



OBSERVATIONAL STUDY OF SEROPREVALENCE OF HUMAN PAPILLOMA VIRUS (HPV) BY ELISA METHOD IN FEMALE PATIENTS ATTENDING IN GYNAECOLOGY OPD

Microbiology

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ABSTRACT

Background: Human papilloma virus (HPV) infection has been reported to be frequently occurring sexually transmitted disease. Although most HPV infections regress, persistent HPV infection, however is strongly associated with the risk of cervical cancer and genital warts. Present study was an observational study of seroprevalence of Human Papilloma Virus (HPV) by ELISA method in female patients attending in Gynaecology OPD. **Material and Methods:** Present study was cross-sectional study, observational study, conducted in female patients attending Gynecology OPD with clinically suspected cases of HPV. Each patient underwent ELISA Test for HPV. **Results:** Among 160 patients, majority of were from age group 31-40 years (39.38%), having education of secondary schooling (38.13%), were office workers (32.5%) followed by house-wife 25.63%. The majority of patients were in Class III (38.75%) followed by Class IV (25.62%) Seroprevalence among patients was 23.12%. The patients who used homemade napkins had a highest seroprevalence of HPV 68.18% followed by patients who are suffering from STD's (63.83 %), had a family history of cervical cancer (50 %), women who used to smoke (37.7 %), drink (25 %), had multiple sex partners (25 %) and the least number seropositivity was among patient who had gynecological problems 23.12%. **Conclusion:** The association of demographic profile and seropositivity of HPV among patients showed that educational status, age of marriage, mode of contraception, parity, socioeconomic status, risk factors and clinical symptoms showed statistically significant.

KEYWORDS

seropositivity of HPV, contraception, HPV infection, cervical cancer

INTRODUCTION

Human Papilloma Virus (HPV) is a non-enveloped deoxyribonucleic acid (DNA) virus belonging to the family Papillomaviridae. Human papilloma virus (HPV) infection has been reported to be frequently occurring sexually transmitted disease. Although most HPV infections regress, persistent HPV infection, however is strongly associated with the risk of cervical cancer and genital warts.¹

High-risk strains of the human papillomavirus (HPV) are now well established as the causative agents responsible for cervical dysplasia. Overall, HPVs associated with cervical cancer have been found in 6%-88% of females with cervical cancer in India.² Hospital based studies conducted on cervical cancer patients in south India confirmed HR-HPV types in majority of the samples.³

The prevalence of HPV in the general population is estimated to be between 9-13% worldwide and varies between 1.6-25.6 % countrywise.⁴ The high burden of cervical cancer in developing countries reflects the insufficiency and poor capacity of existing screening programs to detect precursor for or early stage of cervical carcinoma.⁵ So, HPV detection holds the potential to be used as a tool to identify women, at risk of subsequent development of cervical cancer. Present study was an observational study of seroprevalence of Human Papilloma Virus (HPV) by ELISA method in female patients attending in Gynaecology OPD.

MATERIAL AND METHODS

Present study was cross-sectional study, observational study, conducted in department of Microbiology with help from department of obstetrics & gynaecology, at XXX medical college & hospital, XXX, India. Study duration was of 2 years (August 2018 to August 2020). Study approval was obtained from institutional ethical committee.

The study population was female patients attending Gynecology OPD with clinically suspected cases of HPV, willing to participate in present study. While, pregnant patients, patients with bleeding PV (menorrhagia or metrorrhagia) were excluded.

Study was explained to patients in local language & written consent was taken for participation & study. A detailed history including the

demographic profile, presenting complaints, past history and risk factors were elicited for each patient and duly recorded in the case record form.

Each patient underwent ELISA Test, 5 ml of sample blood was collected for the same and subjected for serological testing for HPV antibody as per kit by using commercially available ELISA kits. A cut-off value turns the measured optical densities into positive or negative results. Samples with an OD 450 nm lower than the Cut-Off value below were considered not reactive for IgG specific to the HPV antigens. Samples with an OD 450nm higher than the Cut-Off value above were considered positive for IgG specific to the HPV antigens.

Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi-square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant.

RESULTS

Among 160 patients, majority of were from age group 31-40 years (39.38%) followed by 41-50 years (29.38%) The mean age of patients was 38.23 ± 20.50 years. The majority of patients were married (88.75 %), married at 18-20 years age (54.36 %), had parity of 2-4 (83.75%).

