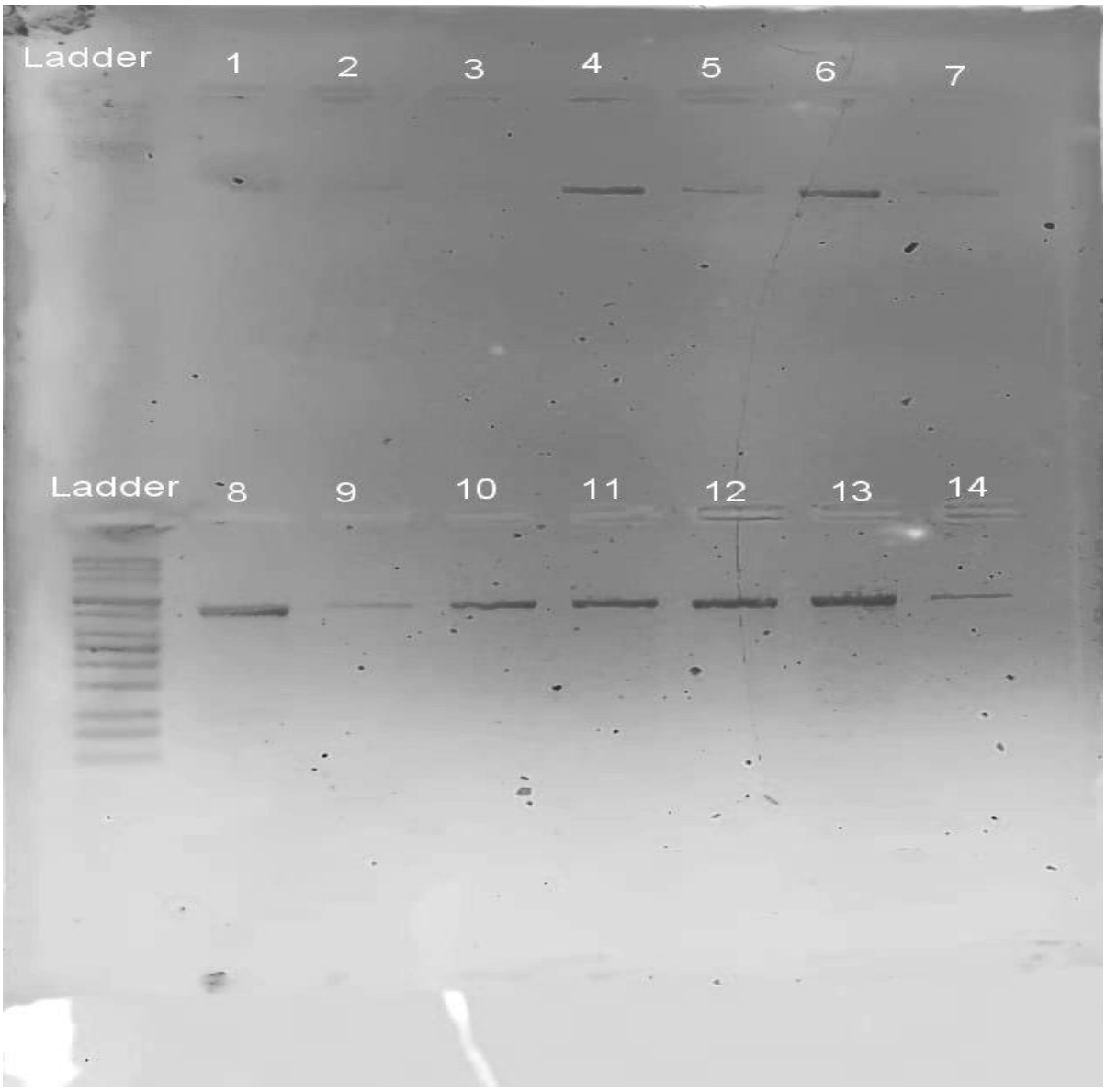


Figure 2 illustrates the PCR amplification of DNA extracted from study participants' samples using internal control primers, with an expected amplicon size of 500 base pairs (bp). Lanes 2–16 confirm the presence of the internal gene, as evidenced by distinct bands at the 500 bp position. A mid-range DNA ladder was used as the molecular weight marker. The successful amplification in these lanes indicates the presence and integrity of the extracted DNA in the tested samples, validating the quality and suitability of the DNA for further molecular analysis.

