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Research Article

FREQUENCY OF HPV INFECTION AND RELATED DYSPLASIA, INCLUDING MALIGNANCY, DETECTED BY PAP SMEAR : A STUDY OF 200 CASES

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A R T I C L E I N F O

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A B S T R A C T

Context: HPV infection is a well recognized risk factor for cervical cancer and its precursors. HPV can be detected in pap smears well in advance of development of carcinoma and appropriate action can be taken. However, despite availability of various cervical cancer screening methods, as well as large burden of disease in India, routine screening is still not practised effectively.

Objective : This study was conducted to assess the burden of HPV infection, detected by pap smears and its association with cervical cancer and precursor lesions.

Material and methods: 200 pap smears from various age groups were randomly selected for cytological examination, and examined specifically for HPV induced changes, which was confirmed later by serological testing and the findings were correlated with the Bethseda category.

Results: Out of 200, 72 cases (36%) were HPV positive. Most of the carcinomas (87.5%), HSIL (88.9%) and LSIL (80%) were positive for HPV infection. 64.3% of ASC-US and 50% of ASC-H were also positive for HPV infection.

Conclusion : HPV infection of cervix is quite prevalent among females in India. Pap smear is good screening method to detect these cases, which may result in dysplasia and malignancy.

INTRODUCTION

Cervical cancer is the second most common cancer of the women worldwide. In developing countries, cervical cancer is often the most common cancer in women and may constitute up to 25% of all female cancers [4]. The development of cervical cancer is strongly associated with previous infection with the human papilloma virus (HPV) and cervical cancer represents 53.4% of the total number of HPV-associated cancers in women[1]. Therefore, cervical cancer prevention is closely tied to the prevention of HPV transmission and recognition of patients who are infected with HPV and therefore at higher risk for developing cervical cancer.

Scientists have identified about 30 HPV types that are spread through sexual contact and infect primarily the cervix, vagina, vulva, penis, and anus. Of these, four are most often found within the malignant cells of cervical cancers, with type 16 accounting for about half of the cases in the United States and Europe and types 18, 31, and 45 accounting for an additional 25 to 30% of cases [4]. Decades of studies have confirmed that cervical infection by high-risk HPV types is a precursor event to cervical cancer. The natural history of cervical cancer as a continuous single disease process progressing gradually from mild cervical cervical intraepithelial neoplasia (CIN1) to more severe degrees of neoplasia and microinvasive lesions (CIN2 or CIN3) and finally to invasive disease has been the basis for diagnosis, therapeutic measures, and secondary preventive strategies[5].

HPV infection can be diagnosed by specific morphological changes in the host cells, by the virus infection, through cytology, histopathology, colposcopy, immunocytochemistry and by molecular methods. Non-oncogenic HPVs as well as oncogenic HPVs have the capacity to induce cytologic changes, but only integrated HPVs are able to produce the oncogenic transformation of the cells[7].

The risk of progression of mild dysplasia to severe dysplasia was only 1% per year, while the risk of progression of moderate dysplasia to severe dysplasia was 16% within 2 years and 25% within 5 years. Nonetheless, it is agreed that early detection and subsequent early treatment of HPV in precancerous lesions can prevent progression to cancer[6].

Data suggest that more than half of cervical cancer cases could be prevented through adequate screening. Fifty percent of women diagnosed with cervical cancer have never undergone cervical cytology testing and another 10% have not received screening in the five years preceding their diagnosis[2]. Furthermore, cervical cancer is very rare among screened women (less than 10 per 100,000 annually)[3].

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HPV cannot be cultured in the laboratory from clinical specimens and immunologic assays are not adequate for detection of HPV infections. The primary diagnostic tools have been cytology and histology. Recently, molecular methods to detect HPV DNA sequences in clinical specimens have been introduced.

