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Prevalence of High-risk HPV Infection in Women Presenting with Chronic Leucorrhoea at Kenyatta National Hospital, Nairobi City County, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors Author WOO came up with and designed the study protocols, carried out statistical analysis, and wrote the drafts of the manuscript with aid and support from the author OMM. The author EM assisted in the layout of study procedures and methods. All authors finally read and approved the final manuscript.

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ABSTRACT

Background: Chronic leucorrhoea is the most common symptom caused by infections of the vagina itself or infections/inflammation of the cervix. These inflammations are mostly associated with Candida spp, the parasite *Trichomonas vaginalis* infection, and the human papillomavirus. Oncogenic HPV sub-types 16,18,31,33 and 45 have been greatly associated and contributed to nearly 70% of all cervical cancers. HPV infections may clear within a period of up to 2 years.

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However, for every 1 million women infected with the virus, approximately 10% (100,000) will develop cervical dysplasia. Persistent infections with high-risk HPV lead to chronic inflammation through various mechanisms. This inflammation induces oxidative stress on the infected host cells thus leading to the release of molecules that may cause cellular damage e.g. white, creamy discharge that is known as leucorrhoea.

Objective: This study aimed to investigate the prevalence of high-risk HPV infection in women presenting with chronic leucorrhoea at Kenyatta National Hospital, Nairobi City County in the Gynecology and Obstetrics department.

Study Design: The study adopted a prospective cross-sectional design.

Place and Duration of Study: Department of Gynecology and Obstetrics Kenyatta National Hospital between October and December 2023.

Methodology: The study included 107 women presenting with chronic leucorrhoea aged 18 years and above who were purposely sampled and screened for high-risk HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73 using real-time PCR.

Results: Among the 107 women in this study, 38 (35.5%) tested positive for high-risk HPV strain whereas 69 (64.5%) tested negative. The highest positives were observed in HPV 16 (42.1%), then other hrHPV (36.8%), and finally HPV 18 at 21.0% Out of all the risk factors analyzed (age, history of substance abuse, number of lifetime sexual partners, age at sexual debut, marital status, level of education, status of income, and choice of family planning) none was found to have a statistically significant association with HPV infection.

Conclusion: The prevalence of high-risk HPV infection in women presenting with chronic leucorrhoea was 35.5% in this study with genotype 16 being the most prevalent. Of all the risk factors analyzed, none had a statistically significant association with high-risk HPV infection.

Keywords: Leucorrhoea; dysplasia; colposcopy; hysterectomy.

1. INTRODUCTION

According to estimates from the World Health Organization, around 530,000 women globally receive a cervical cancer diagnosis each year, with 275,000 of them losing their lives to the illness. It is well known that cervical cancer ranks third among cancers that affect women worldwide, with about 70% of cases occurring in underdeveloped nations. Current estimates in Kenya indicate that every year 5236 women are diagnosed with cervical cancer and 3211 die from the disease [1].

There is a lack of comprehensive diagnosis in women presenting with leucorrhoea as only high vaginal swabs and PAP smears are performed during laboratory investigations forgetting about testing for high-risk HPV genotypes. These genotypes have been found to contribute to chronic vaginal discharge. In the event of chronic infection by high-risk HPV types, the body's immune system will trigger inflammation. Depending on the immune-competence of an individual the inflammation might be acute or chronic. Due to incomprehensive diagnosis in women presenting with chronic discharge in Kenya, high-risk HPV goes undetected causing chronic inflammation. This in turn causes oxidative stress which results in the release of molecules that cause cellular damage hence

producing signs such as whitish discharges (leucorrhoea). HPV infection induces oxidative stress which may bring about the persistence of the infection by interfering with redox homeostasis of the host cells.

The prevalence of high-risk HPV genotypes in women from the general public in Kenya stands at 9.1% as of 2023 i.e. this proportion is for those women who do not have any clinical presentation like vaginal discharge (leucorrhoea).

It has been established that certain high-risk human papillomavirus (HPV) genotypes are the cause of cervical cancer. Geographically limited heterogeneity exists among the many kinds of HPV, and the relationship between HPV and cervical cancer is a worldwide reality [2].

