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Research Article

Infection to Malignancy: the Role of Hpv Dna Pcr In Targeted Screening Strategies

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ABSTRACT
Background: Human papillomavirus (HPV) ranks as the most prevalent sexually transmitted infection globally. Cervical cancers and anogenital cancers are the major public health concerns heavily associated with HPV infections like HPV-16 and 18. Early detection of HPV is very essential particularly in high-risk individuals like men who have sex with men (MSM), transgender population and female sex workers (FSW) allows the clinicians to initiate prompt management.
Materials and Methods: This was a cross-sectional, observational study done in a 200 high-risk individuals in a tertiary care setting. Endocervical, urethral, and rectal swab specimens were collected from them and were screened for the presence of HPV 16 and 18 using HPV DNA qualitative PCR test.
Results: Among the 200 specimens collected, 60 samples were taken from men who have sex with men, 26 samples from transgender populations and 114 samples from female sex workers. The overall prevalence of HPV-16 and 18 was 2.0% (4/200). HPV 16-only infections occurred in 1.0% (2/200), HPV 18-only in 0.5% (1/200), and co-infection in 0.5% (1/200).
Conclusion: This study supports the implementation of HPV DNA qualitative PCR test as a targeted screening tool for high-risk individuals. This approach can assist clinicians in identifying and monitoring individuals early to effectively manage precancerous lesions.

Keywords: Human Papillomavirus, Female Sex Workers, Men Who Have Sex with Men, Transgender.

INTRODUCTION

The most common sexually transmitted infection (STI) globally is caused by the virus that belongs to the *Papillomaviridae* family called as Human papillomavirus (HPV) (1) which is responsible for causing cervical and anogenital tract infection particularly in high-risk individuals like men who have sex with men (MSM), transgender population and female sex workers (FSW). (2) Globally, STI presents a major health challenge with the data from World Health Organization (WHO) reporting that above 1 million individuals are getting affected daily (3) whereas epidemiological data from ICMR indicates that 6% of the adult population in our country is diagnosed annually with a STI. (4)`

HPV infections are always asymptomatic, but sometimes cause malignancy. (5) There are many HPV serotypes identified but the highest risk variants are HPV 16 and HPV 18 which are more potential to cause cancer cervix in females and also associated with causing penile, anogenital, and oral cancers in males. (6) On a global scale, the prevalence of HPV among high-risk individuals is estimated to be between 11% and 12%. By comparison, the burden of HPV within the Indian population showed substantial variation, with reported prevalence rates ranging widely from 2.3% to 36.9%. (7, 8)

To tackle this public health issue, a global initiative was started in the year 2020 by World Health Organization (WHO) called as Cervical Cancer Elimination Programme which

aims to reduce the global cervical cancer incidence below 4 cases per 100,000 women-years. (9) The global "90–70–90" target indicates that by the year 2030 (10), 90% HPV vaccination rate for girls by age 15, a 70% lifetime PCR screening rate for women by age 45 (minimum two tests), and priority treatment for 90% of patients with precancerous or cancerous cervical lesions. Data from registries like the National Cancer Registry Programme (NCRP) is essential for assessing the long-term impact of these screening and vaccination policies. (11)

While statistics in general population and to some extent in FSW population are well documented, there is a significant gap in targeted surveillance for marginalized, high-risk individuals in certain areas. Implementing effective prevention and control measures could greatly decrease the impact of HPV related cancers and improve patient outcomes.

Objective

To determine the prevalence of HPV 16 and HPV 18 among men who have sex with men, transgender population and female sex workers in a tertiary care setting in Chennai

MATERIALS AND METHODS

The present study was carried out as a cross-sectional and observational study in a tertiary care setting in Chennai. The study was duly approved by the Institutional Ethics Committee (IEC/2014/051). All participants received explanation about the study procedures in their preferred language, and written consent form was obtained from them before collecting samples with the confidentiality of the participants strictly maintained.

The study involved collecting 200 urethral and endocervical specimens from the study population. Participants who were willing to give written informed consent were included in the study. Individuals who were not willing to provide consent form and those who were pregnant, or having active vaginal bleeding or those diagnosed with any other co-morbid conditions such as HIV, syphilis were excluded from the study.

Method of Specimen Collection:

Endocervical Swab from Fsw Populations

Endocervical swabs were collected by an Obstetrician & Gynaecologist as per standard guidelines using sterile vaginal speculum & sterile cotton wool swabs by passing the swab tip through endocervix and pressed against its wall and rotated.

Urethral Swab from MSM Populations

Urethral specimens were collected under strict aseptic precautions after cleaning the urethral meatus with normal saline. Purulent discharge, when present, was collected using a sterile cotton wool swabs. In the absence of visible discharge, urethral milking was performed to obtain the specimen.

Rectal Swab from Transgender Populations

Rectal swabs were collected by the surgeon as per standard guidelines using sterile cotton wool swabs by gently inserting the swab tip through the anus into the rectum and pressed against the rectal wall and rotated.

All the swab specimens were collected in a viral transport medium and transported to lab in proper cold storage for viral isolation.