The primary method for detection of high-risk HPV is still the Papanicolaou-stained (Pap) smear. The Pap smear is a screening tool that looks for changes in cells of the transformation zone of the cervix. Often these changes are caused by HPV. In Papanicolaou (Pap) smears diagnosis of HPV infection is based on classical changes (CC) (koilocyte) as well as on non-classical cellular changes (NCC). Diagnostically, koilocyte is an excellent indicator of HPV infection where intermediate and superficial cells show paranuclear halo with eccentric single or binucleated hyperchromatic nuclei with mild irregularity of outline. These are characteristic of the last stage of viral replication and infective genome released as koilocytes are shed, so koilocyte is regarded as the most pathognomic feature with high degree of specificity. However, it is not always present in HPV infected cells, especially those from dysplastic lesion[8]. Non-classical features, which are reflected in cells like rounding of cell margin, mild nuclear hyperchromasia and eccentric nuclei, might be indicator for HPV infection[9].

The current reporting system for pap smears is the Bethesda System (Table 1).

MATERIALS AND METHODS

The study was conducted at the Department of Pathology, Northern Railway Central Hospital, New Delhi, from 2015-2016.

Case selection: 200 cases were randomly selected for the study, from pap smears received for cytological examination for various reasons.

Light microscopic examination of the pap stained slides was done and reporting was done based on Bethesda system (Table 2). Also HPV induced cytopathic changes were noted (both classical and non-classical) (figures).
Suspected cases for HPV induced changes were sent for HPV serology, for confirmation.
Confirmed cases with HPV infection were categorized as follows (Table 3)

- HPV changes with mild dysplasia.
- HPV changes with moderate dysplasia.
- HPV changes with severe dysplasia.

These findings were then correlated with the Bethesda category **(Table 4).**

RESULTS

Table 2**:** Out of 200 cases, 103 cases were reported as negative for intraepithelial lesion/malignancy, 42 cases were reported as ASC-US, 04 cases were reported as ASC-H, 25 cases were reported as LSIL, 18 cases as HSIL and 08 cases were reported as carcinoma (Bethesda system).Out of 200 cases, 114 cases showed no detectable HPV induced changes and remaining 86 cases showed HPV induced changes (classical and non-classical), with/without dysplasia. 72 out of these 86 cases, were serologically positive for various strains of HPV. Remaining 14 cases which were serologically negative for HPV were then described as HPV like changes.

Table 3**:** Out of 72 serologically confirmed cases, 19 cases were having mild dysplasia, 30 cases were having moderate dysplasia and rest 23 were having severe dysplasia.

Table 4**:** Out of 23 cases with HPV and severe dysplasia, 7 were carcinoma and 16 cases were HSIL. Out of 30 cases with HPV and moderate dysplasia, 17 were LSIL and 02 were ASC-H and 11 were ASC-US. Out of 19 cases with HPV and mild dysplasia, 16 were ASC-US and 03 were LSIL. 1/7 case of carcinoma, 1/18 case of HSIL. 4/25 cases of LSIL and 5 /25 cases of ASC-US (41/52) didn't show any HPV change.
To summarise, 36% cases (72/200) were HPV positive. Most of the carcinomas (87.5%), HSIL (88.9%) and LSIL (80%) were positive for HPV infection. 64.3% of ASC-US and 50% of ASC-H were also positive for HPV infection.

DISCUSSION

In today's era, in spite of the availability of HPV vaccines and affordable and effective methods for early detection and treatment of cervical cancer precursor lesions, cervical cancer still continues to be a public health problem in India. The high burden of cervical cancer in India and Southeast Asian countries is due to poor to moderate living standards, a high prevalence of HPV (more than 10% in women aged more than 30 years) and due to lack of screening[10].

Table 1 Various systems for pap smear reporting

Bethesda system 1999	Bethesda system 1991	Cin system	Interpretation
Negative for intraepithelial lesions or malignancy	Within normal limits	normal	No abnormal cells
ASC-US (atypical squamous cells of undetermined significance)	ASCUS (atypical squamous cells of undetermined significance)		Squamous cells with abnormalities greater than those attributed to reactive changes but that do not meet the criteria for a squamous intraepithelial lesion
ASC-H (atypical squamous cells, cannot exclude HSIL)			
LSIL (low-grade squamous intraepithelial lesions)	LSIL (low-grade squamous intraepithelial lesions)	CIN 1	Mildly abnormal cells; changes are almost always due to HPV
HSIL (high-grade squamous intraepithelial lesions) with features suspicious for invasion (if invasion is suspected)	HSIL (high-grade squamous intraepithelial lesions)	CIN2/3	Moderately to severely abnormal squamous cells
Carcinoma	Carcinoma	Invasive squamous cell carcinoma Invasive glandular cell carcinoma (adenocarcinoma)	The possibility of cancer is high enough to warrant immediate evaluation but does not mean that the patient definitely has cancer

Table 2 Categorization of cases by Bethesda system.