The primary cause of cervical cancer is HPV infection of the cervix, and nearly all cases of invasive cervical cancer will have high-risk HPV DNA detected in them thanks to modern testing methods. Ineffective cell-mediated immunity is one of the main factors contributing to the development and progression of cervical neoplasia in women who are frequently infected [3].

More than 40 HPV genotypes have been found in anogenital mucosa samples, and they are sexually transmitted. The high-risk (HR) group of HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 is associated with an increased risk of cervical cancer [4].

The main stages of cervical oncogenesis are invasion, viral persistence, clonal development of the persistently infected epithelium to cervical pre-cancer, and infection of the metaplastic epithelium of the cervical transformation zone with high-risk HPV infection [5].

One of the leading causes of illness and death in sub-Saharan Africa is HPV-associated cervical cancer. In most countries, the high incidence rate of cervical cancer has been linked to a lack of effective efforts and long-term programs and services for prevention. In Eastern Africa, the prevalence of cervical HPV infection is estimated to be 35.8% among women at any given time. and HPV 16 or 18 is linked to 76.5% of invasive cervical cancer cases. Furthermore, the fact that just 0.6% of Kenyan women between the ages of 18 and 69 are checked every three years-that is, 1.6% of urban women and 0.4% of rural women-indicates that screening procedures are inadequate and that overall coverage is extremely low [6]

Significant information was generated by this investigation, including pertinent data on the prevalence of high-risk HPV infection.

2. MATERIALS AND METHODS

2.1 Study Design

This was a prospective cross-sectional study where 107 women presenting with chronic leucorrhoea at Kenyatta National Hospital gynecology and obstetrics department were purposely sampled and screened for high-risk HPV genotypes using real-time PCR between October to December 2023. The current study included women who were at least 18 years of age and had given consent to participate. Demographic data collected was by administering a questionnaire among the study participants. Women who were on their periods, who had undergone a hysterectomy, or refused to give consent were excluded from the study.

2.2 Obtaining Cervical Samples

Before the collection of cervical samples participants were thoroughly informed and instructed on all procedures to be carried out so that they could give informed consent. A singleuse, sterile speculum of appropriate size was lubricated using plain tap water and gently introduced into the vagina. To visualize the cervix, the speculum was manipulated so that it expounded and opened the vaginal canal. Scrapping of the endocervix and ectocervix was done using a cytobrush by rotating it thrice. The brush was then vigorously stirred in a cartridge containing HPV fixative then capped, labeled, and sent to the molecular laboratory for analysis

2.2.1 Processing of samples for HPV DNA detection

High-risk HPV DNA detection was done using real-time PCR. The detection protocol was based on the commercial Kit V31-100/F FRT. All the reagents were prepared following the manufacturer's instructions on the assay day. Extraction of DNA material and amplification was done as per the standard operating procedures and results interpretation was done.

2.2.1.1 Data analysis

The HPV DNA results were first recorded in an Excel spreadsheet and then imported into a software program (IBM SPSS Inc. application software version 21.0.1.2010) for analysis of the dependent and independent variables. Summarized results were tabulated to display proportions and P values. A Chi-square test was performed to establish if there was a significant association between high-risk HPV infection and the demographic factors considered in this study. A P value of less than 0.05 was considered statistically significant at a confidence interval of 95%.

3. RESULTS

In this study, a total of 107 women were enrolled. Their ages ranged from 24 - 57 years. Out of the 107 women enrolled, 38 (35.5%) tested positive for high-risk HPV infection while 69 (64.5%) tested negative (Fig. 1). Out of the 38 women who tested positive for high-risk HPV infection, 16 (42.1%) tested positive for genotype 16, 14 (36.8%) were positive for other hrHPV genotypes and 8 (21.1%) tested positive for genotype 18. (Fig. 2).

Of all the risk factors analyzed, none was found to have any statistically significant association with high-risk HPV infection. Table 1 shows a cross-tabulation of age of participants against high-risk HPV infection. The highest prevalence of HPV infection was noted in the age bracket of 30-34 years (44.7%). The brackets of 50-54 years and 55-60 years each recorded the lowest each recording only 1 woman (2.6%). However, there was no statistically significant association between the age of the participants and high-risk HPV infection (p = .619) (Table 1).

