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Follow up Biopsy Interpretation of Cytologically proved Cervical HPV Infection

KEYWORDS: Koilocyte, HPV, cervical carcinoma

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ABSTRACT *Human papilloma Virus (HPV) is a dominant factor in cervical dysplasia and carcinogenesis. The morphological hallmark of HPV infection of cervical squamous epithelium is koilocytoosis, a change that is related to expression of the viral E4 protein and the disruption of the cytoplasmic keratin matrix. Koilocytes indicate the presence of productive HPV infection in exfoliated cells and biopsy specimens. The topic has been under taken with aim to study the HPV related change in uterine cervical tissue by routine H& E satin that have been already proved to be infected by HPV in the respective cervical scrapping. The result of biopsy revealed presence of koilocytes in the respective tissue sections which has already been HPV positive; hence it may be concluded that without undergoing specific molecular test for HPV, cytology and histopathology may help to detect HPV related change in smear as well as tissue section.*

Introduction:

HPV infection is very common in sexually active women. The infection by HPV has been established as a major cause for development of cervical carcinogenesis[1]. More than 100 HPV types exist out of which, HPV 16, 18, 31, 33 and 35 have been detected in association with up to 80% of high grade lesions (CIN 2and 3) and with up to 90% of invasive cervical cancers [2, 3]. CIN is a spectrum of dysplasia confined within the epithelium that begins in the basal layers and progressively involves other layers to form carcinoma in situ. The major steps known to be necessary for cervical carcinogenesis include HPV infection, persistence of that infection, progression to precancerous lesions and eventually invasion. Provided that the latter step has not taken place, this process is reversible by the clearance of HPV infection and regression of pre cancer, which happen in many women who have ever experienced HPV infection[4]. HPV infection may be differentiated into low-viral load infections that cause no microscopically evident abnormalities and higher-viral load infections that do [4].

The morphological hallmark of HPV infection of cervical squamous epithelium is koilocytoosis, a change that is related to expression of the viral E4 protein and the disruption of the cytoplasmic keratin matrix [5]. The koilocyte was first described by Koss and Durfee [6], and its association with exophytic and flat wart virus(HPV) infections of the female genital tract was established on cytology by Meisels and Fortin in 1976[7] and Purola and Savia in 1977 [8] with colposcopic and histological confirmation following in 1977[9]. Although molecular methods are available for detecting papillomavirus infections of the female genital tract, the presence of koilocytes in Pap smears and cervical biopsies remains fundamental to pathological diagnosis [10].

The Bethesda System recommends a low-grade/high grade approach to grading SIL/CIN. This is based on the evidence that most low-grade squamous intraepithelial lesion is transient infections that carry little risk for oncogenesis, whereas most HSILs are associated with viral persistence and a significant potential for progression to invasive cancer [11].Cytomorphology of LSIL is characterized by presence of intermediate-sized cells showing nuclear atypia and enlargement, irregular contour, hyperchromasia, slight chromatin coarseness, cytoplasmic cavities (koilocytes) and sometimes individual keratinization[12]. Histologically, these lesions have atypia in the form of either i) Flat condyloma, ii) Exophytic condyloma & iii) CIN1. All of these low-grade lesions are the result of productive viral infections in which large numbers of viral particles are generated.

Cytomorphology of HSIL usually involves parabasal-sized cells showing marked nuclear atypia and enlargement, marked irregularity in contour, marked hyperchromasia , marked chromatin coarseness and sometimes indivudal keratinization is present. HSIL is usually a lesion of immature squamous cells. It shares variable morphology, especially nuclear atypia in all layers of squamous epithelium, at least one portion of the lesion.ie presence of immature cells. There is marked disarray of epithelium. Lesion with koilocytic atypia with marked nuclear hyperchromasia & smaller size of cells confirm the images of CIN-2 [11]. In biopsy, CIN 2 and CIN 3 are distinguished from one another by the degree of discernible cell maturation. In CIN 2, immature cells occupy the lower two-thirds of the epithelium but some degree of maturation or koilocytic atypia is present towards the surface. In CIN 3, there is absence of maturation, as immature cells occupy the full-thickness epithelium, often associated with a layer of atypical parakeratotic cells on the surface[12]. Cytomorphology of squamous cell Carcinoma is characterized by HSIL features including macronucleolus, irregular chromatin distribution, tumor diathesis, "tadpoles" and "fiber cells" (keratinizing type)[11]. invasive squamous cell carcinoma with morphological features of HPV infection has been called condylomatous or warty squamous cell carcinoma[12] .

Aim: On this background the present study has been under taken with aim to study the HPV related change in uterine cervix by routine H& E stain that have been already proved to be infected by HPV in the respective cervical scrapping.

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The methodology:
The methods included cervical scrapping of 226 no. of married non pregnant women attending gynecology OPD of Gauhati Medical College hospital, Guwahati for Pap test during the period from January 2011 till June2012. At the same time cervical scraping from the posterior vaginal pool were smeared and dried on to Whatman 3MM filter paper, and stored individually at room temperature after labeling for HPV DNA testing by PCR.

The dry paper smears were carried to the regional research medical center at Dibrugarh, Assam for detection of HPV DNA by PCR. The Nested PCR using primer for L1 consensus gene with My9/My11 & GP6+/GP5+ followed by multiplex PCR is carried out to detect HPV 16 & HPV18 by respective primers. The procedure was done according to the method described by Kailash et al. [13] and Sotlar K et al. [14] .