Fig. 3. Agarose Gel Electrophoresis Image Showing Amplified Products of the Herpes simplex virus-2 DNA

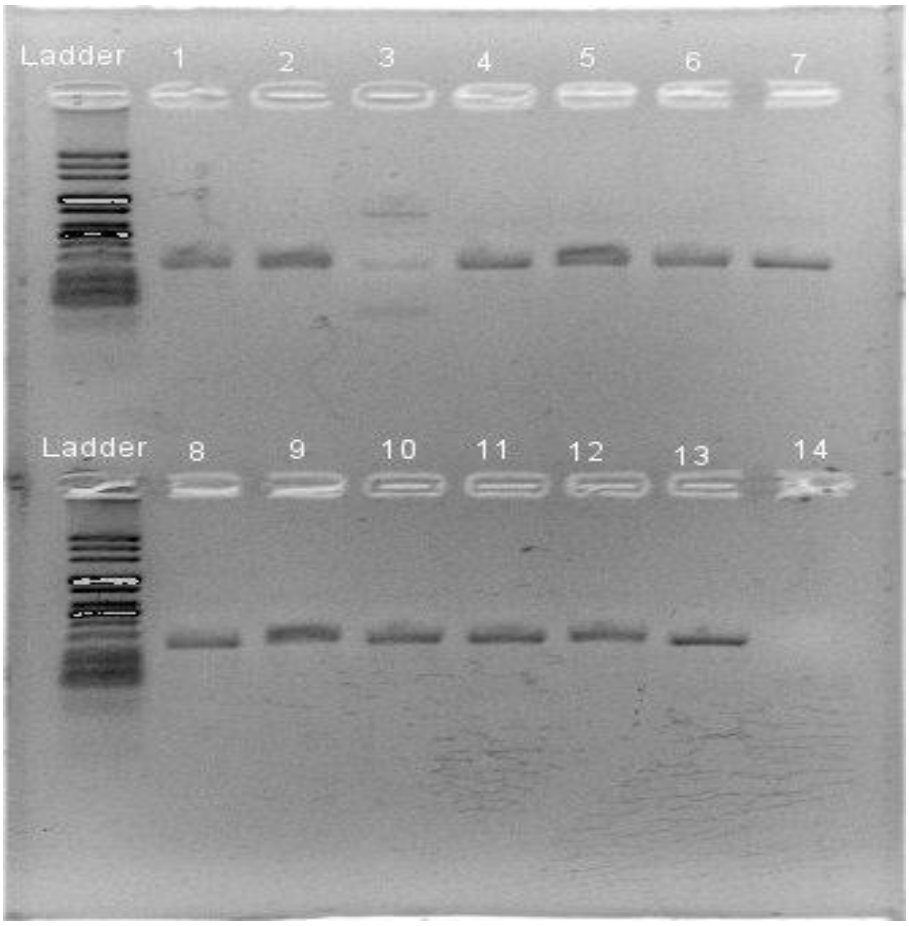
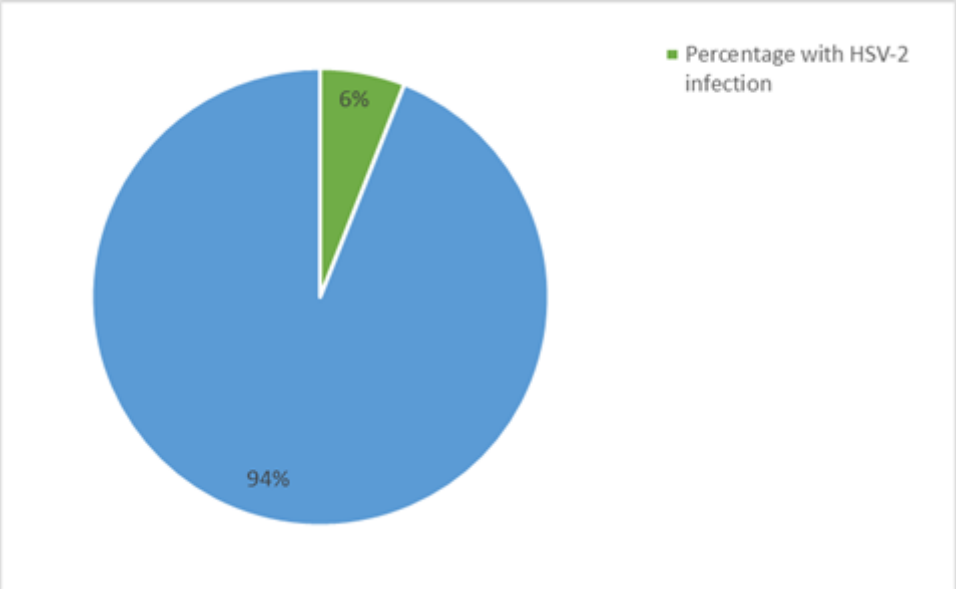


