

Original article:

p16 immunopositivity in anogenital warts

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Abstract:

Aim: To study the utility of p16 and MIB-1 immunohistochemistry in anogenital warts.

Materials and Methods: Histopathological examination was done on 31 consecutive untreated cases of anogenital warts presenting to the sexually transmitted disease clinic. Immunohistochemistry for p16 and MIB-1 was performed on these excision biopsies and correlated with the histological features.

Results: Three cases out of 31 (9.7%) showed band like nuclear and cytoplasmic immunostaining for p16 protein.

Conclusion: A high incidence of p16 immunopositivity amongst anogenital warts mandates a detailed clinical examination and follow up of these patients and their sexual contacts.

Key words: Anogenital warts, condyloma acuminata, p16, MIB-1.

INTRODUCTION

Anogenital warts are cutaneous and mucosal sexually transmitted diseases of anal and/or genital mucosa and its adjoining areas caused by human papilloma virus (HPV). It is one of the commonest sexually transmitted diseases and its incidence is on the rise. Most common HPV associated with genital warts are HPV 6, 11 & 53. Moderate risk HPV type 31, 33 and 35 and high risk HPV type 16 & 18 are also seen in genital warts.[1,2] Differential diagnosis of anogenital warts are skin tag, nevi, condylomata lata, seborrheic keratosis, molluscum contagiosum, lichen planus and psoriasis.

On histopathology anogenital warts show viral changes (intranuclear inclusions & koilocytes), which is the most characteristic feature. Hyperkeratosis, papillomatosis, acanthosis, parakeratosis, apoptotic bodies and mitosis can be seen in anogenital warts. Dysplasia seen in warts need to be categorised into high-grade and low-grade intraepithelial lesions as the former have been reported to recur more than low grade lesions.[3]

The cellular tumor suppressor protein p16INK4a (p16) has been identified as a biomarker for transforming HPV infections. There have been conflicting reports on expression of p16 in anogenital warts mainly due to difference in the interpretation of focal immunostaining for p16. If only band like nuclear and cytoplasmic immunostaining for p16 is considered positive then the correlation with high risk HPV infection is better.[4,5]

Because HPV-associated lesions show increased cellular proliferation, MIB-1 immunostaining has been evaluated as an aid in the differential diagnosis of cases equivocal for condyloma. MIB-1 immunopositivity in the upper two thirds of the thickness of epithelium can be used as an adjunct to histomorphology in the diagnosis of condyloma acuminatum.[6]

This study was designed to evaluate the incidence of p16 immunopositivity in anogenital warts in Indian population since recent studies have highlighted the fact that a significant proportion of anogenital warts harbour high risk HPV types either alone or in combination with low risk types.[7].

MATERIALS AND METHODS

The study was conducted in the department of pathology in collaboration with the department of dermatology at University College of Medical Sciences, Delhi, after obtaining necessary ethical clearance .It was a prospective study of 31 consecutive, untreated cases of anogenital warts presenting to the Sexually Transmitted Diseases clinic. Informed consent was taken and the clinical details were recorded in a predesigned case record . Adequate tissue material was biopsied for histological and immunohistochemical studies. The routinely processed tissue sections were stained with hematoxylin and eosin .

Immunohistochemistry was performed on poly-L-lysine coated slides by labelled streptavidin biotin (LSAB) technique for p16 and MIB-1. All the cases were evaluated by p16 and MIB1 immunohistochemistry. The results were reported as-

1. MIB-1- Percentage of positive cells.(Nuclear staining)
2. P16-Negative, focal positive and band positive.(Both nuclear and cytoplasmic)

RESULTS

Age of the patient ranged from 17 years to 51 years (mean of 29 years, median of 28 and S.D of 8.7). Males constituted 74% (23/31) of the cases while 8 cases were females. The most common site amongst males was the penile shaft (13/23) and amongst females, it was the vulva (6/8). Size of the lesions ranged from 8 mm to 18 mm.

On histopathological examination koilocytes were seen in all the cases. Intranuclear inclusions were seen in 21 of the 31 cases. Fifteen cases had mild dysplasia [Fig.1] while the rest of the cases showed no dysplasia . Of the ten flat warts mild dysplasia was seen in seven (70%) cases while five of the twenty one condylomatous warts showed mild dysplasia .

P16 immunostaining was performed on 31 cases and showed a band of nuclear and cytoplasmic staining in three cases [Fig.3]. MIB1 labelling index did not correlate with the p16 pattern of immunostaining . It showed no correlation with the presence or absence of mild dysplasia.

Follow up data was not available for the patients. HPV genotyping could not be done for these three cases due to lack of resources.

