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Original Research Article

Screening in female patients for HPV and STI for early diagnosis of cervical cancer

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ABSTRACT

Background: High-risk human papillomaviruses (HPV) infection has been the most common sexually transmitted infection worldwide. Moreover, it is a necessary factor for the development of cervical cancer. Several studies have been carried out that screen HPV and other sexually transmitted infections (STI)s occurring in patients at the same time. Timely screening can help early diagnosis of pre-cancerous lesions and assist in prompt treatment to reduce mortality in such patients. The objective of the present study was to screen women for the presence of high-risk HPV (hrHPV) for the early diagnosis of cervical cancer. Some women were additionally screened for STIs. We also aimed to establish an association between the presence of HPV and STI.
Methods: A total of 40 women aged 20-67 years participated in this study. Cervical and or vaginal swabs were collected in liquid-based cytology containers. The samples were tested for fourteen HPV genotypes by USFDA-approved Cobas HPV test. Out of these 40 women 26 were also tested for STI panel.
Results: All women participants were screened for hr-HPV. A total 65% of the study population underwent both the HPV test and the STI test. 7.5% of total women were positive for hrHPV. 30.76% of women tested positive for *Ureaplasma* and *Gardnerella vaginalis* in the STI panel. Some women also showed simultaneous presence of STI and HrHPV.
Conclusions: The results of this study will help in better and early diagnosis of women at risk of cervical cancer. The detection of HPV and STI present simultaneously can further help in establishing the role of these two conditions in the development of cervical cancer. Such studies are an encouragement to the HPV elimination programme and vaccination drive that has taken an impetus in recent times in India.

Keywords: Cervical cancer, High-risk human papillomaviruses, Sexually transmitted infections

INTRODUCTION

Screening in female patients for HPV and STI for early diagnosis of cervical cancer Introduction: The Human Papilloma Virus, a small non-enveloped, double-stranded DNA virus infecting the skin and mucosa has been associated with carcinogenesis and with the use of several tests like Pap test, is found to be one of the most common STI widely studied since 1980s.[1] Cervical cancer is the fourth most common cancer with 6,04,127 new cases and 3,41,831 deaths reported annually. It is the second most common cancer in Indian women with 1,23,907 new cases and 77,348 deaths reported per year.[2] The hrHPVs lead to cancers of cervix, vulva, vagina, anus, penis, larynx oral cavity head and neck.[1] HPV infection frequency differs

according to the anatomical site with higher prevalence shown in the anogenital than oral region. It is noteworthy that 80% of the women acquire at least one of the 15 hrHPV infections owing to the ease of transmission and ubiquitous nature of HPV. Only 10% of these become persistent to develop lesions that may result in a precancers/cancers.[3,4]

The major mechanisms through which HPV contributes to carcinogenesis involve 1. The persistent activity of viral oncoproteins, E6 and E7, which Interfere with major tumor suppressor genes, P53 and Rb 2. Changes in host DNA and virus DNA methylation. Interactions of E6 and E7 with cellular proteins and DNA methylation modifications are associated with changes in key cellular pathways that regulate genetic integrity, cell adhesion, immune response, apoptosis, and cellular control.[5]

The World Health Organisation expects every country to meet the 90-70-90 targets by 2030 to reduce the incidence rate of cervical cancer to less than 4 per 100000 women with 90 % vaccination by 15 years age, 70 % screening and 90 % treatment rate for pre-cancer/invasive cancer of cervix.

A major co-factor for the persistence of HPV is the presence of STI, exaggerating the risk of having cervical neoplasia by facilitating entry of multiple hrHPVs and decreasing local host ability.[6] *Chlamydia trachomatis* has been correlated with the severity of abnormal cervical cytology and HPV infection. *Herpes simplex* virus infection along with HPV increases risk of invasive cervical cancer as do *Trichomonas vaginalis*, *Mycoplasma* spp., and *Ureaplasma* spp. Thus screening for STIs along with HPV testing is of utmost importance especially in countries like India where the occurrence of cervical cancer has been reported to be common in the age group of 15-44 years and cancer diagnosis in 55 to 59 years. According to a study, only 19% of the population in low-middle income countries is targeted for HPV screening as compared to 63% in the developed countries.[7]

The current screening techniques include cytology evaluation, visual inspection, HPV testing, co-testing and the use of some protein biomarkers and next-generation sequencing (NGS)-based tests for integration of the viral genome.[2] Also, HPV vaccine is an important preventive tool against HPV.

Currently around 226 HPV genotypes have been identified with not all HPV genotypes being carcinogenic.[8] The IARC-WHO has classified the genotypes concerning carcinogenicity such that genotypes 6,11 are low-risk HPV, Group 1 hrHPV have genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (high-risk carcinogenic), Group 2A with HPV 68 (probably carcinogenic) and group 2B with HPV 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, and 97 (possibly carcinogenic).[1]

Considering the high prevalence of HPV worldwide and in India, and the expensive screening strategies, we decided to carry out HPV/STI panel screening in some patients at the Cama and Albless Hospital in Mumbai, Maharashtra for identifying the carcinogenic traits in the patients as a preventive measure against developing cervical cancer.