The pap smears were stained with papanicoaloau stain and reported according to the Bethesda system 2001[15].

On the basis of clinical presentation and cytology reports, total 30 no. of patients underwent follow up biopsy examination, either by wedge biopsy, punch biopsy or hysterectomy. The biopsy tissues were fixed in 10% formalein and for overnight. The tissues were processed routinely and stained with Hematoxylein & eosin stain. For reporting, CIN systems are followed.

Results:
Out of 226 cases, 30 cases were available for histological study (13.3%).

Table1: Results of Cervical biopsy (n=30)

Clinical feature	Histology diagnosis	Total No.	%
Cervical erosion	Chronic Cervicitis	4	13.3
Warty lesion	Condyloma	7	23.3
U. V.Prolapse	CIN1	3	10
U. V.Prolapse	CIN2	4	13.3
U.V. Prolapse	CIN3	5	16.7
Growth	SCC	7	23.3

Table 2: Cytology Report of Cervical scrapping

Cytodiagnosis		Number of patients (30)
NILM (3)	Benign reactive change including metaplsia	01
	Atrophic Vaginitis	02
ECA(n=27)	ASCUS	03
	LSIL	08
	HSIL	08
	SCC	08

Table 3: Summary of Cervical cytology & Histology Report (30 n)

Cytology / Histology	NILM	ASCUS	LSIL	HSIL	SCC	Total
Cervicitis	3	1	0	0	0	4
Condyloma	0	1	5	1	0	7
CIN1	0	0	3	0	0	3
CIN2	0	0	0	3	1	4
CIN3	0	1	0	3	1	5
SCC	0	0	0	1	6	7
Total	3	3	8	8	8	30

Table4: Result of HPV status according to Histology report

HPV Status / Histology	Cervicitis (0/4) %	Condylloma (4/ 7) 0%	CIN1 (2/3) %	CIN 2 (4/4) 100%	CIN3 (5/5) 75.0%	SCC (7/7)	Total (22/30)
HPV 16	0	0	1	2	3	6	12 (54.5%)
HPV 18	0	1	0	2	2	1	6(27.3%)
HPV Other than 16/18	0	3	1	0	0	0	4(18.2%)
HPV positivity	0	4	2	4	5	7	22

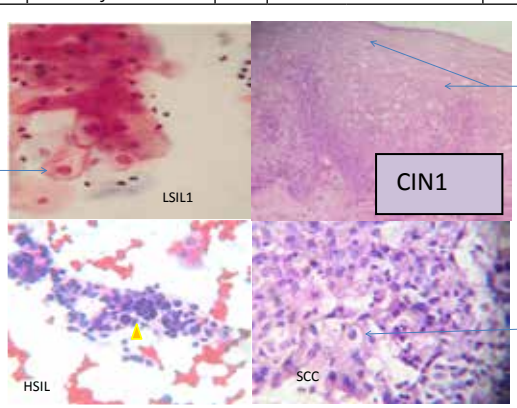

Fig.1:Koilocyte (arrow) in cytology smear(1), biopsy tissue (2) & (4), cell with enlarged hyperchromatic nuclei, scant cytoplasm and nuclear irregularity(yellow arrow head)

Discussion:
The result of biopsy coincides with the cytology finding in case of NILM and LSIL. Out of 3 samples of ASCUS group, histologically one patient turned out to be inflammatory (cervicitis), one - CIN3 and the other as condylloma. Among the HSIL group one case was confirmed as condylloma and the other being invasive SCC. Even 2 cases of cytologically reported SCC have been confirmed as CIN2 and CIN3 each.

Regarding the HPV status, all the higher grade lesion showed H-R HPV positivity in the corresponding cytology smears. HPV 16 prevalence is higher in SCC cases than HPV 18 subtype and 4 cases the HPV sub typing could not been done as we did the tests using primers for HPV16 & HPV18 only. In low grade lesion HPV positivity is noted in 60% of the samples studied. We have not found any single case of multiple infections. Our data on HPV prevalence in the cytology specimens are similar to the study carried out by Walboomer et al who established the presence of HPV in virtually all cervical cancers they studied using PCR based test[1]. Detection of HPV in cervical cancer specimens from different parts of India indicates that HPV 16 is the most predominant type[16]. We could not perform immune- histo-chemistry to establish HPV in biopsy specimen due to unavailability of specific antigen . Further, facility for HPV detection and studying it at molecular level is not available in this institute. Yet cytology smears were carried to R.M.R.C. (ICMR) situated at Dibrugarh which is about 500 Km away from our institute for HPV DNA detection. The histopathology showed the evidence of HPV related tissue change.

Conclusion:
Though the study includes less numbers of samples, it helps to correlate the presence of HPV in cervical intraepithelial neoplasia and squamous cell carcinoma. In medical center where facility for HPV detection at molecular level is not

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available, cytology and histopathology may help to detect HPV related change in smear as well as tissue section. The data generated by the present study will definitely help the medical institute, irrespective of having HPV detection facility, to suspect HPV infection based on the cytology and histological findings.

Abbreviation used

HPV	Human Papilloma virus
DNA	De oxy ribo nucleic acid
PCR	Polymerase Chain Reaction
SIL	Squamous intraepithelial lesion
NILM	No intra epithelial lesion/malignancy
CIN	Cervical Intra epithelial neoplasia
SCC	Squamous cell carcinoma
HSIL	High Grade squamous intraepithelial lesion
LSIL	LOW Grade squamous intraepithelial lesion

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