Seroprevalence of Human Papillomavirus Infection among Female Sex Workers in a Tertiary Care Hospital in Southern India

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Microbiology Section

ABSTRACT

Introduction: Sexually Transmitted Infections (STIs) are generally acquired through sexual contact, and the risk is very high for Female Sex Workers (FSWs). The most common STI in FSWs is Human Papillomavirus (HPV) infection. Majority of the HPV infection is asymptomatic, while a minority of the infection is responsible for causing cervical cancer. High-Risk HPV (HR-HPV) serotypes, HPV 16 and HPV 18, are known to cause cervical cancer.

Aim: To determine the seroprevalence of HPV infection among FSWs attending STD clinic at a tertiary care hospital in Southern India.

Materials and Methods: This cross-sectional study was done during the period between September 2015 and August 2016 in a total of 65 FSWs in Govt. Stanley Medical College, a tertiary care hospital in Chennai, Southern India. The serum was separated after 5 mL of blood was drawn to test for the presence of HPV 16 and 18, Human Immunodeficiency Virus (HIV), and syphilis. Endocervical and vaginal swab specimens were taken to look for the presence of other STIs. Necessary

socio-demographic and clinical information such as mode of sex, any sexual exposure, extramarital or premarital, protected sex or not, history of associated STIs, and any history of blood transfusion, i.v. drug abuse, or surgery was also obtained. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) 20.0 software. Probability value (p-value) <0.05 was considered statistically significant.

Results: Analysis of the blood samples showed that four samples were found positive for HPV infection, while two samples each were positive for HIV and syphilis. Analysis of the swab specimens showed the presence of other associated STIs in 20 samples. Among the four HPV positive samples, three were found positive for HPV 16, and one was positive for HPV 18. Risk factors such as age, marital status, HIV, and syphilis had a very strong association with HPV infection (p-value<0.01).

Conclusion: The prevalence of HR-HPV serotype HPV 16 was higher than HPV 18. Hence, education about STIs, safer sexual practices, and HPV vaccination are essential in this population in order to prevent the transmission of infection to the general population.

Keywords: Cervical cancer, Human immunodeficiency virus, Sexually transmitted infections, Syphilis

INTRODUCTION

The STIs are infections that are spread by sexual activity but are often asymptomatic, which results in a high-risk of passing on the infection to others. According to the World Health Organisation (WHO), more than 1 million people worldwide acquire STIs every day [1]. As per the ICMR, 6% of the adult population in India is diagnosed with an STI every year [2]. Globally, HPV infection is the most common STI [3]. HPV is a small, non enveloped, doublestranded DNA virus belonging to the Papillomaviridae family of viruses responsible for causing infections of the genital tract, particularly in sexually active adults like FSWs [4]. The majority of HPV infections are asymptomatic, but some can persist and cause cervical cancer [5]. Furthermore, HPV can be transmitted from FSWs to the general population via their clients [6]. Many HPV serotypes are identified, and among them, the most prevalent HPV types among FSWs are HR-HPV serotypes, HPV 16 and HPV 18, which are known to cause more than 70% of cervical cancer [7]. Cervical cancer is the third most common cancer in women globally and second most common cancer in women in India, accounting for more than 60,000 deaths each year [8]. The estimated global prevalence of HPV ranges from 11-12% [9], whereas the prevalence of HPV in India ranges from 2.3-36.9% [10]. The median overall prevalence of HPV infection in FSWs worldwide was estimated to be around 42.7%, with the prevalence of HR-HPV serotypes HPV 16 and HPV 18 being 38.9% and 23.1%, respectively [11].

STIs impose a major health and economic burden globally. If left untreated, STIs may cause some serious complications, leading to increased morbidity and mortality. Unfortunately, the general public is unaware of HPV infection, and it is even lower in high-risk populations such as FSWs. Hence, this study was planned with an aim to determine the seroprevalence of HPV infection in FSWs attending an STD clinic in a tertiary care hospital in Southern India and with the main objective of emphasising the necessity of HPV vaccination among FSWs to prevent the risk of developing cervical cancer.

MATERIALS AND METHODS

This was a prospective, observational, cross-sectional study done on a total of 65 blood samples collected from FSWs attending an STD clinic in a Tertiary Care Hospital in Chennai, Southern India during the period between September 2015 and August 2016. FSWs are women who provide sexual services for monetary compensation. Before commencing the study, ethics committee clearance was obtained from the institution (IEC No.: ECR/131/Inst/TN/2013/ RR-22). Details about the study and study procedures were clearly informed to the patients in their native language, and informed consent was obtained. The personal identity of the patients was not revealed in any part of the manuscript.

