



STUDY OF HUMAN PAPILLOMA VIRUS (HPV) & PAP SMEAR IN CLINICALLY SUSPECTED CASES OF HPV IN A TERTIARY CARE HOSPITAL IN MUMBAI

Clinical Microbiology

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ABSTRACT

Background: Cervical cancer is the commonest cancer amongst Indian women and the second most common cancer in the women worldwide. Persistent infection with high-risk HPV (HR-HPV) is the most important risk factor for cervical cancer precursors and invasive cervical cancer. Present study was conducted to study prevalence of Human Papilloma Virus (HPV) & correlation of PAP smear in clinically suspected cases of HPV. **Material and Methods:** Present study was prospective, observational study, conducted in 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:** In the present study, majority of patients were in age group 31-40 years (39.38%), married (88.75%), were married at 18-20 years age (54.36%) & had parity of 2-4 (83.75%). 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%), family history of cervical cancer (50%), women who used to smoke (37.7%). It was observed that serological test and PAP smear was positive among 03 patients with 34 false positive tests. Comparison between ELISA and cytological study showed that ELISA test was better, and finding was statistically significant; p value <0.001 (p= Fischer Exact test). **Conclusion:** Out of 160 PAP smears 2.20% were positive. The PAP smear positive patients were also seropositive of HPV by ELISA method.

KEYWORDS

PAP smear, HPV, ELISA, Cervical Cancer.

INTRODUCTION

Cervical cancer is the commonest cancer among Indian women and the second most common cancer in the women worldwide. Persistent infection with high-risk HPV (HR-HPV) is the most important risk factor for cervical cancer precursors and invasive cervical cancer. High-risk strains of the human papillomavirus (HPV) are now well established as the causative agents responsible for cervical dysplasia.^{1,2} Several epidemiological studies have confirmed that one or more of the oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 should be considered as a necessary cause for cervical neoplasia.³ 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 Protection against genital warts caused by HPV types 6, 11, 16, or 18 was likewise high, with the vaccine preventing 99% of these infections.⁴

With the advent of molecular techniques, particularly PCR (Polymerase Chain Reaction), it is possible to detect very low quantities of HPV and to subtype the commonly occurring HPV in cervical scrape smears.⁵ 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 in a tertiary care hospital to study prevalence of Human Papilloma Virus (HPV) & correlation of PAP smear in clinically suspected cases of HPV.

MATERIAL AND METHODS

Present study was conducted in a Tertiary Care Hospital in Mumbai, Maharashtra. It was a prospective, comparative, parallel-group and observational study, conducted in Department of Microbiology with help from Department of Obstetrics & Gynaecology. Study duration was of 2 years (August 2018 to August 2020). All the cases were followed up and records were kept for clinical features and test results and analyzed. Study approval was obtained from Institutional Ethical Committee.

Inclusion Criteria

- All female patients clinically suspected of having HPV infections like bleeding per vagina, irregular menstruation, pain in lower abdomen or post-coital bleeding.

- Any abnormal findings on per vaginal examination.
- Willing to participate in present study

Exclusion Criteria

- Pregnant patients.
- Patients who do not give consent.
- Bleeding PV patients like PPH, menorrhagia or metrorrhagia patient.

Written informed consent was taken from each patient for participation in the study after explaining the protocol to the patient in the language that the patient best understood. 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.

ELISA Test was performed on 5 ml of blood was collected from suspected HPV infected patients, serum was separated at 3000 rpm for 15 minutes in centrifuge and subjected for serological testing for HPV antibody as per kit by using commercially available ELISA kits.

For Cytological Study cervical swab was collected by gynaecologist and smear was prepared, fixed over slide by BIO FIX SPRAY and sent for cytological section for staining. The reporting was done by cytopathologist according to Bethesda system.

Data were entered into Microsoft Excel Worksheet. Statistical analysis was performed using IBM Statistical Package for the Social Sciences version 20 (SPSS v20, IBM). Categorical variables between two groups were compared with Chi-square test and Fisher exact test. A p value of <0.05 was considered significant.

RESULTS

In present study, majority of patients were in 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%. The unmarried and divorced/single patients were 6.88% & 4.37% respectively. Majority were married at 18-20 years of age (54.36%) & had parity of 2-4 (83.75%).

Table 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%.

