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ORIGINAL RESEARCH

Utility of biomarkers p16,p53 and Ki-67 in detection of cervical intraepithelial lesion and carcinoma of cervix

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ABSTRACT

Cervical cancer is one of the leading cancers in India; crude incidence rate is 20.2 in 100000.[1]The primary pathologic process is reversible, so early detection of precancerous lesion i.e.cervical intraepithelial neoplasia (CIN) is one of the most effective ways of preventing this disease and thus reducing the mortality rate. CIN and cervical carcinoma can be diagnosed morphologically. However, identification of specific biomarker is important to diagnose the difficult cases. To find a specific biomarker, we conducted a crosssectional study with 60 cases, including both CIN and cervical carcinoma. Histomorphology was studied and p16, p53, and Ki-67 expression on immunohistochemistry was correlated with histological findings. Out of total 60 cases, 45(75%) were positive for p16 biomarker, 10/18(55.55%) were of CIN and 35/42 (83.3%) were of cervical cancer. p53 was expressed in 42(70%) cases. Of these 16/18[88.88%] cases were of CIN and 26/42 [61.9%], cases were of cervical cancer. Ki-67 was found positive only in 28(46.7%) cases, 3/18 [16.7%] of CIN and 25/42 [59.52%] of cervical cancer. p16 and p53 showed statistical significance ($p<.005$) in the detection of cervical lesions. p53 showed statistical significance ($p<.005$) in detection of CIN and p16 showed statistical significance ($p<.005$) in detection of cervical cancer in particular. Thus, we conclude that p16 and p53 demonstrate a significant association with the CIN and cervical carcinoma. p53 emerged as a single sensitive biomarker for detection of CIN and p16 was a sensitive marker for detection of cervical carcinoma cases. Thus to conclude p16 and p53 can act as adjuncts for detection of CIN and cervical carcinoma at an early stage.

Key-words: Cervical intraepithelial neoplasia (CIN), Cervical cancer, p16, p53, Ki-67.

INTRODUCTION

Cervical cancer is one of leading cancer in India with crude incidence rate of 20.2 in 100000.[1] The primary pathological process is reversible so early detection is the most effective way of preventing this disease. Still, in India 70% or more of these cases are diagnosed in late stage (stage III).[2] This probably is due to lack of awareness about the disease and difficulties in early diagnosis. The role of HPV in cervical intraepithelial neoplasia (CIN) and cervical carcinoma is well established. HPV oncoproteins E_6 and E_7 cause alterations in the expression of cell cycle proteins such as p16, p53, and Ki-67.[3] However these proteins have not yet been used as markers for either diagnosis or prognosis , particularly in cases of CIN. So we evaluated the role of these markers in detection of CIN & cervical carcinoma.

MATERIAL AND METHOD

The study was conducted in the department of pathology V.M.M.C & Safdarjung hospital over a period of two years from 2013-15. Study was approved by the institutional ethical committee. Sixty cases clinically suspicious of CIN and cervical carcinoma were studied. Patient on radiotherapy and chemotherapy were excluded from the study. Tissues were processed routinely and sections were stained with H&E. The lesions were classified as CIN and cervical carcinoma. CIN cases were further graded as CINI, CINII, and CINIII. Carcinoma cervix cases were further classified as well differentiated squamous cell carcinoma (WDSCC), moderately differentiated squamous cell carcinoma (MDSCC) and poorly differentiated squamous cell carcinoma (PDSCC). Immunohistochemical staining was

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performed for p16 (rabbit clonal antibody, ready to use; DB Biotech Ltd.), Ki-67 (clone DO-7, 1:50; Leica Biosystems, New castle Ltd) and p53 (clone MM1, 1:50; Leica Biosystems, New castle Ltd) and evaluated under a light microscope. Total of 1000 cells were assessed for intensity and type of staining.

p16 immunostaing[4] was evaluated as follows:-

- Negative: <1% of cytoplasmic and nuclear positivity
- Sporadic: 1% to 10% of cytoplasmic and nuclear positivity
- Moderate: 10% to 30% of cytoplasmic and nuclear positivity
- Diffuse: >30% of cytoplasmic and nuclear positivity

It was considered positive if it was seen in more than 10% cells and staining was moderate or diffuse.

Ki-67[5] **and p53 immunostaining**[6]: for Ki-67 and p53, the staining was interpreted as positive if more than 10% cells showed positivity nuclear positivity.The tumors with only focal or no nuclear staining were regarded as negative.

The results of H&E staining and p16, p53, Ki-67 immunohistochemistry were correlated.

Statistical analysis

To analyze the data, we used SPSS software (Statistical Package for the Social Sciences, version17; SSPS Inc., Chicago, IL, USA) and to compare categorical variable NPar test was applied. P value of less than 0.05 was taken as the cut-off value of significance.