Figure 3 shows the PCR amplification of Herpes Simplex Virus Type 2 (HSV-2) DNA from selected positive samples, with an expected amplicon size of 227 base pairs (bp). Lanes 1–12 represent HSV-2 positive samples, Lane 13 contains the positive control, and Lane 14 contains the negative control. A mid-range DNA ladder was used as the molecular weight marker. Distinct bands at 227 bp in the positive samples and positive control confirm the presence of HSV-2 DNA.

Figure 4. Pie chart showing the prevalence of HSV-2 among study participant



Pie chart illustrating the prevalence of Herpes Simplex Virus Type 2 (HSV-2) among study participants. Out of 232 samples tested, 14 (6.0%) were positive for HSV-2 by PCR.

Discussion

This study aimed to investigate the prevalence of human papillomavirus (HPV) and Herpes simplex virus 2 (HSV-2) infection among young female students in a tertiary institution in Oyo State. Two sets of HPV primers were used to test for the presence of HPV DNA in 232 samples, and no HPV was detected. This finding indicates no prevalence of HPV infection among this population compared to the adjusted global HPV prevalence of 10.4%, 11.7% and 18.6% reported in previous studies by [26] [27]and [28] respectively. It is suggested that the absence of HPV DNA in this study is due to increased knowledge about HPV infection among the female undergraduate students and the lack of identified risk factors for HPV infection in this study population. The level of study of the students was significantly associated with knowledge of HPV infections, with students in higher levels of study having greater exposure to information about HPV. This finding is consistent with previous research that has shown an association between higher education levels and greater knowledge of HPV [29]. Furthermore, the majority of the study population 188(81%) reported not being sexually active, and among those who were sexually active 44(19%), condom use was reported by 33(75%) of the sexually active participants. From this study only 4(9%) among the sexually active participants have more than one sexual partner. These findings suggest that the absence of HPV infection in this population is due to a lower frequency of sexual activity and the use of condom during sexual intercourse. This is consistent with previous studies that have identified unprotected sexual intercourse and multiple sexual partners as significant risk factors for HPV infection [30].

This study suggests that early education about HPV infection and cervical cancer should be provided to female children in primary schools, culminating in the administration of the HPV vaccine before entering secondary school. This strategy could potentially reduce the risk of HPV infection and associated cervical cancer among young women in Nigeria. The study also tested for HSV-2 among the study participants using PCR technique with HSV-2 specific primers. Among the 232 students screened, 14 individuals (6%) tested positive for HSV-2, as depicted in Figure 9. The remaining 218 students (94%) were found to be negative for HSV-2 infection. Table 4.2 presents the distribution of HSV-2 occurrence based on factors such as age group, sexual activity, condom use, and number of sexual partners among the study participants. Significant differences ($P<0.05$) were observed in HSV-2 occurrence with respect to these variables. Notably, the findings of this study differ from previous research. While the prevalence rate of HSV-2 infection in this study (6%) is higher than the rates reported by [31] in women in Iraq (3.2%), it is considerably lower than the rates reported by [32] among HIV co-infected patients in Gwagwalada, Nigeria (36.4%) and Naga *et al.,* 2015 in Eastern India (61.5%). Furthermore, the prevalence of HSV-2 in this current study (6%) is significantly lower than the rate of 31.5% reported by [34] among women attending routine cervix care clinics in Ghana.

