



CLINICAL STUDY OF PREVALENCE OF HUMAN PAPILLOMA VIRUS (HPV) BY ELISA METHOD IN FEMALES ATTENDING GYNAECOLOGY OPD

Medical Microbiology

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ABSTRACT

Background: Women with normal cervical cytology, who are infected with high-risk HPV type, have an approximately 100-fold increased risk of developing cervical cancer compared with uninfected women. Present study was conducted to study seroprevalence of Human Papilloma Virus by ELISA method in female patients attending in Gynecology OPD of in a tertiary care hospital. **Material and Methods:** Present study was cross-sectional study, observational study, conducted female patients clinically suspected of having HPV infections like bleeding per vagina, irregular menstruation, pain in lower abdomen or post-coital bleeding or had any abnormal findings on per vaginal examination. **Results:** Among 160 patients, majority of were from age group 31-40 years (39.38%), married (88.75 %), married at 18-20 years age (54.36 %), had parity of 2-4 (83.75%). All females 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%. **Conclusion:** The sero-prevalence among patients for HPV was 23.12%. 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. ELISA method is an important diagnostic method in future in HPV infection detection as it has several advantages over other existing diagnostic methods in respect of early detection, reliability, cost effectiveness and patients compliance as blood can be easily accessible.

KEYWORDS

sero-prevalence, HPV, ELISA method, cancer cervix

INTRODUCTION

Cervical cancer is the commonest cancer among Indian women and the second most common cancer in the women worldwide. Women with normal cervical cytology, who are infected with high-risk HPV type, have an approximately 100-fold increased risk of developing cervical cancer compared with uninfected women.¹

According to the Indian Council of Medical Research (ICMR), the incidence of cervical cancer of India varies from 20-30/100,000 women between the age group of 35-64 years, while in developed countries, it is as low as 1-8/100,000 women.² The prevalence of HPV in the general population is estimated to be between 9 and 13% worldwide and varies between 1.6 and 25.6 % country wise.³

High-risk strains of the human papillomavirus (HPV) are now well established as the causative agents responsible for cervical dysplasia. HPV 16 is the most oncogenic, accounting for almost half of all cervical cancers, and HPV 16 and 18 together account for approximately 70% of cervical cancers.⁴ In India, strategies such as cervical cancer screening with combination of cytology, visual inspection methods, serological tests and prophylactic vaccination programs should be focused to reduce disease burden due to oncogenic HPV infection and cervical cancer. Therefore, the present study was conducted to study seroprevalence of Human Papilloma Virus by ELISA method in female patients attending in Gynecology OPD of in a tertiary care hospital.

MATERIAL AND METHODS

Present study was cross-sectional study, observational study, conducted in Department of Microbiology, in a Tertiary Care Hospital in Maharashtra, India. Study duration was of 2 years (August 2018 to August 2020). The study was approved by the Ethical Committee of the institute.

Inclusion Criteria

- female patients clinically suspected of having HPV infections like bleeding per vagina, irregular menstruation, pain in lower abdomen or post-coital bleeding or had any abnormal findings on

per vaginal examination, willing to participate in present study.

Exclusion Criteria

- Pregnant patients.
- Bleeding PV patients like PPH, menorrhagia or metrorrhagia patient.

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.

For ELISA Test 5 ml of blood was collected. Serum was separated at 3000 rpm for 10 minutes in centrifuge and subjected for serological testing for HPV antibody as per kit by using commercially available ELISA kits. Microplates are coated with recombinant VLPs derived from HPV Type 6, 11, 16 & 18. In the 1st incubation, the solid phase is treated with diluted samples and anti-HPV IgG are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2nd incubation bound anti-HPV IgG are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-HPV IgG antibodies present in the sample. 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 were entered into Microsoft Excel Worksheet. Statistical analysis was performed using IBM Statistical Package for the Social Sciences version 20 (SPSS v20, IBM). Statistical analysis was done using descriptive statistics.

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

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

Table No 2: Serological Test Results

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

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 %).

