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Early Detection of Carcinoma Cervix and Role of Human Papilloma Virus and Chlamydia Trachomatis in Etiology of Cervical Intraepithelial Neoplasia and Carcinoma Cervix

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ABSTRACT

Aims: To study the various methods available for early detection of carcinoma cervix along with role of HPV and Chlamydia trachomatis as cofactor in causing CIN and Carcinoma cervix. METHODS: This was a prospective study from January 2009 to July 2010 which was conducted at G.S.V.M. Medical College, Kanpur. The present work was carried out on 74 women presenting to the out patients department of Upper India Sugar Exchange Maternity Hospital, Kanpur. Cytology, colposcopy directed biopsy, HPV DNA detection by Polymerase Chain Reaction and Chlamydia trachomatis antigen detection was done in all 74 patients in a subsequent manner. After analysing, the result was obtained by various methods. Conclusion: Maximum number of patients i.e. 39 (52.66%) were in the age group 31-40 years. All patients were multiparous. Most common presenting symptom was vaginal discharge, present in 41 patients (55.41%). According to cytological findings, maximum number of cases i.e. 30 (40.54%) had inflammatory picture and 13 (17.58%) cases showed koilocytic changes which indicate HPV infection. It was also seen that HPV positivity was significantly associated with cytological findings suggestive of pre-malignant and malignant lesions. (P value is 0.0001) Histologically, 43 cases (58.10%) showed changes of CIN. The Chlamydia was found positive in 8 (50%) HPV DNA positive cases and 2 cases (3.45%) were HPV DNA negative. Thus it is concluded from this study that HPV infection is sexually transmitted disease acting like a agent along with cofactors (C.Trachomatis) in the development of preneoplastic and neoplastic lesion of the cervix. Thus follow up of the patients by HPV DNA detection alongwith Chlamydia antigen as cofactor can be used as an adjunctive to the cytology preventing the requirement of the unnecessary interventions.

KEYWORDS Cervical intraepithelial lesion(CIN), human papilloma virus(HPV)

Introduction

Carcinoma cervix is the second most common cancer among women. It is the disease which has been well known before Christ and yet its pathogenesis remains enigma. Combined efforts of Gynaecologist, Pathologist, and Oncologists led to the recognition of precancerous lesion possible. This leads to possibly diagnosing cancer cervix in its early stages. According to Singh et al [1] it has been estimated that annually about 90,000 women develop cervical cancer in India, which contributes to about 16% of the world's annual incidence. The reported incidence of pre invasive carcinoma in India is around 1.5/1000. Hunter et al [2] reported that over the last 2 decades the incidence of the cervical carcinoma has increased particularly in the young females.

Screening for the cervical cancer is the most prevalent, cost effective and successful public health measure for the prevention of cancer.

A screening programme directed to the precursor lesion can be expected reduce the incidence of the cervical lesion, as well as mortality and morbidity of the disease. Screening procedure is most effective in early detection of malignancies in which the transit time from low grade to invasive carcinoma is more. The transit time from low grade CNI to high grade CINII is about 5-6 years, and from high grade to invasive carcinoma is approximately 10years.

Elovanio et al [3] reported that PAP smear has become a routine method of cervical cancer screening and its clinical utilization has certain limitations like false negative rate. Colposcopy introduced by Hans Hinselmann et al [4] as an adjunct to cytology possibly increases the accuracy of cervical cancer detection to almost 100%.

Even after ten decades, cervical biopsy continues to be gold standard. It is done to confirm the results of cytology and colposcopy but is also indicated in a woman with negative smear where symptoms or signs suggestive of the cancer or pre-cancer are present.

A high prevalence of Human Papilloma Virus infection is reported in healthy women, especially those over 40years. It has been found to be associated with cervical dysplasia.

Screening is normally done by cytological study of a PAP smear which detects only 50-80% of the abnormality found on histological examination of cervical biopsy. According to Lorinez et al [5] pap smear combined with identification of HPV DNA, augments our ability to identify women who are infected with HPV and those that are at high risk for CIN.