Differences observed between the prevalence rates reported in this study and previous studies can be attributed to differences in cultural and socio-economic backgrounds and geographic locations of the study participants. The lower prevalence rate found in this study compared to previous research may be attributed, in part, to the fact that a majority of the participants (81%) reported being virgins and having no sexual partners or engaging in sexual intercourse. Since HSV-2 is primarily transmitted through sexual contact, individuals who are not sexually active are less likely to have detectable HSV-2 DNA. Abstinence has been well-established as a 100% effective measure for controlling and preventing all types of sexually transmitted infections, including HSV-2. Regarding age distribution, the study revealed a significantly higher prevalence of HSV-2 infection among participants aged 21-25 years compared to other age groups examined. This age range corresponds to a period of heightened sexual activity, particularly among undergraduate female students who may engage in frequent sexual intercourse in exchange for money or academic benefits, thus making them more vulnerable to sexually transmitted diseases, including HSV-2 infection. In industrialized countries, approximately 15% to 30% of sexually active adults have HSV-2 infection, with the risk factors based on the number of sexual partners. This study identified certain risk factors associated with the prevalence of HSV-2,

including lack of knowledge or awareness about the infection, increasing numbers of sexual partners, and engaging in unprotected sex. These findings align with previous research by [35], who reported similar risk factors. In epidemiology, knowledge and information play a crucial role in disease prevention and control. A significant proportion of participants in this study demonstrated a lack of awareness regarding HSV-2 and its associated infection, highlighting the need for enhanced public awareness initiatives targeting the study population.

Conclusion

This study has demonstrated the absence of Human papillomavirus (HPV) infection among young female students. The low prevalence of HPV infection in this population suggests that there is a need to promote adequate knowledge and understanding of the risk factors associated with HPV infection as an approach to prevent the infection. To increase awareness of HPV infection and cervical cancer, education on these topics should be integrated into the curriculum of all primary, secondary and tertiary institutions in Nigeria. Parents and caregivers should be encouraged to ensure their children receive HPV vaccination before they become sexually active.

References

1. Ryndock, E. J., and Meyers, C. 2014. A risk for nonsexual transmission of human papillomavirus?. *Expert review of anti-infective therapy*, 12(10), 1165-1170.
2. Helt, A. M., and Galloway, D. A. 2001). Destabilization of the retinoblastoma tumor suppressor by human papillomavirus type 16 E7 is not sufficient to overcome cell cycle arrest in human keratinocytes. *Journal of Virology*, 75, 6737-6747.
3. Bernard, H. U., Burk, R. D., Chen, Z., van Doorslaer, K., Hausen, H. Z., and de Villiers, E. M. 2010. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Journal of Virology*, 401(1), 70–79.
4. Roman, A., and Munger, K. 2013. The papillomavirus E7 proteins. *Virology*, 445(1-2), 138-168.
5. Fathi, R., and Tsoukas, M. M. 2014. Genital warts and other HPV infections: Established and novel therapies. Clin Dermatol, 32, 299-306.
6. La Rosa, G. 2016. Papillomavirus. In J. B. Rose and B. Jiménez Cisneros (Eds.), Part 3 Viruses (Meschke, J. S. and Girones, R. (Eds.), Global Water Pathogen Project). *Michigan State University.* http://www.waterpathogens.org/book/papillomavirus
7. Gallay, C., Miranda, E., Schaefer, S., Catarino, R., Jacot Guillarmod, M., Menoud, P. A., Guerry, F., Achtari, C., Sahli, R., and Vassilakos, P. 2016. Human papillomavirus (HPV) contamination of gynaecological equipment. *Sexually Transmitted Infections*, 92, 19-23.
8. Boda, D., Neagu, M., Constantin, C., Voinescu, R. N., Caruntu, C., Zurac, S., Spandidos, D. A., Drakoulis, N., Tsoukalas, D., and Tsatsakis, A. M. 2016. HPV strain distribution in patients with genital warts in a female population sample. *Oncology Letters*, 12, 1779–1782.
9. You, W., Li, S., Du, R., Zheng, J. and Shen, A. 2018. Epidemiological study of high risk human papillomavirus infection in subjects with abnormal cytological findings in cervical cancer screening. *Experimental and Therapeutic Medicine,* 15, 412-418. https://doi.org/10.3892/etm.2017.5424
10. Chesson, H. W., Ekwueme, D. U., Saraiya, M., Dunne, E. F., and Markowitz, L. E. (2011). The cost effectiveness of male HPV vaccination in the United States. Vaccine, 29(46), 8443-8450. https://doi.org/10.1016/*journal of vaccine*.2011.08.114
11. Ojiyi, E. C., Dike, E. I., Okeudo, C. 2018. Prevalence and predictors of high-risk human papillomavirus infection among women attending routine cervical cancer screening in southeastern Nigeria. *Infect Agent Cancer*, 13, 32. https://doi.org/10.1186/s13027-018-0204-0