Table No 1: General Characteristics

Characteristics	No. of patients (n=160)	Percentage (%)
Age group (years)		
15-20	01	00.63
21-30	23	14.38
31-40	63	39.38
41-50	47	29.38
51-60	26	16.25
Marital status		
Unmarried	11	06.88
Married	142	88.75
Divorced/single	07	04.37

Age of marriage		
<18 yrs.	13	08.72
18-20 yrs.	81	54.36
21-25 yrs.	34	22.82
>25 yrs.	21	14.10
Parity		
Nullipara	09	05.63
Primipara	17	10.62
Multipara	134	83.75

The majority of patients were having education of secondary schooling (38.13%), were office workers (32.5%) followed by house-wife 25.63%. The majority of patients were in Class III (38.75%) followed by Class IV (25.62%)

Table No 2: Sociodemographic Characteristics

Characteristics	No. of patients (n=160)	Percentage (%)
Educational status		
Illiterate	25	15.63
Primary schooling	31	19.37
Secondary Schooling	61	38.13
Higher Secondary	32	20
Graduate & above	11	6.87
Occupational status		
Laborer	16	10
Office workers	52	32.5
Unemployed	23	14.37
Self employed	28	17.5
Housewife	41	25.63
Socioeconomic status	No. of patients	Percentage (%)
Class I	15	09.38
Class II	21	13.13
Class III	49	30.63
Class IV	52	32.5
Class V	23	14.38

Among 160 patients, it was observed that the seroprevalence among patients was 23.12%.

Table No 3: Serological Test Results

Serological test	No. of patients	Percentage (%)
Reactive	37	23.12
Non-reactive	123	76.88

Majority of women among 160 patients had symptoms of abnormal discharge (37.5 %), followed by vaginal complaints like itching, rash, ulcer (31.25 %), lower abdominal pain (19.37 %) and menstrual disturbances (16.87 %) respectively. The seroprevalence of HPV among women having symptoms of postcoital bleeding and dyspareunia was 100% followed by abnormal discharge (35.48 %), vaginal complaints like itching, rash, ulcer (33.33 %), menstrual disturbances (25.93 %).

Table no 4: Distribution of Clinical symptoms

Symptoms	No. of patients (n=160)	Percentage (%)	Positive patients (n=37)	Percentage (%)	p value
Abnormal discharge	60	37.5	22	35.48	<0.001
Vaginal complaints itching /rash/ulcer	50	31.25	20	33.33	
Pain lower abdomen	31	19.37	6	19.35	
Menstrual disturbances	27	16.87	7	25.93	
Genital wart	01	0.6	0	00.00	
Post-coital bleeding	01	0.6	1	100.00	
Dyspareunia	01	0.6	1	100.00	

Among 160 women, all had gynecological problems followed by women who were smoker (33.13 %), suffering from STD's (29.37 %), had alcohol drinking habit (25 %), had multiple sexual partner (5 %) and family history of cervical cancer (2.5 %).

The patients who used homemade napkins had a highest seroprevalence of HPV 68.18% followed by patients who are suffering from STD's (63.83 %), had a family history of cervical cancer (50 %), women who used to smoke (37.7 %), drink (25 %), had multiple sex partners (25 %) and the least number seropositivity was among patient who had gynecological problems 23.12%.

Table No 5: Prevalence Of Risk Factors

Risk factors	No. of patients (n=160)	Percentage (%)	Positive patients (n=37)	Percentage (%)	p value
Gynecological complaints	160	100	37	23.12	0.02
STD's	47	29.37	30	63.83	
Smoking	61	33.13	23	37.70	
Drinking	40	25	10	25	
Homemade napkins	22	13.75	15	68.18	
Multiple sex partner	08	5	02	25	
Family H/O of cervical cancer	04	2.5	2	50	

In present study, majority of patients were not using any contraception (68.12%), using OC pills (11.87 %), condoms (10.62%). HPV seroprevalence were maximum among OCP users (36.84 %), followed by IUCD users (36.36 %), condom use by husband (35.29 %) and no contraception user (18.34 %).

Table No 6: Contraception-use:

Contraception status	No. of patients	No. of positive patients	Percentage (%)	p value
None	109	20	18.34	0.005
OCP	19	7	36.84	
IUCD	11	4	36.36	
Condoms	17	6	35.29	
Others	04	0	0	
Total	160			

DISCUSSION

In the past decade aetiological association between infection with high-risk human papilloma virus and development of cervical cancer has been increased. A study conducted in 22 countries disclosed that 99.7% of invasive cervical cancer contained HPV-DNA, indicating that HPV may be a necessary cause of cervical neoplasia.^{1,6} Due to the central role of HPV in the carcinogenesis of cervical cancer, HPV testing has now been included in current clinical guidelines for the management of cervical disease in women.⁴

Determinants for seroreactivity against HPV include the number of lifetime sexual partners, HPV viral load and persistence of HPV infection.⁷ Advantages of serology include the ability to measure current and past infections, and the allowance for retrospective studies on serum banks when tissue samples are not available.