Out of the 107 women recruited, 92 (86.0%) had no history of drug abuse whereas 15 (14.0%) had a run-in with a substance in the past. The majority of the women reported to have only one sexual partner (96.3%). Most of the participants (66) reported to have engaged in their first intercourse at the age of 18 years (Table 2).

Among the 107 women recruited, 47 (43.9%) were married, 42 (39.3%) were single, 13 (12.1%) were widowed and 5 women (4.7%) were divorced. There was no statistically significant association between the marital status of the participants and high-risk HPV infection (p=.112). On assessing the level of education, the majority of the participants (47) had attained secondary education level (43.3%) while only 26 had gone up to college level (24.3%). Under the type of contraceptive use, the most preferred method for family planning was condoms with 38 women (35.5%) while pills were the most unpopular with only 3 women (2.4%) (Table 3).

Table 1. Comparison of age versus high-risk HPV infection

Age (years)	Final HPV Result					
	Total	Negative	Positive	P-value		
18-23	0 (0.0%)	0 (0.0%)	0 (0.0%)	.619		
24-29	2 (1.9%)	2 (2.89%)	0 (0.0%)			
30-34	42 (39.3%)	25 (36.23%)	17 (44.73%)			
35-39	22 (20.6%)	16 (23.18%)	6 (15.78%)			
40-44	20 (18.7%)	11 (15.94%)	9 (23.68%)			
45-49	17 (15.9%)	13 (18.84%)	4 (10.52%)			
50-54	2 (1.9%)	1 (1.44%)	1 (2.63%)			
55-59	2 (1.9%)	1 (1.44%)	1 (2.63%)			
Total Number	107(100%)	69 (100%)	38 (100%)			

 Table 2. History of Substance Abuse, number of sexual partners, age at first intercourse versus high-risk HPV infection

	Final HPV result					
History of substance abuse	Total	Negative	Positive	P-value		
NO	92 (86.0%)	61 (88.4%)	31 (81.57%)	.330		
YES	15 (14.0%)	8 (11.59%)	7 (18.42%)			
Total number	107(100.0%)	69 (100%)	38 (100%)			
Number of sexual partners						
One	103 (96.3%)	66 (95.6%)	37 (97.36%)	.654		
More than one	4 (3.7%)	3 (4.34%)	1 (2.63%)			
Total number	107 (100.0%)	69 (100%)	38 (100%)			
Age at first intercourse						
16	2 (1.9%)	1 (1.44%)	1 (2.63%)	.202		
17	7 (6.5%)	5 (7.24%)	2 (5.26%)			
18	66 (61.7%)	44 (63.76%)	22 (57.89%)			
19	19 (17.8%)	9 (13.04%)	10 (26.31%)			
20	12 (11.2%)	9 (13.04%)	3 (7.89%)			
21	1 (0.9%)	1 (1.44%)	0 (0.0%)			
Total number	107 (100%)	69 (100%)	38 (100%)			

	HPV result				
Marital status	Total	Negative	Positive	P-value	
Single	42 (39.3%)	26 (37.68%)	16 (42.1%)	.112	
Married	47 (43.9%)	29 (42.02%)	18 (47.36%)		
Divorced	5 (4.7%)	2 (2.89%)	3 (7.89%		
Widowed	13 (12.1%)	12 (17.39%)	1 (2.63%		
Total number	107(100%)	69 (100%)	38 (100%)		
Education					
Primary	34 (31.8%)	25 (36.23%)	9 (23.68%)	.202	
Secondary	47 (43.9%)	26 (37.68%)	21 (55.26%)		
College	26 (24.3%)	18 (26.08%)	8 (21.05%)		
Total number	107 (100%)	69 (100%)	38 (100%)		
Family planning method					
None	22 (20.6%)	14 (20.28%)	8 (21.05%)	.675	
Pills	3 (2.8%)	3 (4.34%)	0 (0%)		
Implant	20 (18.7%)	11 (15.94%)	9 (23.68%)		
Condom	38 (35.5%)	26 (37.68%)	12 (31.57%)		
Injection	14 (13.1%)	8 (11.59%)	6 (15.78%)		
IÚD	10 (9.3%)	7 (10.14%)	3 (7.89%)		
Total number	107 (100%)	69 (100%)	38 (100%)		