Dna Extraction

DNA extraction from all the swab specimens was done by using a silica-based spin column method by using QIAamp DNA Mini Kit.

All the swab specimens were incubated by using lysis buffer, proteinase K and ethanol. The resultant was then shifted into a spin column and centrifuged at the rate of 8,000 - 15,000 rpm. The spin column was then washed with wash buffers 1 and 2 and then an empty centrifugation step was performed to remove trace ethanol. Finally, an elution buffer was added to the centrifuge tube.

Master Mix Preparation

The master mix was prepared by combining 10 µL of commercial master mix with 10 µL of HPV 16 and HPV 18 primer mix. A 20 µL aliquot of this mixture was dispensed into 0.2 mL PCR tubes, followed by the addition of 5 µL of the sample template, positive control, or negative control.

The primer sequences for HPV 16 were as follows: forward (5'–3'), GCAACAGTTACTGCGACGTGAGGT; and reverse (5'–3'), CACACAACGGTTTGTTGTATTGCTGT. For HPV 18, the primer sequences were: forward (5'–3'), TGATCTGTGCACGGAACTGAACAC; and reverse (5'–3'), TCAACGGTTTCTGGCACCGCAG.

Temperature Conditions

The 0.2 ml tubes containing the positive control, negative control, and samples were then placed into a thermal cycler which was preprogrammed with the following parameters: initial denaturation at 95°C for 30 s, annealing at 54°C for 30 s, and extension at

72°C for 30 s. The amplification cycle was repeated for a total of 35 cycles.

Gel Electrophoresis

A micropipette was used to load 5 µl of DNA ladder, 20 µl of the PCR product, 20 µl of the negative control, and 20 µl of the positive control into individual gel wells. A 1.5 % agarose gel was prepared by melting 0.6g of agarose in 40 ml of 1x TAE (Tris-Acetate-EDTA) buffer and it was boiled until it became clear indicating complete melting of agarose. Then the agarose was allowed to come up to 50 °C and to that 2 µl of (10mg/ml) the reagent called ethidium Bromide was added. The agarose solution was poured into the casting tray with required comb and allowed to solidify. After solidification, the gel was placed in 1x TAE electrophoresis tank. Two µl of loading dye along with 4 µl of PCR product was mixed and loaded in the well. The tank was then connected with electrical leads and a constant voltage of 150V was applied. The run was terminated when the loading dye traveled approximately three-fourths of the distance down the gel. After that, UV transilluminator was used to visualize the PCR products. The fragments were compared against the DNA ladder to determine their size. Presence of bands at 200 bp and 300 bp confirmed the presence of HPV 16 and HPV 18, respectively and the bands were visualized and documented in UVP gel documentation system.

RESULTS

The study population involved 200 high-risk individuals and was consisted primarily of female sex workers (57%), followed by men who have sex with men (30%) and transgender individuals (13%). Their distribution is tabulated below in table 1.

Table 1: Summary of Frequency and Percentage Analysis of the Study Population

Type of population	Frequency	Percentage (%)
FSW	114	57
MSM	60	30
Transgender	26	13
Total	200	100%

Analysis of the participant's baseline characteristics showed that nearly half of them (49%) fell within the age group range of 21–30 years, with a vast majority participants identified as unmarried (92%). In terms of educational status, 77% of them had completed secondary or higher education.

Behavioral analysis indicated that 69% of participants reported a history of protected sexual intercourse. Notably, the clinical prevalence of HIV and syphilis co-infection within this population stood at 1%. The summary of the baseline and behavioral characteristics of the participants is detailed below in Table 2.

Table 2: Summary of Baseline and Behavioral Characteristics of the Participants

Characteristics	Frequency	Percentage (%)
Age		
18-20 years	4	2
21-30 years	98	49
31-40 years	78	39
41-50 years	20	10
Marital status		
Married	16	8
Unmarried	184	92
Protected sex		
Protected	138	69
Unprotected	62	31
Education status		
Primary	46	23
Secondary / higher	154	77
HIV		
Reactive	2	1

Non-reactive	198	99
Syphilis		
Reactive	2	1
Non-reactive	198	99
HPV		
Positive	4	2
Negative	196	98

Our study revealed that out of the 200 samples, 2% (4 samples) tested positive for the presence of HPV. Among them, HPV 16 subtype was found in 1% (2/200) of the population whereas HPV 18 was found in 0.5% (1/200) of the population. Both HPV 16 and 18 co-infections were found to be present in 0.5% (1/200) of the population. The summary of the HPV positivity in different category of study population is summarized below in table 3.

Table 3: Summary of HPV Positivity in Various Study Population

HPV	FSW (114)	MSM (60)	Transgender (26)	Total
16 Positive	2	0	0	2/200 (1%)
18 Positive	1	0	0	1/200 (0.5%)
16 and 18 Positive	1	0	0	1/200 (0.5%)
Total	4	0	0	4/200 (2%)

Among the baseline characteristics of the participants, Age was highly associated with HPV positivity (P=0.006), despite a uniform distribution of positive cases across various age groups. Educational status of the participants was also significantly associated with HPV positivity (P=0.04), with individuals having primary level of education showed very high prevalence rate (75%). Conversely, no statistically significant link was observed between marital status and HPV positivity.