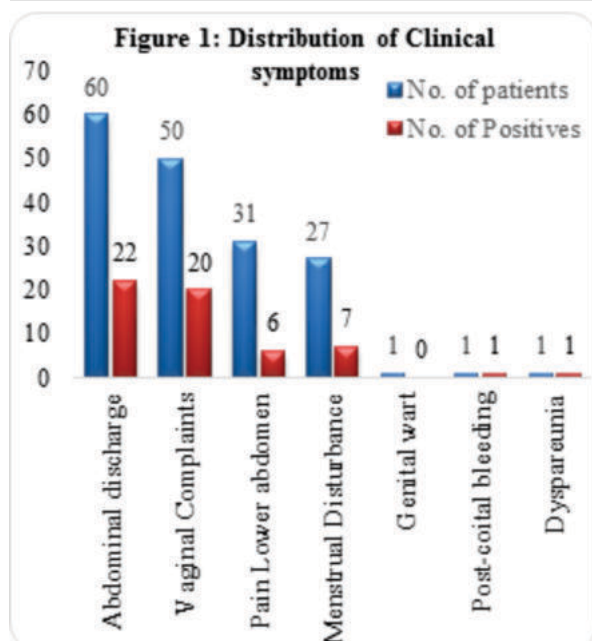
Table 2: Serological Test Results

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

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

Table 3: Distribution of Clinical symptoms

Symptoms	No. of patients	Percentage (%)	No. of positive patients	Percentage (%)
Abnormal discharge	60	37.5	22	35.48
Vaginal complaints	50	31.25	20	33.33
Pain lower abdomen	31	19.37	6	19.35
Menstrual disturbance	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 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%), family history of cervical cancer (50%), women who used to smoke (37.7%).

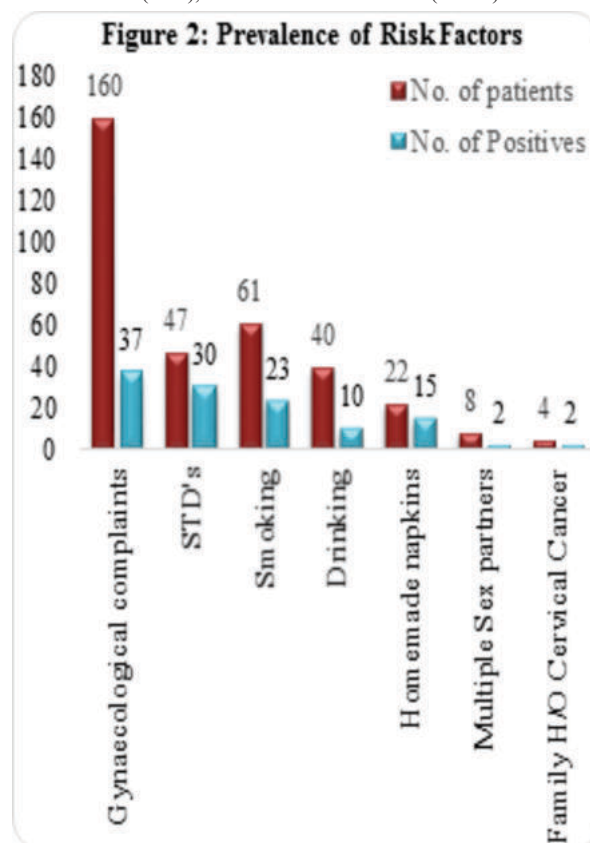


Table 4: Prevalence Of Risk Factors

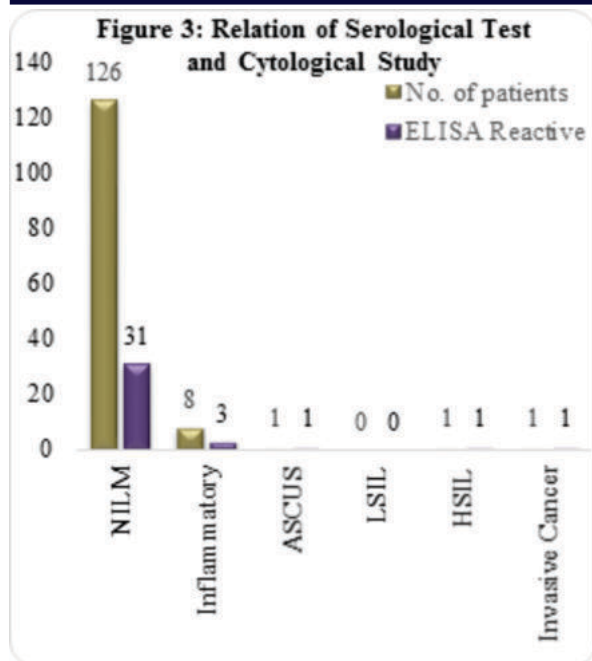
Risk factors	No. of patients	Percentage (%)	No. of positive patients	Percentage (%)
Gynecological complaints	160	100	37	23.12
STD's	47	29.37	30	63.83
Smoking	61	33.13	23	37.70
Drinking	40	25	10	25
Homemade napkins	22	13.75	15	68.18
Multiple sex partners	08	5	02	25
Family H/O cervical cancer	04	2.5	02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

Among 160 patient's PAP results were available to 137 patients by the Bethesda system as 14.37% patients' smears were inadequate for opinion due to unsatisfactory smear. Among the normal pap smear NILM (no intraepithelial lesion or malignancy) HPV positivity by ELISA were 24.6% and inflammatory group 37.5% whereas among the positive smears ASUS (atypical squamous cells of undetermined significance), HSIL (high grade squamous cells intra epithelial lesion) & squamous cell carcinoma all 100% positivity by ELISA.

It was observed in the study that serological test and PAP smear was positive among 03 patients with 34 false positive tests. Comparison between ELISA and cytological study showed that ELISA test was better than cytological study and finding was statistically significant; p value <0.001 (p= Fischer Exact test).