Estimation of sample size: The prevalence of HPV in India ranges from 2.3-36.9% [10]. Considering the prevalence of HPV to be 2.3%, then for 5% margin of error snd 95% CI, only 35 subjects were required but, this number was increased to 65 subjects in order to achieve good statistical power.

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Inclusion criteria:

- FSWs aged between 18 and 50-year-old.
- Those willing to give written, informed consent.

Exclusion criteria:

- Pregnant females.
- Those who are not willing to give written, informed consent.

Relevant socio-demographic and clinical information was obtained from all the participants. Then, under aseptic conditions, 5 mL of blood was drawn from each participant, and the serum was separated to detect the presence of HIV, syphilis and HPV using the following kits. All the tests were done as per manufacturer's instruction.

Combaids: RS Advantage-ST: HIV 1+2 immunodot test kit: It is used for the detection of HIV 1 and/or HIV 2 antibodies in whole blood or serum. Manufactured by Span Diagnostics Ltd.

Carbogen: Rapid Plasma Reagin (RPR) card test for syphilis: It is a test which was used for the detection and quantitation of antilipoidal antibodies. The serum is mixed with the Carbogen reagent and allowed to react for eight minutes. If antilipoidal antibodies are present in the specimen, they will react with the Carbogen reagent forming visible black floccules. If antilipoidal antibodies are not present in the specimens, there will be no flocculation. Manufactured by Tulip Diagnostics Ltd.

HELINI HPV 16/18 PCR kit used for the detection of HR-HPV serotypes, HPV 16 and HPV 18. The kit components include PCR master mix, HPV 16 and 18 primer mix, endogenous primer mix, HPV 16 and 18 positive template and nuclease free water. The kit contains all reagents and enzymes for the specific amplification of E6/ E7 region of the HPV genome. In addition, it also contains an endogenous control system to identify possible PCR inhibition and to validate DNA purification. Manufactured by Helini Biomecules Pvt., Ltd.

Endocervical and vaginal specimens were also collected to test for the presence of any other associated STIs.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS 20.0 software. The results for all the categorical variables were expressed as percentages and then comparison was done using Chi-square test. The p-value <0.05 were considered statistically significant.

RESULTS

A total of 65 FSWs were included in the study of which 51% of the study population was in the 31-40 age groups; 92.3% were married; 69.2% had a history of protected sex; and 77% had a secondary or higher level of school education [Table/Fig-1]. Analysis of the samples for the presence of associated STIs showed that 31% (20/65) of the study population was affected by STIs such as trichomoniasis (n=2/20), bacterial vaginosis (n=8/20), candidiasis (n=8/20), genital warts (n=1/20), and genital ulcers (n=1/20). A 3% (2/65) of the population were found co-infected with HIV and syphilis each. HPV infection was found to be present in 6% (4/65) of the population [Table/Fig-2]. Among four HPV positives, three were found to be positive for HPV 16, and one was found positive for HPV 18.

Despite the fact that all age groups had a similar number of HPV positive individuals, present study findings revealed that the age of the participants had a very significant association with HPV seropositivity (p-value<0.01). The marital status of the women was found to have a very significant association with HPV seropositivity (p-value<0.01). Women who were not married had a 75% HPV prevalence. Protection during sexual intercourse was found to be positively associated with HPV infection (p-value=0.05). Women who had not had protection during sexual intercourse showed a higher HPV prevalence of 75% when compared to those who were protected (25%).

Characteristics	Frequency	Percentage (%)			
Age (in years)					
18-20	2	3			
21-30	28	43			
31-40	33	51			
41-50	2	3			
Marital status					
Married	60	92.3			
Not married	5	7.7			
Protected sex					
Yes	45	69.2			
No	20	30.8			
Education status					
Primary	15	23.1			
Secondary	45	69.2			
Higher	5	7.7			
[Table/Fig-1]: Frequency analysis for various socio-demographic characteristics of the study participants.					

Characteristics	Frequency	Percentage (%)			
Associated STIs					
Yes	20	30.8			
No	45	69.2			
HIV					
Positive	2	3			
Negative	63	97			
Syphilis					
Positive	2	3			
Negative	63	97			
HPV					
Positive	4	6			
Negative	61	94			
[Table/Fig-2]: Frequency analysis of the associated STIs and co-infections in the study participants.					