Bethesda category (1999)	No. Of cases (out of 200)	Percentage
Negative for intraepithelial lesion/ malignancy	103	51.5%
ASC-US	42	21%
ASC-H	04	02%
LSIL	25	12.5%
HSIL	18	09%
Carcinoma	08	04%
Total	200	

Table 3 HPV changes detected by light microscopy, in 72 serologically confirmed cases.

Category	No. of cases	Percentage
HPV change with mild dysplasia	19	26.4%
HPV change with moderate dysplasia	30	41.6%
HPV change with severe dysplasia	23	31.9%
Total	72	

Table 4 Correlation of Bethesda category with HPV findings.

Bethesda Category	HPV Changes					Total
	Not detected	Detected but serologically negative (HPV like changes)	Detected and serologically positive			
			Mild dysplasia	Moderate dysplasia	Severe dysplasia	
Negative for ntraepithelial esion/malign ancy	103					103
ASC-US	05	10	16	11		42
ASC-H		02		02		04
LSIL	04	01	03	17		25
HSIL	01	01			16	18
Carcinoma	01				07	08
Total	114	14	19	30	23	200

Following observations were noted

- Out of 200, 72 cases (36%) were HPV positive.
- Most of the carcinomas (7/8, 87.5%) were HPV positive.
- Most of the HSIL (16/18,88.9 %) were HPV positive.
- Most of the LSIL (20/25, 80%) were HPV positive.
- ASC-H had HPV in 50% cases.
- ASC-US had HPV in 64.3% cases.
- All the cases reported as negative for intraepithelial lesion/malignancy (103/103) were HPV negative.

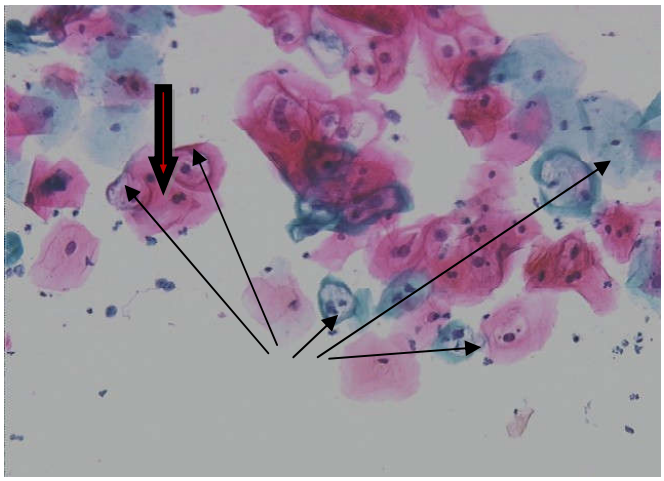

Figure 1 HPV induced change with mild dysplasia

Here we see, koilocytes with heterogenous cytoplasm, perinuclear halo and binucleation (thin arrows). Hyperchromatic nuclei (thick arrow) and mild nuclear pleomorphism, suggestive of mild dysplastic changes (pap, 40x).

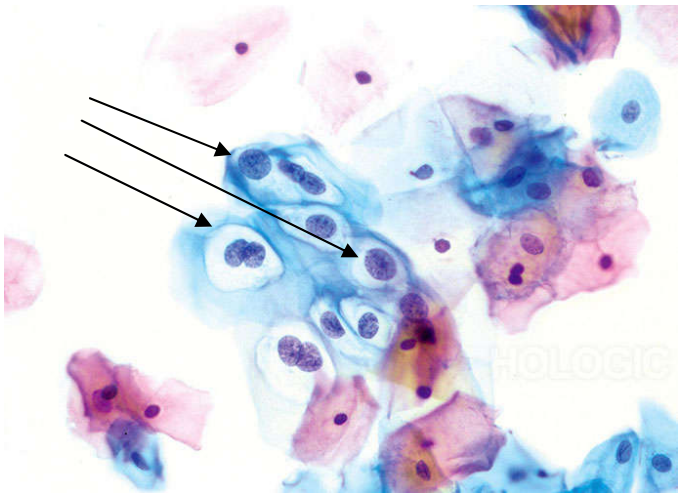

Figure 2 HPV induced changes with moderate dysplasia

Here we see, typical koilocytes with enlarged nuclei (arrow) (pap, 40x)