Regarding the baseline characteristics of the participants, Consistent use of barriers during sexual intercourse showed a strong protective effect (P=0.009); notably, the entire unprotected individuals (100%) tested positive for HPV. A significant correlation was also identified between HPV and dual co-infection with HIV and syphilis (P=0.04).

A comparative evaluation was performed for the baseline and behavioral characteristics among HPV positive and negative individuals and given below in table 4.

Table 4: Distribution of Participant Characteristics across HPV Status

Characteristics	HPV positive (4)	HPV negative (196)	Total (200)	P value
Age				
18-20 years	1 (25%)	3	4	0.006
21-30 years	1 (25%)	97	98	
31-40 years	1 (25%)	77	78	
41-50 years	1 (25%)	19	20	
Marital status				
Married	1 (25%)	15	16	0.28
Unmarried	3 (75%)	181	184	
Protected sex				
Protected	0 (0%)	138	138	0.009
Unprotected	4 (100%)	58	62	
Educational status				
Primary	3 (75%)	43	46	0.04
Secondary / higher	1 (25%)	153	154	
HIV				

Reactive	1 (25%)	1	2	0.04
Non-reactive	3 (75%)	195	198	
Syphilis				
Reactive	1 (25%)	1	2	0.04
Non-reactive	3 (75%)	195	198	

DISCUSSION

Cervical cancer caused by Human papilloma virus is the most common sexually transmitted infection (STI) globally especially in high risk individuals like FSW, MSM and transgender population. They can transmit the infection to the general population and the risk is more particularly in individuals who were positive for HPV 16 and HPV 18. Hence, identifying them is very essential which can lead to a decrease in the morbidity and mortality associated with the HPV infection.

Prevalence of HPV:

In this study, the overall prevalence of HPV among high risk individuals was 2% with HPV 16 and 18 variants being prevalent in 1% and 0.5% of the study population respectively. These frequencies are in consistent with the findings of Shah A et al (12) in which he showed the prevalence of HPV 16 and 18 variants to be 1.08% and 0.27% respectively. When compared with the Indian literatures the frequencies noted in our study is less (13, 14) but the global literatures reported higher prevalence of HPV infection in high risk individuals ranging between 1.4 and 26%.

An analysis of the study population indicated that HPV prevalence was significantly linked to age, educational status, and use of protective barriers during sexual intercourse, and concurrent infections with HIV and syphilis. A highly significant statistical correlation was observed between age and HPV positivity. The age groups 21–30 years emerged as the most prominent population infected with HPV and it aligns closely with previous research findings by Devi et al. (17), Shukla et al. (18), and Singh et al. (19). Conversely, marital status did not exhibit a significant relationship with HPV positivity ($P > 0.05$). This finding diverges noticeably from the results reported by Shukla et al. (18) and Dandona et al. (20), both of whom reported strong correlation between marital status and HPV positivity. The use of protective barriers during sexual intercourse was also significantly associated with HPV positivity, with a higher prevalence of HPV detected among individuals reporting unprotected intercourse. This matches with the observations made by the authors Devi et al. (17), Shukla et al. (18), and Ouattara et al. (21). Additionally, educational status was also found to have very strong correlation with HPV positivity, with participants who had only completed a primary level of education showed an increased risk of HPV infection. This is inconsistent with findings of Velazquez-Hernandez et al. (22), Solomon et al. (23), and Sohoo et al. (24), where the major proportion of infected individuals had not progressed to secondary/higher education. Finally, a statistically significant association was established between HPV positivity and dual co-infection with HIV and syphilis.

Detecting the high-risk HPV DNA in asymptomatic high-risk individuals will help clinicians to follow up by periodic cytology studies, such as anal pap smear in males and endocervical smear in females. Furthermore, they can be periodically screened by physical examination for any newly developing cancerous mass lesions. Thereby, we can identify and prevent them progressing from precancerous to cancerous stage. We can also educate the individuals about the symptoms and also how to self-examine themselves for any newly developing lesions.

Though India has launched a nationwide HPV vaccination campaign, it is targeting only the girls aged 9–14 yrs to prevent cervical cancer. The government is offering only a single dose of HPV vaccine covering only the 4 HPV high-risk types responsible for 80-85% of cervical cancers in India. As a support to the global Cervical Cancer Elimination Programme lot of surveillance studies have to be conducted in different locations to identify the exact high-risk HPV type that is prevalent in that particular area and vaccines covering that type are to be administered in that geographical area.

CONCLUSION

HPV Screening in the high risk asymptomatic individuals could identify the individuals who are harboring HPV virus without any clinical symptoms. By doing so, targeted follow-up of those high-risk individuals by anogenital clinical examination and cytology studies can be done for a longer duration to diagnose at the precancerous stage & they can be very

well managed beforehand developing the highly progressing cancer. The quadrivalent vaccine contains particles derived from HPV types 6, 11, 16, and 18 vaccines are effective at preventing persistent infections by the high-risk HPV and the development of HPV-related genital precancerous lesions. HPV vaccination can be recommended for boys aged 9-14 yrs to prevent the anogenital cancers.

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