Table 5: Relation Of Serological Test And Cytological Study

	Cytological test (PAP smear)	No. of patients	Percentage (%)	ELISA Reactive	Percentage (%)	p value
Normal	NILM	126	14.37	31	24.6	≤0.001
	Inflammatory	8	91.97	3	37.5	
	ASCUS	1	5.83	1	100	
	LSIL	0	0.72	0	0	
	HSIL	1	0	1	100	
Positive	Invasive Cancer	1	0.72	1	100	



ELISA	PAP smear		Total	p value
	Positive	Negative		
Reactive	03	34	37	≤0.001
Non-reactive	00	100	100	
Total	03	134	137	

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. 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.⁶

There are different methods for diagnosis of HPV but ELISA method has good sensitivity and specificity when it is done along with other diagnostic procedures. Although ELISA test is not for diagnostic purpose and limited to research purpose only but this test could be used for diagnostic purpose if sensitivity and specificity increases.

Because of the use of PAP smear screening programs, the incidence of cervical cancer had declined over the past 50 years but has now stabilized.^{5,7} However, in a primary screening mode, the sensitivity of the conventional PAP test for detecting cervical cancer precursors taken at any given time has been shown to average 50%. Repeated PAP tests are associated with poor patient compliance with return visits; thus, the conventional PAP test is cost-ineffective for cervical cancer screening.

Most of the women are asymptomatic with HPV infection in the general population and they do not seek any medical advice. Only a portion of symptomatic patients attend gynecological consultation and symptoms vary widely from abnormal discharge, pain abdomen, irregular menstruation, vaginal complaints like ulcer, itching, genital warts etc.

A study by Aminu M et al.,⁸ shows abnormal discharge (37.7%), followed by abnormal vaginal symptoms included itching, rash, and ulcer (36.6%) which were similar to our studies. In a study by Chandana V. et al.,⁹ the chief complaints with which the study population attended were white discharge (32.95%), menstrual disturbances (15.64%) and pain abdomen (18.35%) which coincides our study. A study by Ghosh S et al.,¹⁰ shows among the clinical symptoms post coital bleeding and dyspareunia were 0.8% and 0.6% respectively while study by Angela Pista et al.,¹¹ showed 0.9% had genital warts which coincides our study.

In our study, seroprevalence of HPV were 23.12%. Studies conducted by MP Singh et al.,¹² show prevalence 27.5% in FSW (female sex workers) and 23.5% in HC (healthy controls) whereas studies from

Colapani V et al.,¹³ show prevalence of 24.5% which is concordance of our study. However, studies conducted by M Aminu et al.,⁸ and Chandana V et al.,⁹ show higher prevalence of HPV 42.9% and 43.3% respectively.

Enlargement, hyperchromasia, irregular membranes and double nucleation of the superficial and intermediate cells are the hallmark of HPV infection. Malignant change occurs in transitional zone where intra-cervical glandular columnar cell joins with ecto-cervical squamous cell. The sensitivity of conventional cytology smears in detecting significant cervical lesions ranges from 29% to 80%, a relatively poor level of sensitivity that is partially compensated by repeated testing after short intervals.^{14,15,16}

Study by S Franceschi et al.,¹⁷ shows a variation in unsatisfactory smear which varies high as 11% to as low as 2.54%. Our study shows 14.37% were inadequate. This might be related to method of collections and staining techniques as well as experience of cytopathologist. However, 5.83% were inflammatory, this may be due to complaint of abnormal discharge due to infection other than HPV.

In a study by Chandana V et al.,⁹ reported the positive pap smear was 2.3% where a study by Angela Pista,¹¹ shows pap smear positivity 6.2% which is in concordance of our study. PAP smear usually positive in chronic stage of disease.

In a study by Chandana V. et al.,⁹ showed correlation of cytology with HPV ELISA 37.3% HPV ELISA positivity were among inflammatory group whereas 100% positivity in HSIL and squamous cell carcinoma and the study by Angela Pista et al.,¹¹ shows 100% ELISA positivity of HPV among ASC-H (atypical squamous cells where HSIL cannot be excluded) and HSIL which coincides with our study.

PAP test is the end result, which may be positive when there is chronic local inflammation in the cervical mucosa which may leads to abnormal cell changes. Whereas, ELISA detects IgG antibody for HPV which cannot differentiate between chronic and acute infection. Conventional cytology methods need highly skilled person for collection, staining and reporting. While ELISA test is commercially available and easy to run and interpret the result. ELISA test is more sensitive and specific than cytology method. The patient's compliance is more in serological tests as blood is easily accessible.

CONCLUSION

Out of 160 PAP smears 2.20% were positive and 97.80% were negative. The PAP smear positive patients were also seropositive of HPV by ELISA method. There is a great difference of seropositivity of HPV and PAP smear positive which is 23.12% and 2.20% respectively. The study results reflect the need for further studies in understanding what lies between the causal exposure and the disease end point. HPV testing in cervical screening programs has an important role to play; the management of HPV seropositive cases need further attention as it progresses to cervical cancer.

Conflict of Interest: None to declare

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