Infection caused by Chlamydia trachomatis is one of the most common sexually transmitted disease over worldwide, which may be asymptomatic in more than 50% of cases.

Hare MJ & Coworkers et al [6] said that the prime target of Chlamydia infection in the lower genital tract of females is columnar epithelial cells lining of endocervical canal.

Madeleine et al [7] found Chlamydia trachomatis as a cofactor with HPV infection in its association with cervical intraepithelial neoplasia and carcinoma cervix. Epidemiological data support the role of HPV and Chlamydia trachomatis in carcinoma cervix not as the sole causative agent but rather as initiating agent in the multistep process of neoplastic transformation. Simonetti et al [8] had similar findings.

Methods

The present work was carried out on 74 women presenting to the out patients department of Upper India Sugar Exchange Maternity Hospital, whose complaints or findings were of an unhealthy cervix warranting pap smear examination. The common complaints were persistent vaginal discharge, post coital bleeding, post menopausal bleeding, leukoplakia, pruritis vulvae, dyspareunia, backache and irregular bleeding etc.

All women were subjected to detailed history, clinical examination including general examination, local examination, per speculum and bimanual pelvic examination to note any abnormality on cervix, uterus and adenexa.

All women were subjected to cervical cytology and then colposcopy which provides an optical method for examining the illuminated cervix.

Cervical biopsy was done after defining the abnormal areas colposcopically.

HPV DNA detection was done in this study by the polymerase chain reaction. Chlamydia antigen detection was done by taking Cervical swab using the VIDAS Chlamydia specimen collection kits and (enzyme linked fluorescent assay- ELFA) was applied to detect the antigen.

Aims

1) To study the various method available for the early detection of the carcinoma cervix.
2) To study the human papilloma virus as an etiological factor in the causation of CIN lesions and carcinoma cervix.
3) To study the role of Chlamydia trachomatis as a cofactor of HPV in the etiological role of CIN lesions and carcinoma cervix.
4) To study the various strain of human papilloma virus most commonly associated with CIN lesions and carcinoma cervix.

Results

Total number of patients included in the study was 74. Most of the cases (52.66%) were found in age group of 31-40 years. All patients were multipara, and the majority (82.44%) had 3 or more children. 83.78% were from low socio-economic strata with poor hygiene maintenance. In our study the most common presenting symptom was vaginal discharge in 55.41% cases followed by the menstrual disturbance in 22.98% cases and then backache in 12.16% patients. According to the cytological finding, 40.54% had an inflammatory picture which includes nonspecific, trichomonal and monilial infections. 17.58% cases showed koilocytic changes which indicate HPV infection. Total premalignant and malignant cases detected by cytology (Low and high grade squamous in-situ lesion and carcinoma in-situ) were 21(28.38%). 3 smears were unsatisfactory. Colposcopy was performed in all the 74 patients. Commonest finding was acetowhite epithelium in 33.75% cases, while mosaic was seen in 4.05% and punctuate was present in 2.70% patients. Atypical vessels were seen in 1 case only (1.35%). All the patients underwent PCR to detect HPV DNA. 78.38% cases were HPV DNA negative and the rest were HPV DNA positive. 10 cases were such which showed HPV related changes on colposcopy but PCR could not detect HPV DNA in them. On histopathological examination, it was found that 58.10% patients showed changes of cervical intraepithelial neoplasia (grade I, II and III included), 32.44% were inflammatory, 8.11% were normal. 1 case out of the 74 (1.35%) showed invasive disease i.e. carcinoma. There was 100% HPV DNA positivity with invasive disease. 4.17% patients with inflammatory changes had HPV DNA positivity. HPV DNA was strongly associated with all the grades of CIN. 50% of the HPV DNA positive patients also depicted Chlamydia infection. In our study 96.55% cases had both HPV and Chlamydia negative.