Educational status was found to have a significant association with HPV seropositivity (p-value=0.01), women with a primary level of education had a higher HPV prevalence of 75% when compared to women with a secondary or higher level of education (25%). The presence of other STIs such as trichomoniasis, bacterial vaginosis, candidiasis, genital warts, and genital ulcers was found to have a statistically significant association with HPV seropositivity (p-value=0.05). Those infected with the above mentioned STIs had a higher prevalence of 75%. Co-infection with HIV and syphilis was found to have a very high and statistically significant association with HPV seropositivity (p-value <0.01). Those who were infected with HIV or syphilis showed a higher HPV prevalence of 75%. The summary of the comparison of various characteristics between HPV positive and HPV negative individuals is given in [Table/Fig-3].

Characteristics	HPV positive (4)	HPV negative (61)	Total (65)	p-value (Chi-square test)		
Age (in years)						
18-20	1 (25%)	1	2			
21-30	1 (25%)	27	28	<0.01		
31-40	1 (25%)	32	33			
41-50	1 (25%)	1	2			
Marital status						
Married	1 (25%)	59	60	-0.01		
Not married	3 (75%)	2	5	<0.01		
Protected sex						

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Yes	1 (25%)	44	45	0.05			
No	3 (75%)	17	20	0.05			
Educational stat	Educational status						
Primary	3 (75%)	12	15				
Secondary/ higher	1 (25%)	49	50	0.01			
Associated STIs							
Yes	3 (75%)	17	20	0.05			
No	1 (25%)	44	45	0.05			
HIV							
Positive	1 (25%)	1	2	0.01			
Negative	3 (75%)	60	63	<0.01			
Syphilis							
Positive	1 (25%)	1	2	-0.01			
Negative	3 (75%)	60	63	<0.01			
[Table/Fig-3]: Comparison of various characteristics between HPV positive and HPV negative individuals.							

DISCUSSION

The HPV infection is the most common STI in the world, causing cervical cancer, especially in FSWs. They are at high-risk of transmitting the infection to the general population through their clients, particularly those with HR-HPV serotypes, HPV 16 and HPV 18. Hence, identifying this high-risk population can lead to a decrease in the morbidity and mortality associated with HPV infection.

Seroprevalence of HPV: The present study showed that the overall prevalence of HPV in FSWs was 6%, with the prevalence of HPV 16 being 4.6% and HPV 18 being 1.5%. This prevalence rate is much lower when compared to the studies done in different parts of India [Table/Fig-4]. One of the main reasons for this low prevalence of HPV in present study could be attributed to the fact that 69.2% of the study population had a history of protected sex. The HPV seroprevalence studies done on FSWs in various parts of India are tabulated below in [Table/Fig-4] [12-16].

Author	Publication year	Place	No. of subjects	HPV prevalence (%)
Hemalatha R et al., [12]	2011	Andhra Pradesh, South India	3200	16.3
Ghosh I et al., [13]	2012	West Bengal, East India	45	73.3
Shukla P et al., [14]	2015	Uttar Pradesh, North India	288	35.8
Singh MP et al., [15]	2016	Chandigarh, North India	120	27.5
Parwez A et al., [16]	2022	Bihar, East India	197	16.7
Present study	2023	Chennai, Tamil Nadu, South India	65	6
[Table/Fig-4]: Seroprevalence studies of HPV infection in Female Sex Workers				

(FSWs) done in India [12-16].

Studies done in other countries showed that the prevalence of HPV infection in FSWs ranges between 5.5-85%. When compared with those studies, present study HPV prevalence was much more comparable to that range. The HPV seroprevalence studies done in other countries are tabulated below in [Table/Fig-5] [17-31].

Author	Publication year	Place	No. of subjects	HPV prevalence (%)
Kjaer SK et al., [17]	2000	Denmark, Europe	182	32.4
Juárez-Figueroa LA et al., [18]	2001	Mexico, North America	495	48.9
Hernandez BY and Vu Nguyen T, [19]	2008	Southern Vietnam	282	85
del Amo J et al., [20]	2009	Spain	549	28

Menon S et al., [21]	2017	Western Kenya	599	14.5
Rodriguez-Cerdeira C et al., [22]	2012	Spain	208	31
Montano SM et al., [23]	2011	Lima, Peru	87	50.6
Hoang HT et al., [24]	2013	Northern Vietnam	281	49.5
Hooi DJ et al., [25]	2018	Curacao, Netherlands	76	25
Jia H et al., [26]	2017	North-eastern china	309	61.9
Abdoulaye O et al., [27]	2015	Ivory coast, West Africa	350	51.5
Diop-Ndiaye H et al., [28]	2019	Senegal, West Africa	436	79.8
Adams AR et al., [29]	2019	Ghana, West Africa	100	26
Velazquez-Hernandez N at al., [30]	2019	Mexico, North America	217	5.5
Ferre VM et al., [31]	2019	Togo, West Africa	310	32.9
Present study	2023	South India	65	6

The analysis of various characteristics in the study population showed that HPV prevalence was significantly associated with factors such as age, marital status, educational status, associated STIs, and the presence of co-infections such as HIV and syphilis.