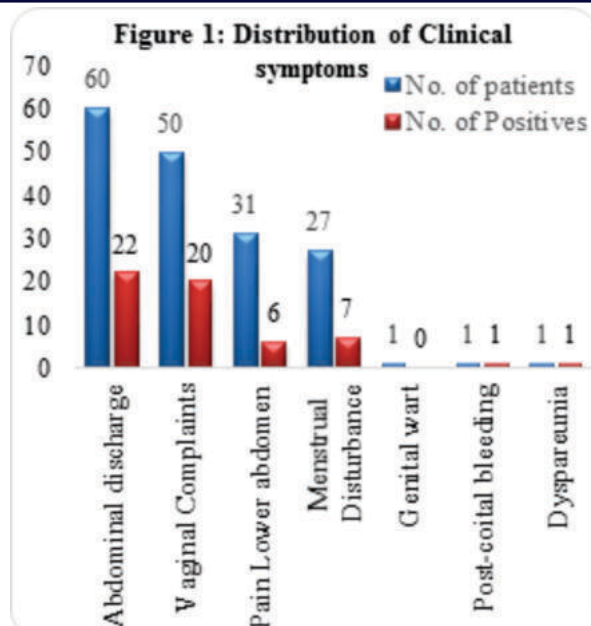
Table No 3: Contraception-Use

Contraception status	No. of patients	Percentage (%)	No. of positive patients	Percentage (%)
None	109	68.12	20	18.34
OCP	19	11.87	7	36.84
IUCD	11	06.87	4	36.36
Condoms	17	10.62	6	35.29
Others	04	02.50	0	0

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	Percentage (%)	No. of positive patients	Percentage (%)
Abnormal discharge	60	37.5	22	35.48
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

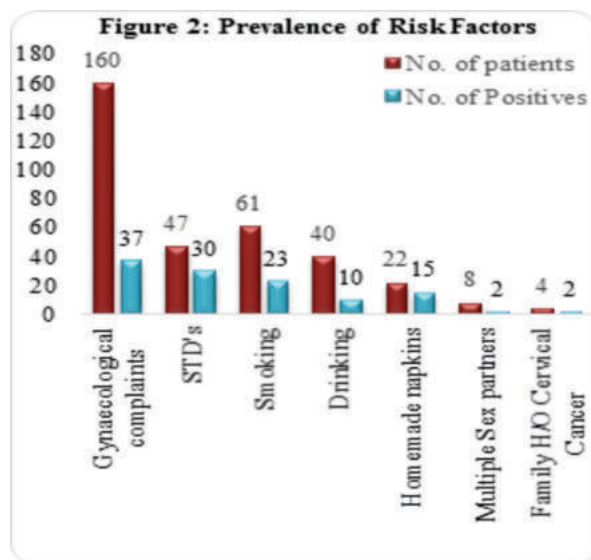


Among 160 women, all had gynecological problems followed by women who were smoker (33.13 %), suffering from STD's (29.37 %), had alcohol drinking habit (25 %), had multiple sexual partner (5 %) and family history of cervical cancer (2.5 %).

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	Percentage (%)	No. of positive patients	Percentage (%)
Gynecological complaints	160	100	37	23.12
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



DISCUSSION

HPV is widely prevalent sexually transmitted virus. Although the majority of infections are benign and transient, persistent infection is associated with the development of carcinoma cervix. In the past decade aetiological association between infection with high-risk human papilloma virus and development of cervical cancer has been increased.

Molecular methods for HPV detection offer promising alternatives as screening tools to improve the sensitivity of conventional cytology to detect prevalent high-grade lesions and provide near 100% negative predictive values. In turn, this could allow for better compliance at lower costs and increased screening intervals safely. Molecular methods for HPV detection are now accepted as tests for the triage of women with abnormal Pap smears for invasive diagnostic procedures.