Discusssion

Cervical cancer is the commonest malignancy found amongst Indian women and the third most common cancer in the world. Over 5,00,000 new cases of invasive cervical cancer are diagnosed annually worldwide. Although the incidence of cervical cancer has decreased in developed countries as a result of screening by cytology, it is still the single largest cause of lives lost to cancer in developing countries as cytology based screening programmes are almost non-existent and ineffective.

Table 1 shows cervical lesions were found mostly above the age of 30years (70.3%). The mean age of women with cervical lesions was 34.54 +_ 2 S.D. No cases were recorded below 20 years of age. Our findings are in accordance with that of Bhatia and Iyer [9] in which the 78% cases were above the age of 30 years.

Most of the cases were having three or more children indicating that trauma following childbirth can predispose to cervical cancer. According to the Wahi [10], incidence of the dysplasia increases with the parity. Invasive carcinoma most frequently occurs in multiparous women as according to Jeffcoate et al[11]. In present study, no case was recorded amongst the nulliparous group. So it is evident that parity affects the incidence of cervical cancers and our findings are consistent with those other authors.

In our study maximum cases 62(83.78%) belonged to the lower socio economic status. Te Linde et al[12] stated that the risk of cervical cancer is highest amongst the Latin American, Caribbean, sub-Sahara Africa, southern and South east Asia, poor and uneducated population. This probably is due to the poor standard of hygiene, which facilitates infections by other organisms as well.

Table 2 shows that most common presenting symptom in this study was the vaginal discharge 41(55.35%). Next common presenting symptom was menstrual disturbance, which occurred in 17(22.95%) cases followed by backache in 9 cases(12.15%) and post coital bleeding in 5(6.75%). Sasieni et al[13] stated the vaginal bleeding is the most common symptom occurring in patents with carcinoma of cervix. Often it is post-coital bleeding. Patients with advanced disease present with vaginal malodourous discharge, weight loss or obstructive uropathy.

Table 3 suggests that cytological findings with maximum number of the cases 30 (40.5%) had inflammatory picture on cytology. 13(17.55%) cases showed koilocytosis suggestive of HPV infection. Total premalignant and malignant cases detected by cytology were 21 (28.38%) cases while 3(4.05%) cases show unsatisfactory smear because of drying effect and presence of excess necrosis and blood. These results were in close conformity with the other studies. A study conducted by Enrique Boccardo et al [14] showed that by targeting different innate and adaptive immune signalling pathways, as well as by interfering with cell cycle and death cycle, high risk HPVs are strong carcinogens to humans. Infact cervical cells co-infected with C. Trachomatis and HPV secrete large amounts of pro-inflammatory cytokines which may be the consequence of more profound inflammatory state

Most of the inflammatory smear group includes nonspecific and specific bacterial infections like Chlamydia, trichomonial, HPV, monilial infections. Our findings are comparable to a study by Philip Castle et al [15].

The cytology can help in identifying the women at risk of development of pre malignant and malignant lesions of the cervix, which can be further assessed by colposcopy directed biopsy and HPV DNA testing for the proper management. Cytology class normal and inflammatory were taken as cytological negative and rest taken as cytologically positive. A comparison between HPV DNA positivity and cytological positive and negative cases yielded a P-value of 0.0002 which is suggestive of a highly significant statistical association. A relative risk of 15 was found with 95% confidence interval between 2.086 to 107.86. Pilch H et al [16] showed that HPV DNA was detected by PCR in 150 of 203 (73.4%) tissue specimens of cervical cancer patients. DNA sequence analysis revealed the presence of HPV 16 (n = 68, 45.3%), HPV 18 (n = 49, 32.6%) and rare HPV types (n = 33, 22.1%).