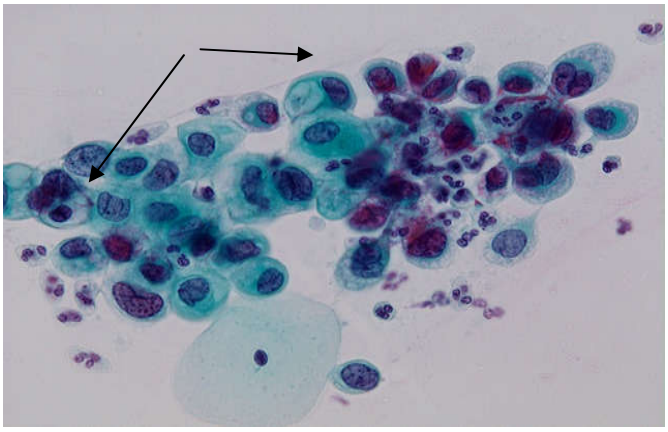

Figure 3 HPV induced changes with severe dysplasia.

Here we see, highly dysplastic squamous cells, with few HPV infected cells (arrow) (pap, 40x).

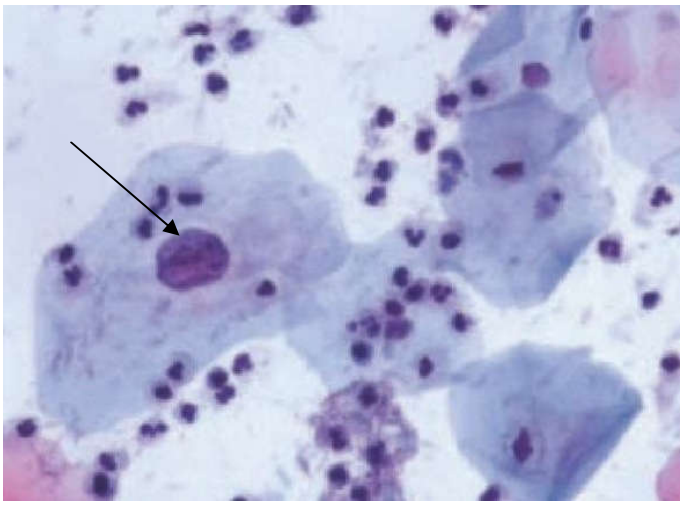

Figure 4 ASC-US

Here we see, a single atypical cell with enlarged and hyperchromatic nuclei (arrow) (pap, 40x).

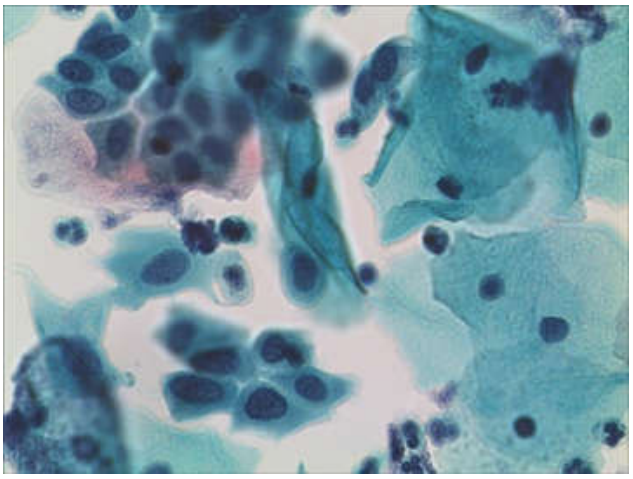

Figure 5 ASC-H

Here we see, cells with hyperchromatic, enlarged nuclei, but nuclear contour is regular HSIL can't be ruled out (pap, 40x)