Dysplasia	P16			Total
	neg	Focal +	Band +	
No dysplasia	8	8	0	16
Mild dysplasia	6	6	3	15
Total	14	14	3	31

Table 1: Cross tabulation of p16 immunohistochemistry with presence or absence of mild dysplasia

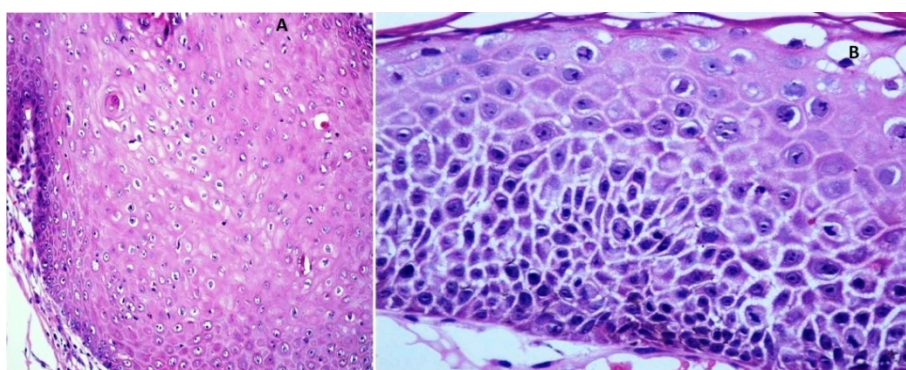


FIG 1

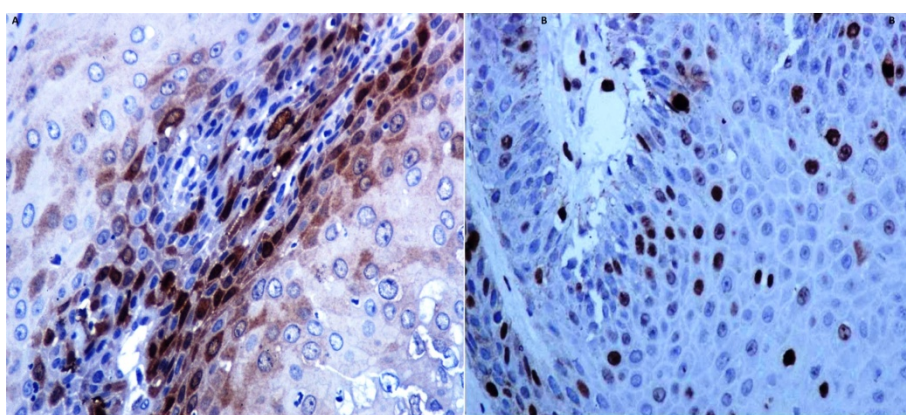


FIG 2

DISCUSSION

The role of Human Papilloma virus in cervical carcinogenesis and recently in squamous cell carcinomas of the upper aerodigestive tract has sparked tremendous interest in cancer risk reduction by cervical screening and HPV vaccination. However the so called low risk lesions like warts have not been given due attention and they are still treated by destructive chemical methods without histopathological examination or HPV testing. Recently there has been renewed interest in genotyping of the viral anogenital warts . Mixed infection by both low and high grade HPV types in the same wart are fairly common. Recently Park SJ et al , in a study of 150 male genital warts demonstrated combined infection by both high and low risk HPV in 27 (20.5%) cases and only high risk HPV in 4 cases.[7]

In another study the incidence of HPV-16 in genital warts was 9.8% .[8] Few other studies have also emphasized the higher incidence of coinfection by low and high risk type HPV in anogenital warts.[9,10] However mere demonstration of two different genotypes of HPV in the same wart does not confirm their etiological role.

.In countries where genotyping for HPV is not readily available, a histopathological examination followed by p16 immunohistochemistry may be a cost effective method of screening the patients for high risk HPV. The patients thus identified can be followed up more closely and their sexual contact(s) screened .[11]

Immunohistochemistry for p16 has been used as a marker for transforming HPV infection and has been extensively studied in cervical carcinogenesis. Its expression increases as the lesion progresses from Low grade dysplasia to High grade dysplasia to squamous cell carcinoma.[12] Hence p16 can be a useful adjunct to histopathological examination as a surrogate for HPV genotyping. Numerical percentage cut off has not been of much help in reporting p16 immunostaining though initial articles mentioned cut offs of 80% and 70%.[13,14]. A band like pattern is considered more useful and correlates better with high risk HPV types.[5]. In our study this band like positivity was found in three cases which showed mild dysplasia.

MIB-1 immunostaining has been reported to aid in the diagnosis of viral warts in equivocal cases. In our study also there was no statistically significant correlation between dysplasia and MIB-1 immunostaining.

This study makes a strong case for routine histopathologic examination of atleast a part of the lesion in all cases of anogenital warts. Similar recommendations have also been made by other authors.[16]

Conclusion:

A band like p16 immunopositivity is seen in a significant proportion of anogenital warts .These patients need careful follow up and contact screening . Necessary clearance was taken from the institutional ethics committee for undertaking this study. Informed patient consent was also taken.

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