12. Onyekwuluje, J. M., Ogunwale, A. B., Oluwasola, O. A., Fasola, F. A., and Ayoola, O. O. 2020. Prevalence of Human Papillomavirus and its genotypes among women in Nigeria. *J Med Virol*, 92(11), 3343-3349. https://doi.org/10.1002/jmv.26192

13. ICO/IARC HPV Information Centre, 2023. Institut Català d'Oncologia Avda. Gran Via de l'Hospitalet, 199-20308908 L'Hospitalet de Llobregat (Barcelona, Spain). *info@hpvcentre.net.*

14. Ryan, K. J., and Ray, C. G. 2004. Sherris Medical Microbiology (4th ed.). McGraw Hill.

15. James C., Harfouche, M., Welton, N. J, Turner, K. M., Abu-Raddad, L. J, Gottlieb, S. L., Looker, K. J. 2020. Herpes simplex virus: global infection prevalence and incidence estimates, 2016. Bull World Health Organ. May 1;98(5):315-329. doi: 10.2471/BLT.19.237149. Epub 2020 Mar 25. PMID: 32514197; *PMCID:* PMC7265941.

16. Schiffer, F., Johnston, A. L., Ravichandran, C., Polcari, A., Teicher, M. H., Webb, R. H., and Hamblin, M. R. 2009. Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety. *Behavioral and Brain Functions*, 5, 46. doi: 10.1186/1744-9081-5-46.

17. Mathew J. J., Sapra A. 2021. Herpes Simplex Type 2. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, Available from: *https://www.ncbi.nlm.nih.gov/books*/NBK554427.

18. Burd, E. M. 2003. Human papillomavirus and cervical cancer. *Clinical Microbiology Reviews*, 16(1), 1-17. doi: 10.1128/CMR.16.1.1-17.2003.

19. Smith, J. S., Herrero, R., Bosetti, C., Muñoz, N., Bosch, F. X., Eluf-Neto, J., Castellsagué, X., Meijer, C. J., Van den Brule, A. J., Franceschi, S., Ashley, R., and International Agency for Research on Cancer (IARC) Multicentric Cervical Cancer Study Group 2002. Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. *Journal of the National Cancer Institute,* 94(21), 1604-1613. doi: 10.1093/jnci/94.21.1604

20. McCloskey, J. C., Kast, W. M., Flexman, J. P., McCallum, D., French, M. A., and Phillips, M. 2017. Syndemic synergy of HPV and other sexually transmitted pathogens in the development of high-grade anal squamous intraepithelial lesions.

21. Golais, F., and Mrázová, V. 2020. Human alpha and beta herpesviruses and cancer: passengers or foes? *Folia Microbiologica*, 65(3), 439-449. doi: 10.1007/s12223-020-00780-x.