Table 4 shows that after excluding negative cases (inflammatory cases), the commonest finding in dysplasia and carcinoma in situ was acetowhite epithelium 25 (33.75%) followed by mosaic 3 (4.05%) and punctuate in 2 (2.70%) cases. Atypical vessels were found in 1(1.35%) case suggestive of severe dysplastic or invasive change. In our study, colposcopy was unsatisfactory in 5(6.75%) cases. These were either due to postmenopausal picture or there was a polyp present. Eileen M. Burd [17] showed that patients with abnormal Pap smear findings who do not have a gross cervical lesion are usually evaluated by colposcopy and colposcopy-directed biopsy. Acetowhitening and the vascular patterns characteristic of dysplasia or carcinoma can be seen.

The clinical usefulness of colposcopy in diagnosing HPV infection was also evaluated in our study. Only 5 out of 16 HPV positive cases (31.25%) were truly positive cases showing HPV changes in colposcopy. The specificity of colposcopy was found to be 82.76% with a false negative rate of 14.86% (11 cases with HPV DNA out of 74 patients had normal colposcopy). A study was conducted by Anuradha et al [18] for comprehensive evaluation of cervical cancer. The study was carried out on 100 patients with abnormal pap smear. On initial pap smear, 62% were inflammatory, 23% low grade, 14% high grade and one case was suspicious of malignancy. On further comparison with colposcopic findings, it was found that the initial pap smear results were only 50% sensitive and 78% specific. The sensitivity of repeat smear was 80% and specificity was 78%. Colposcopic examination was as such 93% sensitive and 93% specific. Combining cytology and colposcopy, sensitivity was 93% and 95%.

Table 5 shows the correlation of histopathological findings with HPV DNA Positivity. 26.67% cases of CINI were positive for HPV. 50% cases of CINII, 33.34% cases of CINIII and 100% cases of carcinoma in situ were positively associated. There was statistically significant association between histological findings of premalignant and malignant lesions of cervix and HPV DNA positivity P value 0.0046.

Hopman et al[19] and Nobbenhius et al[20] showed that over 90% of patients who developed Pap smear abnormalities had persistent high risk HPV infection.

The 100% positive rate in our study can be a very well overestimate due to the small sample size.

Moreover in our study, the prevalence of HPV16 is more (13.53%) as compared to HPV 18(6.75%). Only single case was found positive for other type of HPV54. This data is well supported by the study conducted by BAUER et al[21], in which HPV16 is the most common HPV found in premalignant and malignant lesions of cervix. It is also the most common HPV found in normal cytology.

HPV DNA increased with the cervical lesion severity, ranging from 30% among women with normal cytology to 99–100% among women with HSIL or ICC. ***C. trachomatis*** DNA prevalence increased from low levels in women with normal cytology (11%) to 47% in those with HSIL, but was uncommon among ICC patients (20%). Among women with normal cytology, ***C. trachomatis*** prevalence was higher in HPV DNA positive (12.5%) than HPV DNA negative women (10.9%), but this difference was not significant.

Table 6 shows that Chlamydia antigen was found positive in 8 (50%) HPV DNA Positive cases and in 2 (3.45%) HPV DNA negative cases. This suggests a high correlation between HPV DNA Positivity and Chlamydia antigen. A relative risk of 14.5 was found with 95% confidence interval between 3.41 to 61.66. Our study is well supported by the study conducted by Koskelaet al [22] which provides sero-epidemiological evidence that infection with C.Trachomatis along with HPV confers an increased risk for subsequent development of invasive squamous cell carcinoma of the cervix.

Conclusion

Thus it is concluded from this study that HPV infection is a sexually transmitted disease acting like a agent along with cofactors (C.Trachomatis) in the development of preneoplastic and neoplastic lesion of the cervix. Thus follow up of the patients by HPVDNA detection alongwith Chlamydia trachomatis antigen can be used as an adjunctive to the cytology preventing the requirement of the unnecessary interventions.