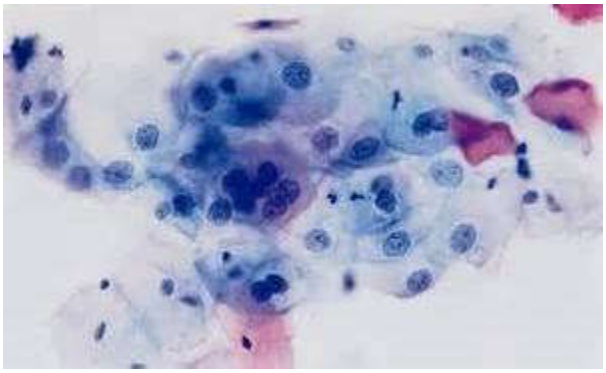

Figure 6 LSIL

Here we see, cells with nuclear enlargement and few hyperchromatic nuclei (pap, 40x)

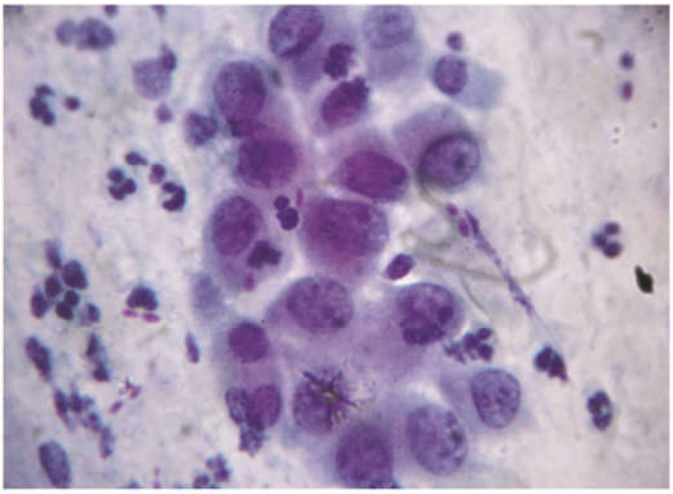

Figure 7 HSIL

Here we see, moderate to severe cytological atypia (pap, 40x).

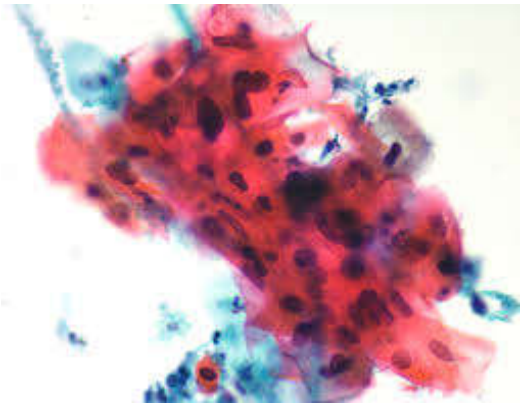

Figure 8 carcinoma

Here we see, keratinized highly dysplastic squamous cells, typical of squamous cell carcinoma (pap, 40x).

Two meta-analyses study was conducted worldwide in which ***Bruni et al.*** found that an estimated 7.9% Indian women had HPV infection (4.8%-36.8%)[11]. In our study, we found 36% of the women were HPV positive. This higher prevalence may be due to the fact that it was conducted in a hospital setting, so more chances of high risk patients to be included in the study. ***Guan et al.*** found that Indian women with cervical disease had higher incidence of any HPV (normal cytology 7.6%, CIN1 42.3%, > CIN2 87.5%)[12] and this proportion increased with severity of disease[13]. In our study, we also found similar results, with negative HPV serology in all cases with normal cytology (100%), reported as negative for intraepithelial lesion/malignancy and increasing trend towards higher grade lesions (carcinoma 87.5%, HSIL 88.9%, LSIL 80%, ASC-H 50% and ASC-US 64.3%).

Findings in our study can contribute to previous studies published in the literature regarding the prevalence of HPV infection in cervical smears with abnormal cytology and emphasize the importance of screening methods to detect cervical cancer at the earliest, even before the development of frank malignancy. Routine screening should be promoted more meticulously in India and awareness of general public is must in this regard.

CONCLUSION

Based on the results in our study, we can conclude that HPV is a major contributor in the development of cervical carcinoma and its precursor lesions. Pap smear is a good screening tool to detect HPV changes, which should be confirmed by serological testing or other methods.

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