HPV seropositivity was found to have a very high statistical association with the age of the FSWs (p-value<0.01). The most common age group observed among FSWs is 31-40 years, which was in accordance with the studies of Shukla P et al., and Singh MP et al., [14,15].

The marital status of the FSWs seems to have a very significant association with HPV seropositivity. FSWs who were not married showed higher HPV prevalence when compared to those who were married (p-value<0.01). This was consistent with the findings of the studies by Shukla P et al., and Dandona R et al., [14,32].

Protected sex was found to have a statistically significant association with HEV seropositivity (p-value=0.05). FSWs with unprotected sex were found to have higher HPV seroprevalence. This finding was similar to the findings observed by Shukla P et al., Abdoulaye O et al., and Devi SA et al., [14,27,33].

Educational status was strongly associated with HPV seropositivity (p-value=0.01), which shows that FSWs with a primary level of education had increased HPV prevalence. This observation was in concordance with studies done by Soohoo M et al., Velazquez-Hernandez N et al., and Solomon MM et al., where majority of the FSWs had completed only primary school level of education [11,30,34].

Vaginal swab and endocervical swab specimen taken from all 65 FSWs showed the presence of concomitant other STIs in 20 FSWs. The common STIs found in present study population were trichomoniasis, bacterial vaginosis, candidiasis, genital warts, and genital ulcers (syphilitic). The prevalence of trichomoniasis in present study participants was 3%, which was slightly higher when compared to the study done by Shethwala ND et al., where the prevalence was found to be 2% [35]. The prevalence of bacterial vaginosis in present study participants was 12%, which was slightly lower when compared to the study done by the same author, where the prevalence was 13.3%. The same author in the same study also showed the prevalence of candidiasis to be 10.3%, which was slightly lower when compared to present study findings, where the prevalence of candidiasis was found to be 12%. The prevalence of genital warts, which are the most common non ulcerative STI, was found in one FSW (1.5%) which was very low when compared to the studies done by Devi SA et al., (17.6%) and Kumar B et al., (25.2%) [33,36]. The prevalence of syphilitic genital ulcer, which is the 2nd most common ulcerative STI, was found in one FSW (1.5%), which was very low when compared to the studies done by Manas C and Ramadasan P, (15.6%) and Parmar J et al., (28.1%) [37,38].

A 5 mL of blood was also drawn from the study population for serological testing to detect the presence of STI such as HIV and syphilis (RPR). The test results revealed that two FSWs were reactive for HIV and two FSWs were positive for syphilis. The prevalence of HIV was found to be 3%. This was very similar to the recent National AIDS Control Organisation (NACO) statement, which documented that the HIV prevalence among FSW was found to be 2.67% [39]. The RPR test for syphilis was found to be positive in 3% of the study population, which was very low when compared to the study finding of Uribe-Salas F et al., where they reported the prevalence of syphilis in FSWs to be 8.2% [40]. Among the two HIV-reactive FSWs, one was found to be HPV positive, and similarly, among the two FSWs who were positive for syphilis, one was found to be HPV positive. Since HR-HPV serotypes are associated with 70% of cervical cancer, FSWs with serotypes HPV 16 and HPV 18 have to be identified because, even if they are asymptomatic, they can indirectly transmit the HPV infection through their clients to the general population. By screening for HPV 16 and HPV 18 among the high-risk population like FSWs, one can promote active vaccination among them and thereby spread of infection to the general population can be prevented. Since HR-HPV serotypes, HPV 16 and HPV 18 can cause precancerous and cancerous lesions of the cervix, vagina, vulva, penis and anogenital regions, administering vaccine to the high-risk group can help prevent development of malignancy.

Limitation(s)

Difficult to reach the study population, Cancer risk not only for FSWs but also the general population. PCR tests were not performed as they are expensive, time consuming and requires trained personnel.

CONCLUSION(S)

Present study findings showed that the seroprevalence of HR-HPV serotype HPV 16 and HPV 18 was low in FSWs. Age, marital status, and the presence of co-infections such as HIV and syphilis were found to be the most important risk factors for acquiring HPV infection in FSWs. Even though the prevalence rate of HPV as well as HPV 16 and HPV 18 was low in FSWs, there is a need for cervical cancer prevention campaigns especially in developing countries, like India. Educating the high-risk population like FSWs about safer sexual practices, necessary for prompt screening for STIs and taking HPV vaccination prophylactically may not only protect them but also avoid the transmission of infection to the general population.

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Data availability: All the datasets generated during this study were included in this manuscript.

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