22. Zhao, Y., Cao, X., Zheng, Y., Tang, J., Cai, W., Wang, H., Gao, Y., and Wang, Y. 2012. Relationship between cervical disease and infection with human papillomavirus types 16 and 18, and herpes simplex virus 1 and 2. *Journal of Medical Virology*, 84(12), 1920-1927. doi: 10.1002/jmv.23353

23. Depuydt, C. E. 2007. Comparison of MY09/11 consensus PCR and type-specific PCRs in the detection of oncogenic HPV types. Journal of Cellular and Molecular Medicine, 11, 881–891. doi: 10.1111/j.1582-4934.2007.00083.

24. Sotlar, K., Diemer, D., Dethleffs, A., Hack, Y., Stubner, A., Vollmer, N., Menton, S., Menton, M., Dietz, K., Wallwiener, D., Kandolf, R., and Bültmann, B. 2004. Detection and typing of human papillomavirus by e6 nested multiplex PCR. *Journal of Clinical Microbiology,* 42(7), 3176-84. doi: 10.1128/JCM.42.7.3176-3184.2004. PMID: 15243079; PMCID: PMC446280.

25. Ramamurthy, S., Cui, X., Wang, Y., and Vijayendran, R. 2011. Conventional PCR compared to real time PCR for CSF samples. *Neurology,* 78(1), P01.183.

26. de Sanjosé, S., Diaz, M., Castellsagué, X., Clifford, G., Bruni, L., Muñoz, N., and Bosch, F. X. 2007. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis.

27. Bruni, L., Diaz, M., Barrionuevo-Rosas, L., Herrero, R., Bray, F., Bosch, F. X., and de Sanjosé, S. 2010. Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. *The Lancet Global Health,* 4(7), e453-e463.

28. Nejo, Y. T., Olaleye, D. O., and Odaibo, G. N. 2018. Prevalence and risk factors of genital human papillomavirus infection among women in Southwest, Nigeria. *Archives of Basic and Applied Medicine,* 6(1), 105-112. doi: 10.4314/abam.v6i1.15

29. Makwe, C. C., Anorlu, R. I., Odeyemi, K. A. 2012. Human papillomavirus (HPV) infection and vaccines: Knowledge, attitude and perception among female students at the University of Lagos, Lagos, Nigeria. *Journal of Epidemiology and Global Health*; 2: 199-206

30. Winer, R. L., Hughes, J. P., Feng, Q., Xi, L. F., Cherne, S., O'Reilly, S. 2010. Detection of genital HPV types in fingertip samples from newly sexually active female university students. *Cancer Epidemiology, Biomarkers and Prevention*, 19(7), 1682–1685. https://doi.org/10.1158/1055-9965.EPI-10-0226

31. Mohamed, Z. K., Alsamarai, A. M., Najem, W. S. 2013. Seroprevalence of Herpes Simplex Virus type 2 (HSV-2) in women with bad obstetric history. *American Journal of Dermatology and Venereology*.

32. Yunusa, T., Haruna, S. A., and Garba, H. Z. 2019. Seroprevalence of herpes simplex virus among human immunodeficiency virus positive patients in resource-limited setting. *Global Journal of Infectious Diseases*, 11(3), 107-111.

33. Naga, S., Sarkar, S., Chattopadhyay, D., Bhattacharya, S., Biswas, R., Sen Gupta, M. 2015. Seroprevalence of herpes simplex virus infection in HIV co-infected individuals in Eastern India with Risk Factors Analysis. *Advances in Virology.* 7. Article ID 537939.

34. Agyemang-Yeboah, F., Oksana, D., Timmy-Donkoh, E., Asmah, R. H., and Mohammed, M. S. 2019. Commentary: Sero-prevalence of herpes simplex virus type 1 and type 2 among women attending routine cervix care clinics in Ghana. *Current Findings of Infectious Diseases,* (01), RD-INF-10005.

35. Hayatudeen, M. R., Mukhtar, G. L., Aminu, M. 2017. Seroprevalence of immunoglobulins G, M associated with herpes simplex virus type 2 among apparently healthy individuals in Katsina State, Nigeria. *UJMR.* 2(1):186-191.