Human Papilloma Virus & its correlation with CIN lesion of cervix by four different methods

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Summary

Presently there is mounting evidence of strong association of CIN lesions and HPV infections. Screening was performed in 100 cases for HPV infection of cervix. Cytology, colposcopy, and histopathology predicted presence of HPV infection. Predictive value of cytology was 21%, colposcopy 30% and histopathology was 31%. Microscopic diagnosis of HPV infection is highly specific although not very sensitive. All Papilloma viruses share common antigenic determinants making it possible to detect genus specific viral antigen. Immunoperoxidase staining was done for detecting HPV virus. Immunoperoxidase staining could localize 16% cases of HPV. Frequency of HPV positivity increased as the grade of CIN increased.

Introduction

The decline in the morbidity and mortality for invasive squamous cell carcinoma of cervix over past several decades attests the importance of identifying the women who are at greater risk for developing the disease. Ayre (1960) for the first time suggested probable viral etiology of cervical cancer. He postulated that the viral infection might represent "a stepping stone" between normal, premalignant and malignant cells. As there is strong association of CIN lesions and HPV infections, these patients need thorough investigations for the presence of HPV. Thus they can be treated at the premalignant stage itself.

Objective Cytology, colposcopy and histopathological interpretation of tissues are used world wide for diagnosis of HPV infection. Recently it has been possible to detect genus specific antigen in tissues by means of immunologic reagents.

Design – Prospective cross sectional diagnostic test hospital based study.

Methodology – Government Medical College Hospital Nagpur is a tertiary care hospital where 100 patients were screened for presence of HPV infection. Selection criteria were patients of papillary cervical erosion, endocervicitis, and hypertrophied cervix on visual inspection. Patients with persistent white discharge and postcoital bleeding were also considered for the study. Thus all patients were high-risk patients of STD. Exclusion criteria were when any of the tests was inconclusive They were subjected for cytology and colposcopy. Reid's (1984) colposcopic ind

was used for the diagnosis of HPV infection.
Grade I 0-2 score – Subclinical HPV / CIN I
Grade II 3-5 score – CIN I – CIN II
Grade III 6-8 score – CIN II-CIN III

Biopsy was taken in all 100 cases from suspected sites like the acetowhite area or abnormal capillaries. Histopathology and Immunoperoxidase staining of the biopsy specimen were done. Mcleod (1987) wart scoring was used for histological diagnosis of HPV infection. Histologically HPV infection may occur as exophytic venereal wart (condyloma acuminata)- Tissue stalk supporting proliferating epithelium with acanthosis, papillomatosis and hyperkeratosis. Subclinical infection (flat condyloma) is also a variety of HPV. It is slightly thickened epithelium with prominent koilocytosis in the uppermost layer. Nuclei are hyperchromatic with irregular mitosis in the basal and parabasal layer. Coexistent CIN I and II is commonly found in flat condyloma.

Microscopic diagnosis of HPV was based on Koilocytosis and dyskeratocytosis. Koilocyte is the intermediate cell recognized by perinuclear halo. Cytoplasm is pushed to the periphery of the cell with atypical nucleus. Mcleod (1987) distinguished koilocytes in squamous epithelium by their nuclear morphology as members of two populations A & B. In type A koilocytes the nucleus is large bizarre shape and has a granular appearance. While type B Koilocytes have small round nucleus with regular outline with a dense homogenous cytoplasm.

Dyskeratocytosis is a cluster of superficial cells. There is premature keratinisation in the form of dense eosinophilic cytoplasm. Nuclei are opaque and hyperchromatic. Wart score includes acanthosis-epidermal and intermediate cell hyperplasia,

parakeratosis, orthokeratosis, dyskeratosis and submucosal capillary proliferation.

All histologically proven HPV tissue sections cut at 5 micrometer were subjected for Immunoperoxidase staining. Peroxidase – anti peroxidase method was used. Hydrogen peroxidase methanol was used as endogenous peroxidase blocking agent. Substrate utilized for peroxidase staining was 3,3 – diamino benzydine tetrahydrochloride. (DAB). Antibody reaction site was identified as brown black resin like nuclei or granular precipitate in nuclei.

Results

On screening 100 high-risk patients HPV with CIN was detected maximum in age group of 20-30 years with 3 parity. 56% had early marriage at the age of 16 years. Clinically 83% had white discharge and 4% had no symptoms. On visual inspection 33% had cervical erosion and 44% were with hypertrophied unhealthy cervix.

HPV on cytology was 32%, 29% with inflammatory smear and 3% with dysplastic smear (Table 1).

On colposcopy 40% had abnormal transformation zone. Subclinical HPV infection is most common lesion of squamous epithelium of the genital tract. Colposcopy detected 33% cases of HPV and minor group of CIN. There were 4 cases of CIN II and 3 cases of CIN III. Colposcopy observations (Table II) were recorded according to classification by Coppleson and Browne (Reid et al 1980). Reid's wart indexing was done in-group III (40) cases with atypical transformation zone. There was increased wart scoring with increased koilocytic atypia. Table III indicates wart scoring. Biopsy was taken in all 100 cases.

Table I cytology reports

Inflammatory	HPV	Metaplasia	CIN I	CIN II	CIN III
59 (24%)	18 (30.50%)	22	6	2	11

Table II- Colposcopy observations.