METHODS

Study population

The present pilot study included 40 females who visited the Cama and Albless Hospital in Mumbai, Maharashtra with inclusion criteria above 20 years of age, sexually active, with major complaints of itching, irritation, bleeding, and frequent white discharge on whom we performed the test for HrHPV and/or STI panel. Sample collection: A cervical/vaginal swab immediately placed in the liquid-based cytology container and sent to AyuGen Biosciences Pvt. Ltd, Pune for testing was used.

Test procedure

Detection: The HPV test for detecting the presence of hr-HPV is a US FDA-approved Cobas HPV test. The method is based on proprietary Taqman Real-Time PCR chemistry and is processed on a fully automated Roche-Cobas 4800 system. Being one of the very few tests that have received FDA approval, this test has undergone rigorous clinical validation and has more than 50 publications all over the world to its credit.

The test contains primers separately for 1) hrHPV 16, 2) hrHPV 18 and 3) a combined pool to detect hrHPV types 31,33,35,39,45,51,52,56,58,59,66 and 68. The test helps in detecting clinically significant HPV infection and whether the HPV type is 16 or 18 or the 12 other high risk types. For the STI test, we used multiplex real-time PCR using Taqman technology where we used organism-specific primer to detect specific organisms. The STI panel covered in the test involves the following organisms: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, *Ureaplasma urealyticum/parvum*, *Gardnerella vaginalis*, *Herpes simplex* virus 1/2.

RESULTS

The age range for study participants was a minimum 20 years to a maximum 67 with mean age of 41.9 years with their baseline characteristics noted. All 40 women underwent the HPV test, while 26 took both HPV and STI test accounting for 65% of the study population.

In the HPV testing study, out of 40 women only 3 tested positive for hrHPV such that of these 7.5 % cases, 2.5% tested positive for HPV-18, while 5% were positive for the other 12 hrHPV with none positive for HPV-16. The results are depicted in Table 1 and Figure 1.

Table 1: Results of HPV testing.

Test particulars	Number of women	Percentage (%)
Total number of women for HPV test	40	100
Positive for hr HPV 16	0	0
Positive for hr HPV 18	1	2.5
Positive for other hr HPV	2	5

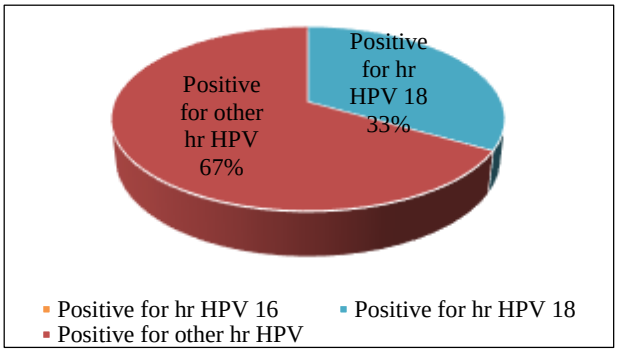

Figure 1: Proportion of HPV positive patients.

From the STI panel that we used in 26 women, we got some interesting findings showing the infections *Ureaplasma urealyticum/parvum* as highest. 13 women out of 26 (50%) tested positive for one or more microbe in the STI panel. Further analysis shows that 8 women out of 26 (30.76%) who took the STI test tested positive for both *Ureaplasma* and *Gardnerella vaginalis*. 4 women out of 26 (15.38%) tested positive only for *Ureaplasma* only while 1 woman among the 26 (3.84%) showed positive results only for trichomonas. Out of the 8 women who tested positive for both *Ureaplasma* and *Gardnerella*, two women also tested positive for hrHPV (one was positive for other hrHPV and one for hrHPV-18). The number of women testing positive for the STI panel is given in detail in Table 2.

Table 2: Results of STI testing.

Test particulars	Number of women	Percentage
Total women tested for STI panel	26	100
Positive for *Ureaplasma urealyticum/ parvum* and *Gardneralla vaginalis*	8	30.76
Positive for *Ureaplasma ureaplasma urealyticum/ parvum*	4	15.38
Positive for *Trichomonas vaginalis*	1	3.84

DISCUSSION

Our study was designed to primarily detect the presence of hrHPV and some women from this group were screened for STI after physically examining the signs and symptoms reported by them in the outpatient department of Cama and Albless Hospital in Mumbai. In the current study, the occurrence of hrHPV-18 was reported in 2.5% and of the other hrHPV was 5% of the study population. This screening acts as triage for cervical cancers helping to know which patients need additional intervention such as colposcopy. The coinfections of HPV and STI have been studied previously in different research groups and the existence is common.[9,10]

Similar findings were reported in this particular study. Our HPV genotyping was mainly focused on hrHPV-16 and hrHPV-18 and some other hrHPV genotypes as they are highly predictive of cancer. Similarly, the STI panel included the most common microorganisms associated with STI of non-HPV nature.[11,12] Our findings go hand in hand with the earlier studies. The high occurrence of *Ureaplasma urealyticum/parvum* and *Gardnerella vaginalis* is similar to the results obtained in another study of similar nature that shows around 60% prevalence of co infection of these two species.[13] *Ureaplasma* and *Gardnerella* along with many other bacteria are present in the commensal flora of the genital tract.[14] Any disturbance in the vaginal ecosystem results in the replacement of the lactobacilli that are most common in the vaginal ecosystem by an abnormally high number of bacteria such as *Ureaplasma* and *Gardnerella*, that may eventually cause an infection.[15]