Serological tests are not presently commercialized or standardized, it is a useful tool for epidemiological studies and vaccine trials, but is of limited value for clinical diagnosis. There are more than 200 types of HPV types all of few of them causes cancer in different organs and their pathogenesis is very slow.^{5,7} Moreover 70 % of HPV infection regress from body within one year and 90% in 2 years. Thus, study of HPV is very important to control the HPV infection by preventive and further management.

In present study, out of 37 seropositive patients majority were from age group of 21-30 years (47.83 %) & from age group of 31-40 years (25.40 %). However, study conducted by MP Singh et al.,⁸ showed prevalence of high-risk human papilloma virus observed mean age of 34.48 years & in a study by Chandana V. et al.,⁹ majority of the subjects belong to age group 30-40 years 42.5%. This finding was in accordance to present study.

Our observed pattern could be attributed to waning of antibodies with time in the older age groups, or a cohort effect, while increased HPV infection exposure among women from the younger cohorts due to their higher-risk sexual practices.¹⁰ The sexual behavior pattern among younger women highlights the necessity to reinforce education on HPV vaccination and safer sexual practices to prevent not only HPV

transmission, but other STIs in general.¹⁰

In the present study the majority of patients were married (88.75 %), similar findings were noted by Chandana V et al.⁹ & Asha A. Elmi et al.⁵ In the present study, possibly indicating a lifespan HPV persistence and/or a reacquisition with increasing age due to changing sexual behavior and age-related changes of mucosal biology and immune competence.⁵ The long-term use of birth control pills appears to increase the risk of developing cervical cancer in women who have HPV, but experts say the risk is eliminated with careful screening.

The study of Bhattacharya A et al.¹¹ shows there was a significant ($p < 0.05$) overrepresentation of HPV prevalence among women who were users of oral contraceptive-pills, while study by Chandana V. et al.⁹ observed seroprevalence among OCP users 36.84% and among IUCD users 36.36% which coincides our study. Studies have shown that use of oral contraceptives is a risk factor for acquiring HPV, and prolonged use of oral contraceptives is associated with development of squamous intraepithelial lesions (29,47) which could be due to change in cervical mucosal epithelium by hormonal changes.

It has been shown that educational level is a socioeconomic factor with an effect on risk of HPV infection. The HPV positivity is inversely proportional to educational status of patients. This observation could be due to early indulgence in sexual activity and lack of awareness of its consequences.

In a study by Chandana V. et al.⁹ on prevalence and determinants of high-risk human papilloma virus observed majority of the study group belong middle class 68.4%. A study by Supriti Ghosh et al.¹² among tribal and general population shows maximum number i.e. 62.8% was in middle socio-economic group followed by low socio-economic. The HPV infection was also higher in the middle socio-economic status group followed by low socio-economic group which coincides our study.

A significant number of risk factors are associated with HPV infection like gynecological problems, smoking, STD's, alcohol drinking, multiple sexual partners, family history of cervical cancers etc, Acquisition of HPV infection has been shown to be strongly related to sexual behavior, and the prevalence of HPV increases with increasing number of sexual partners¹³ as women are constantly exposed to virus by allowing reinfection and persistence of the virus for years.

In our study, seroprevalence of HPV were 23.12%. Studies conducted by MP Singh et al.⁸ show prevalence 27.5% in FSW (female sex workers) and 23.5% in HC (healthy controls) whereas studies from Colapani V et al.¹⁴ show prevalence of 24.5% which is concordance of our study. However, studies conducted by M Aminu et al.¹⁵ and Chandana V et al.⁹ show higher prevalence of HPV 42.9% and 43.3% respectively. The variations in the prevalence may be due to variations in ethnicity and geography.^{16,17}

Advantages of serology include the ability to measure current and past infections. Serological tests are not presently commercialized or standardized. Serology is a useful tool for epidemiological studies and vaccine trials, but is of limited value for clinical diagnosis. ELISA method is more sensitive and specific than cytology method. The patient's compliance is more as blood is easily accessible. The HPV diagnosis by ELISA gives an idea about the seroprevalence of HPV in the general population however this test is for research purpose only and not for in vitro diagnostic use.

CONCLUSION

The association of demographic profile and seropositivity of HPV among patients showed that educational status, age of marriage, mode of contraception, parity, socioeconomic status, risk factors and clinical symptoms showed statistically significant. The prevalence increased with early marriages, high parity, and middle socio-economic status group. So, our study will guide for planning and implementation of governmental HPV infection control measures and the consequences of infection around the world.

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