Colposcopy group Coppleson and Browne	%	-	-	-
Group I Normal	22%	-	-	-
Group II typical	38%	-	-	-
Group III atypical	40%	Reid score	Number of cases	%
		0-1	33/40	82.50%
		3-5	3/40	0.75%
		6-8	4/40	1%

Table III Histopathology observations : CIN lesions with koilocytes

	CIN	CIN I	CIN II	CIN III	Koilocytes
20-30 years	21	15	4	2	8
31-40 years	3	0	2	1	2
41-50 years	0	0	0	0	0

Histopathology could detect HPV in 28 cases, 18 cases with inflammatory lesion and 10 cases with different CIN lesions. A & B koilocytes distribution is shown in table III and IV. Positivity of HPV antigen was associated predominantly with type A koilocytes.

HPV virus location was possible only in 16% by Immunoperoxidase staining (table V). Table VI reveals ten patients of CIN with Immunoperoxidase positive cases to have association with multiple risk factors. It indicates need for proper evaluation of these high-risk cases. Screening should be done by a triaged system of cytology, colposcopy and histopathology for koilocytic atypia. These patients need further investigations like LEEP cone biopsy of cervix to rule out invasive malignancy.

Table IV : A & B koilocytes distribution on histopathology

Koilocytes	CIN I	CIN II	CIN III
A	4	2	1
B	2	0	1

Table V Immunoperoxidase positivity

Histopathology	Total	Immunoperoxidase positivity	Koilocyte A	Koilocyte B
Chronic cervicitis	59	6 (9.83%)		
CIN	24	10 (24%) CIN I	4	2
		CIN II	1	1
		CIN III	1	0
		CIS	1	0
All	83	16 (33%)		

Table VI gives the view of presentation of 10 Immunoperoxidase positive cases in CIN lesions of cervix.

S.N	Marital status	parity	Age at first coitus	Age at first pregnancy	Visual inspection of cervix	Cytology	Colposcopy R-INDEX	Histopathology
1	Married	3	15	16	Hypertropied + Erosion	Inflammatory	0-2 grade III	CIN I + HPV
2	Married	3	14	18	Erosion	HPV	0-2 grade III	CIN I + HPV
3	Married	3	20	22	Hypertrophied	HPV	0-2 grade III	CIN II + HPV
4	Remarried	2	18	19	Erosion	Severe dysplasia	5-8 grade III	CIS + HPV
5	Separated	4	15	18	Erosion	HPV	0-2 grade III	CIN I
6	Married	4	14	16	Erosion	HPV	Grade III	CIN III
7	Married	3	20	22	Erosion	Inflammatory	Grade III	CIN III + HPV
8	Married	2	18	19	Hypertrophied + Erosion	Inflammatory	0-2 grade III	CIN I + HPV
9	Married	4	18	20	Hypertrophied +	Metaplasia	Grade I	CIN II + HPV
10	Married	2	12	16	Hypertrophied + Erosion	Inflammatory	0-2 grade III	CIN I + HPV

Table VII showing test results of cytology, colposcopy and histopathology

		Immunoperoxidase Positive	Immunoperoxidase Negative	Total
Cytology	Positive	7	25	32
Cytology	Negative	9	59	68
	All	16	84	100
Colposcopy	Positive	12	28	40
Colposcopy	Negative	4	56	60
	All	16	84	100
Histopathology	Positive	9	19	28
Histopathology	Negative	7	65	72
	All	16	84	100

Table VIII Accuracy of different tests for HPV infection

	Cytology	Colposcopy	Histopathology
Sensitivity	43	75	56
Specificity	70	66	38
Predictive Value	21	30	32

Subpermissive HPV infection of squamous epithelium give rise to insufficient viral reproductive cycles with low production of viral antigens. Secondly viral expression is a late phenomenon. Thus latent or nonproductive HPV infections are not picked up. Presence of nuclear positivity and cytoplasmic negativity for HPV antigen indicates proliferative lesions, it probably suggests the role of HPV in the natural history of the disease.

Table IX have detected HPV infection by Immunoperoxidase staining

Authors			Immunoperoxidase positive	%
Reid and Stanhope 1987	I	46	22	48.00
	II	22	2	50.00
	BIOPSY		24	48.00
	50			
	I	10	2	20.00
Mecleod 1987	II	14	11	7.14
	III	8	6	75.00
Present 1990	I	15	6	27.77
Study	II	5	2	35.48
	III	3	2	60.00

Discussion

All Papilloma virus share common antigenic determinant making it possible to detect genus specific viral antigen in tissue by means of immunology reagents. Thus in the present study HPV was localized in 16% cases. 10% with CIN and 6% with chronic cervicitis. Other authors (Table IX) have detected HPV infection by Immunoperoxidase staining in the range of 48% by Reid and Stanhope (1987) and 75% in Mecleod study.

Reid and Stanhope (1987) first time studied 50 cases showing predominant koilocytic atypia in cervical cancer, with the help of peroxidase anti-peroxidase technique. In the histopathology study 48% were HPV positive cases. If Viral antigen is expressed in a reasonably high amount only can this technique detect HPV.

Thus, Mecleod (1987) commented that HPV antigen positivity is found in less number of cases.

References

1. Ayre J.E.; Obst. Gyn. 15: 481, 1960.
2. Mecleod K, J. Clin. Path. 40 (3) 323, 1987.
3. Reid R. Laverty C. R., Coppleson M., Ischangkar W, Hill E., Obst. Gyn. 55, 476, 1980.
4. Reid R, Stanhope R,. Herschman B R, Crum C.P, Am. J. Obst & Gyn 149: 815, 1984.
5. Reid R, Stanhope R, Am. J. Obst. Gyn 157: 809, 1987.