HPV prevalence is generally constant across age groups in India, with no clear peak in young females.⁵ In the Osmanabad district study, the prevalence of HR-HPV types in the 30-39, 40-49 and 50-59 age groups were 9.8%, 10.4% and 12.2%, respectively.⁶ In a multicentre cross-sectional study in India, these were 7.0%, 6.8% and 7.5%, respectively.⁶ Also the population-based study in Dindigul, which included a broad age range of females 16-25 years, did not find any peak prevalence in younger age group. 44 Low clearance of incident infections, and underrepresentation of teenagers in study samples have been stated as factors responsible for the constant, steady prevalence of HPV infection in different age groups in India.⁷

Our study showed a decreasing trend in HPV seropositivity for HPV with increasing in age. Higher seropositivity was also previously reported in younger women, although no significant differences were observed across age-groups in that smaller study.⁸ However, study conducted by MP Singh et al.,⁹ showed prevalence of high-risk human papilloma virus observed mean age of 34.48 years & in a study by Chandana V. et al.,¹⁰ majority of the subjects belong to age group 30-40 years 42.5%. This finding was in accordance to present study. 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.⁸

Early marriage is a significant factor that has been known to be associated with HPV infection.¹⁰ The prevalence increased with early marriages, high parity, and low socio-economic status & education.¹⁰ Study emphasizes the aspect of primary prevention by good education, modification of sexual behavior, practice of good genital hygiene.¹⁰

The majority of patients were not using any contraception is 68.12%. The OC pills were used among 11.87% patients followed by condoms 10.62%. The numbers of patients used IUCD 6.87% and other methods 2.50%. In a study by Chandana V. et al.,¹⁰ on prevalence and determinants of high-risk human papilloma virus observed in most of the patients who never used contraception i.e.73.7%. While 13.16% woman used oral contraceptive pills, IUCD 7.52% & 5.64% women gave history of usage of condom by their husbands which coincides with our study. However a study conducted by Elmi A A et al.,¹¹ shows majority of patients were not using contraception.

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 which could be due to change in cervical mucosal epithelium by hormonal changes.^{12,13} Multi-parous women were found to have a higher risk of persistent/recurrent infection. explaining that such association may be due to the eversion of the columnar epithelium on the ectocervix, which renders it more vulnerable to the effects of HPV, and may be due to cervical trauma during delivery, the action of estrogen and progesterone and the physiological immune-suppression during pregnancy.^{14,15,16}

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 partners as women are constantly exposed to virus by allowing reinfection and persistence of the virus for years.^{2,17}

Determinants for seroreactivity against HPV include the number of

lifetime sexual partners, HPV viral load and persistence of HPV infection.¹⁸ 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.

In our study, seroprevalence of HPV were 23.12%. Studies conducted by MP Singh et al.,⁹ show prevalence 27.5% in FSW (female sex workers) and 23.5% in HC (healthy controls) whereas studies from Colapani V et al.,¹⁹ show prevalence of 24.5% which is concordance of our study. However studies conducted by M Aminu et al.,²⁰ and Chandana V et al.,¹⁰ show higher prevalence of HPV 42.9% and 43.3% respectively.

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.

In spite of the fact that India contributes to the maximum number of cervical cancer cases and deaths due to the same, there is a lack of National screening programs. In a resource poor country like India, the women from low socioeconomic areas get only once in a lifetime opportunity to undergo pap smear examination. In many instances, this is reported as obscure due to blood or unsatisfactory smears. Fluid-based thin layer processing of samples is a recently developed upcoming screening technique which reduces sampling error, makes cytological abnormalities clear and can detect multiple infections.²¹

The prospects of HPV vaccination and HPV based screening are being considered as options for cervical cancer control. It might take many years for poor countries to establish organized population-based screening programmes. Empowerment of women, Improvement of living standards and health education coupled with low intensity pap smear and provision of equitable resources for treatment can control HPV infection and bring down the cervical cancer incidence in the poor countries of the world.

CONCLUSION

The sero-prevalence among patients for HPV was 23.12%. 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. ELISA method is an important diagnostic method in future in HPV infection detection as it has several advantages over other existing diagnostic methods in respect of early detection, reliability, cost effectiveness and patients compliance as blood can be easily accessible.

Conflict of Interest: None to declare

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