Table 3. Comparison of marital status, level of education, choice of family planning versus high-risk HPV infection

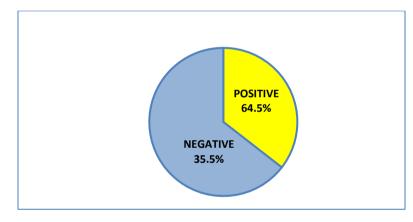


Fig. 1. Prevalence of high-risk HPV

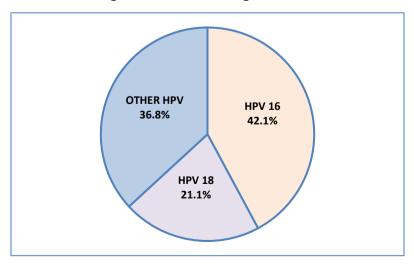


Fig. 2. Frequency of HPV 16, HPV 18, and other hrHPV

4. DISCUSSION

The proportion of women tested who were found to be positive for the high-risk human papillomavirus was 35.5%. Genotype 16 was found to be the most prevalent (42.1%) followed by other hrHPV (36.8%) and lastly HPV 18 (21.1%). Out of these factors (age, history of substance abuse, number of lifetime sexual partners, age at first intercourse, marital status, level of education, status of income, and choice of family planning), none were found to have a significant statistical association with acquiring the HPV infection.

The prevalence of our study was closer to the prevalence of a study done by Vinodhini et al., whose prevalence was 32.1%. Their study also found HPV 16 to be most prevalent in their analysis at 9.5% [7]. Zizipho et al., a study carried out in South Africa also found HPV 16 to be the most prevalent at 35%. Denny et al. study also found HPV 16 to be the most prevalent at 51.2% [8]. However, studies by Okoye et al., showed that HPV 53 and HPV 31 were the most prevalent at 7.3% and 5.3% respectively. This study however only recruited women who were HIV positive [9]. A study done by Meulen et al., in Tanzania found that HPV 18 was more prevalent at 17.5%. This study majorly focused on recruiting women who had already presented with cervical dysplasia and were also HIV positive [10]. Taku et al., study also showed the highest prevalence to be of HPV 35 (23.9%). They also only included women positive for CIN [11].

Our study showed no significant association between age and high-risk HPV infection. Watson-Jones et al., study showed that age had a significant association with contracting HPV because they found that young age at sexual debut played a significant role in getting the infection [12]. This concurred with Sangwa-Lugoma et al., a study that said the younger generation was more at risk than older women. This is because they were more sexually active than the older generation [13]. However, Sinayobye et al. showed a negative association between old age and acquiring HPV infection because, with older age, the immune system weakens leading to the reemergence of previously latent HPV infections [14]. However, Ermel et al., and Kuguyo et al., found that age had no statistically significant association with acquiring HPV infection in the tested women because with advanced age there was reduced

sexual vigor and the number of sexual partners that one woman indulged [15,16].

Our study also showed no association between having a history of substance abuse and acquiring HPV infection. Osazuwa-Peters et al. study also concluded that there was no significant statistical association between drinking alcohol or smoking and HPV infection [17]. Sangwa-Lugoma et al., study showed that smoking or chewing tobacco had a significant association with getting HPV infection. This is because women with a history of smoking or other substances were most likely to engage in earlier sexual debut [13]. Mbulawa et al. and Swai et al. also concurred with this by stating that binge drinking alcohol increased the risk of aetting infected by HPV because alcohol abusers were more likely to falter in practicing safe sex hence a lot of unprotected sex among these groups of women [18,19].