This study can be considered as a contribution to establishing an association between hrHPV and the simultaneous presence of STI as a role in developing cervical cancer in India. The mechanisms involved co-infections of hrHPV and STI have been put forth by researchers and include 1. The interaction of HPV with other microbes present in the vagina that act as an HPV replication enhancer speeding up carcinogenesis.[2]. Inflammation due to STI/HIV increases penetrability of hrHPV. This needs larger clinical studies.

A similar study conducted in Saudi Arabia with 351 participants screened for HPV and STIs. The overall prevalence of STIs was 26.78% (94 positives). The most common pathogens were *Ureaplasma urealyticum/ parvum*, *Mycoplasma hominis*, HPV 6, 11. Of them, 142 previously tested positive for HPV and thus were included in the following analysis of which 49.2% tested positive for HPV 16, 25.3% for HPV 18 and 7% for HPV 31. The results of cytology testing showed that 256 of the cervical samples 72.9% were normal, 1.7% were classified as ASCUS, 3.4% were LGSIL, 6.2% were HGSIL, and 15.6% were cervical cancer. A significant association between cytology grades and HPV status was found ($P<0.005$). Of the 142 HPV-positive samples, 36 tested positive for an STI (odds ratio 25, $P<0.001$). The most common STIs in the HPV-positive samples were *Ureaplasma urealyticum*/*Ureaplasma parvum* (13.38%; n = 19, odds ratio 0.43, $P<0.01$), followed by *Mycoplasma*

hominis (6.3%; n=9), and HPV types 6 and 11 (4.2%; n=6).[16]

Another study conducted in Brazil showed that among 3512 participants, 276 had HPV/STI co-infection. Participants with HPV/STI co-infection were younger at first sexual intercourse than those with HPV only. People with HIV presented higher HPV positivity, with 25 HPV positive amongst 33 HIV cases. The other associated STI was gonorrhoea followed by herpes and syphilis. The study also found other variables like smoking (prevalence ratio (PR)=1.64), illegal drug use (PR=1.58) and same-sex sexual experience (PR=2.15) uniquely associated with coinfection compared to those associated with HPV infection only.[9]

A Greek study evaluated 345 women of which 61 women tested positive for HPV and 22 for STIs such as *Chlamydia*, *Ureaplasma* and *Mycoplasma*. Only 8.5% with an STI had cytology of ASCUS or LSIL.[17] The division of the research subjects according to their symptoms demonstrated that in HPV infection, 24.2% of the subjects were detected as asymptomatic and 35.8% as symptomatic; however, this difference was not statistically significant. Alternatively, STI bacteria were detected in 83.0% of symptomatic subjects, a significantly higher percentage than that of asymptomatic subjects (60.1%).[18]

In another Brazilian study, the total prevalence of HPV was 15.5%, although the prevalence increased significantly to 32.2% of the women aged less than or equal to 25 years of age. 23.8% of the women that reported having first sexual intercourse at less than 15 years of age, 20% of those that reported having more than one sexual partner over their lifetime but used contraceptives. The overall prevalence of sexual infection by C. trachomatis was 4.6% increasing significantly to 16.1% in the young women upto 25 years of age. Prevalence of HPV and C. trachomatis infection were 28% and 12%, respectively, in women who presented cytological alterations.[19]

This demonstration study, though very limited in sample size, shows that a screening programme using simple sample collection techniques can be used in the public institutions like Cama and Albless Hospital with minimal requirement of manpower and training. Public institutions in India are overburdened with gynaecological patients. Performing visual inspection by acetic acid in public institutions, though very cost effective, can be challenging since it requires time and skilled manpower. The method itself has limitations of subjective variation and much lower sensitivity compared to HPV testing. But if an accurate HPV test like Cobas hrHPV test if made available and affordable to the patients in public institutions can significantly help in reducing overall cost and resources in managing such patients. If available at free of cost this can be an opportunity to screen women that are vising the public hospitals. Identifying high risk women in time can help prevent the disease development to herself and also prevent the spread of the infection to others.

CONCLUSION

Our study shows a correlation of STIs primarily ureaplasma with high risk HPV which can increase risk of abnormal cervical cytology in women. These women should be offered treatment for the STI and HPV for better and early diagnosis of women for cervical cancer. The detection of HPV and STI present simultaneously can aid in establishing their role in the development of cervical cancer. Screening using standardized, accurate and automated HPV testing must be included in the HPV elimination programme being advocated in recent times in India. The women with STI and HPV identified in studies such as this would benefit from an increased frequency of follow up to watch for progression to cervical cancer. Adverse outcomes such as cervical cancer can thus be diagnosed early and further complications resulting from it causing death can be prevented. This will help reduce the burden of disease in countries such as India which have a high number of cervical cancer cases with a high risk of preventable mortality.

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