A number of studies have been done in various parts of the world and an effective vaccine has been developed against HPV so we can be hopeful that the combination of vaccination against HPV, screening HPV DNA by polymerase chain reaction and Chlamydia antigen testing, we will be able to prevent more and more number of cases from developing HPV and Chlamydia infection and it's adverse effects such as cervical intraepithelial neoplasia and carcinoma cervix.

TABLE 1 DEMOGRAPHIC PROFILE OF CERVICAL LESIONS

S.no	Age group	No of patients	Percentage	Parity	No of patients	Percentage%	Socio-economic status	No of patients	Percentage %
1.	<20	0	0	P0	0	0	Low	62	83.78
2.	21-30	22	29.79	P1	5	6.75	Middle	10	13.52
3.	31-40	39	52.66	P2	8	10.81	High	2	2.70
4.	41-50	10	13.50	>P3	61	82.44			
5.	>50	3	4.05						
	TOTAL	74	100		74	100		74/100	100

TABLE 2 DISTRIBUTIONS ACCORDING TO THE CHIEF COMPLAINT

S.no.	Chief Complaint	No. Of Patients	Percentage %
1.	Vaginal Discharge	41	55.41
2.	Menstrual Disturbance	17	22.98
3.	Backache	9	12.16
4.	Post Coital bleeding	5	6.75
5.	Dyspareunia	2	2.70
Total		74	100

TABLE3 DISTRIBUTION OF CASES ACCORDING TO THE CYTOLOGICAL DIAGNOSIS AND ASSOCIATION OF HPV INFECTION

S.no.	Cytological Diagnosis	No. of Patients	Percentage	HPV Positivity	Percentage	HPV Negativity	Percentage
1.	Normal	7	9.46	0	0	7	100
2.	Inflammatory	30	40.54	1	3.33	29	96.6
3.	LSIL	15	20.39	9	60	6	40
4.	HSIL	4	5.37	2	50	2	50
5.	Koilocytosis	13	17.58	3	23	10	77
6.	Carcinoma in situ	2	2.70	1	50	1	50
7.	Unsatisfactory	3	4.06	0	0	3	100
TOTAL		74	100	16	100	58	100

TABLE 4 DISTRIBUTION OF CASES ACCORDING TO THE COLPOSCOPIC FINDING AND CORRELATION OF THE CASES WITH THE HPV INFECTION

S.no.	Colposcopic Findings	No. Of cases	Percentage	HPV changes	No. of cases with HPV DNA	No. of cases without HPV DNA
1.	Normal	4	8.11	Present	5 (6.76%)	10 (13.52%)
2.	Unsatisfactory	5	6.75	Absent	11 (14.86%)	48 (64.86%)
3.	Negative	32	43.25			
4.	Aceto white	25	33.78			
5.	Mosaic	3	4.06			
6.	Punctate	2	2.70			
7.	Atypical Vessels	1	1.35			
TOTAL		74	100		16 (21.62%)	58 (78.38%)

TABLE 5 DISTRIBUTION OF CASES ACCORDING TO THE HISTOLOGICAL FINDING AND CORRELATION OF THE CASES WITH THE HPV INFECTION

S.no	Histological finding	No.of Patients	Percentage	HPV DNA Positivity	Percentage	HPV DNA Negativity	Percentage
1.	Normal	6	8.11	0	0	6	100
2.	Inflammatory	24	32.44	1	4.17	23	95.83
3.	CIN I	30	40.54	8	26.6	22	73.33
4.	CIN II	10	13.50	5	50	5	50
5.	CIN III	3	4.06	1	33.34	2	66.66
6.	Carcinoma	1	1.35	1	100	0	0
Total		74/100	100	16	100	58	100

TABLE 6 CORRELATION OF THE CHLAMYDIA TRACHOMATIS ANTIGEN WITH THE HPV DNA INFECTION

S.no	HPV DNA	No. of cases	Chlamydia Positivity	Percentage	Chlamydia Negativity	Percentage
1.	Positive	16	8	50	8	50
2.	Negative	58	2	3.45	56	96.55

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