This study showed no significant inference between the number of lifetime sexual partners and HPV infection. A study in Rwanda by Sinayobye et al. also showed no significant statistical association between the number of lifetime sexual partners and HPV infection [14]. Watson-Jones et al., study also showed that there was no significant statistical association between having several lifetime sexual partners and acquiring HPV infection [12]. Taku et al., study showed that >3 lifetime sexual partners increased the odds of getting HPV infection. This is because women with more than one lifetime sexual partner were more likely to either acquire the infection from their multiple partners or they would spread the infection to their partners [11]. Vinodhini et al. concurred that increased sexual partners also increased the risk of getting HPV infection because of reasons similar to the study done by Taku et al. [7].

Mchome et al., study showed that women who were at an early age at first intercourse were at risk of getting HPV infection [20]. This agreed with a similar study done by Kirui et al., which also concluded that early sexual debut increased the risk of getting HPV. This is because, during puberty, the cervical cells are constantly changing thus making the cervix vulnerable to damage. Also, certain sexual behaviors increased the risk of these women acquiring HPV infection [21]. This study concluded that there was no significant statistical association between early sexual debut and getting high-risk HPV infection. This might have been maybe because

some of the women participants were not very honest during the filling of questionnaires about the age at which they started indulging in sex due to fear of judgment. Houlihan et al., study concurred that there was no association between early sexual intercourse and acquiring HPV infection [22].

Our study found that there was also no association between the women's level of education and acquiring high-risk HPV. Ali et al. study showed that level of education had no statistical significance [23]. Nang et al. said that women who attained secondary education had a reduced risk of contracting HPV infection compared to those with no form of education. This is because with some level of education. these women understand and are aware of the benefits of regular cervical HPV screening and also they understand that there are vaccines that can be taken at early ages to prevent complications brought by the virus [24]. Manga et al., also proved that level of education was significantly associated with HPV infection [25].

This study showed no association between choice of contraceptive use and acquiring HPV infection. Maggwa et al., study also showed no association between the choice of contraceptive use and high-risk HPV infection [26]. Vinodhini et al, study stated that the use of contraceptives contributed to an increased risk of acquiring HPV infection [7]. Molano et al. showed that using oral contraceptives and IUDs increased the risk of acquiring HPV significantly especially if they were used over long periods [19]. Niue et al., study also concluded that the use of hormonal contraceptives increased the risk of attaining high-risk HPV. This is because these specific contraceptives bring about hormonal fluctuations in women which may leave them vulnerable to the high-risk HPV [27].

This study did not find any association between marital status and high-risk HPV infection. Ali et al. study also did not show any significant correlation between the marital status of the women and the high-risk HPV [23]. Sangwa-Lugoma et al., study showed that married women had the highest frequency of HPV positive results [13]. Njue et al., study concurred by stating that the status of a woman's marriage was significantly associated with acquiring HPV infection. This is because married women indulged more in sexual practices compared to other groups e.g. single, divorced, and widowed women [27].

Our study showed no significant association between the women having a means of income and them testing positive for the high-risk HPV. Ali et al. study went in line with our conclusion. Vinodhini et al., study however contradicted this by stating that there was indeed an association. This is because women of lower economic status had less access to recommended routine HPV testing compared to those coming from a higher stature economically [7]. Houlihan et al., added to this by stating that women from poor backgrounds do not have access to important public health information that may be of use to them. This is because they come from rural areas where these services are rarely offered compared to the women who come from urban areas where they get comprehensive medical and diagnostic services [22].

This study had the limitations of time frame, finances, and inaccurate responses given by the study participants during the filling of the questionnaires. Thus, there is a need to increase the study population to encompass a meaningful proportion of the general population so that valid inferences can be deduced from the study population [28-30].

4. CONCLUSION

The prevalence of high-risk HPV infection in women presenting with chronic leucorrhoea was 35.5% in this study with genotype 16 being the most prevalent. None of the risk factors analyzed had a statistically significant association with high-risk HPV infection.

CONSENT

The authors affirmed that all participants were briefed concisely and clearly on all procedures about to be carried out on them after both verbal and written consent were obtained from them.

ETHICAL APPROVAL

The authors also affirmed that the study protocols had been gone through and cleared by the Kenyatta National Hospital Ethical Review Committee (P293/03/2023).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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