RESULT

This was a cross sectional study with a sample size of 60 cases including CIN and cervical cancer. The age of patient was in the range of 30 to 74 years. Out of these 60 cases, 18 (30%) were diagnosed as CIN (GROUP I) and 42 (70%) as cervical carcinoma (GROUPII). The CIN cases were further sub classified according to their histological subtype as CINI (8.3%), CINII (11.7%), CINIII (10.0%), and cervical cancers were further sub classified according to their histological subtype as well-differentiated carcinoma (6.7%), moderately differentiate carcinoma (48.3%) and poorly differentiate carcinoma (15.3%). p16 positivity was observed in 45 (75%) cases and p53 showed positivity in 42 (70%) cases. On the other hand, Ki-67 was positive in only 28(46.7%) cases of cervical lesions. Also, the expressions of these three biomarkers were correlated with the morphological diagnosis. In group one(CIN), out of 18 cases evaluated, p53 showed highest positivity in 89% cases (p<.005) followed by p16 and Ki-67 (Table: 1). In group 2 (cervical cancer), p16 showed maximum positivity is 83% (p <.005) (Table: 1). The control group did not show the expression of these markers.

Expression of these biomarkers was then evaluated in each subcategory of CIN and cervical carcinoma. It was noted that p16 expression increased with the increasing grades of CIN and this was statistically significant (Table: 2, Graph 1). p53 did not show such pattern and Ki-67 was not expressed in CINI cases. However Ki-67 expression was observed to increase with the grade of the cervical carcinoma (Table: 2, Graph 1) although the p value was not statistically significant. Pattern of p53 expression was unpredictable in various categories.

Table 1: Correlation of biomarkers with group1 (CIN) and group2 (cervical carcinoma) lesions:

Group (number of cases)	Group1: CIN (18)			Group2: Cervical cancer (42)			Total (CIN & Cervical cancer) (60)		
	p16	p53	Ki-67	p16	p53	Ki-67	p16	p53	Ki-67
Positive	10(56%)	16(89%)	3(17%)	35(83%)	26(62%)	25(60%)	45(75%)	42(70%)	28(46%)
Negative	8(44%)	2(11%)	15(83%)	7(17%)	16(38%)	17(40%)	15(25%)	18(30%)	32(54%)
P value	>0.005	**<0.005**	>0.005	**<0.005**	>0.005	>0.005	**<0.005**	**<0.005**	>0.005

Table 2: p16, p53 and Ki-67 expression in CIN & cervical carcinoma

Marker	p16						p53						Ki-67					
Subtype	CINI	CINII	CINIII	WDSCC	MDSCC	PDSCC	CINI	CINII	CINIII	WDSCC	MDSCC	PDSCC	CINI	CINII	CINIII	WDSCC	MDSCC	PDSCC
Total case	5	7	6	4	29	9	5	7	6	4	29	9	5	7	6	4	29	9
Positive	2	3	5	2	24	9	4	7	5	3	18	5	0	2	1	1	17	6
Positivity percenta	40	43	83	50	83	100	80	100	83	75	38	56	0	29	17	25	59	67

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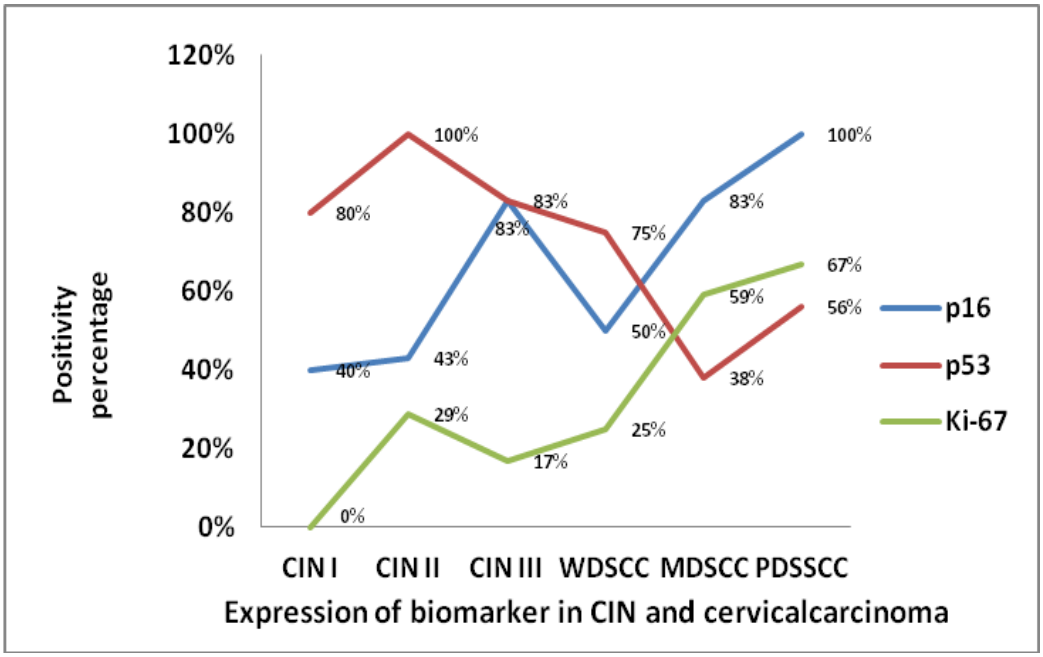
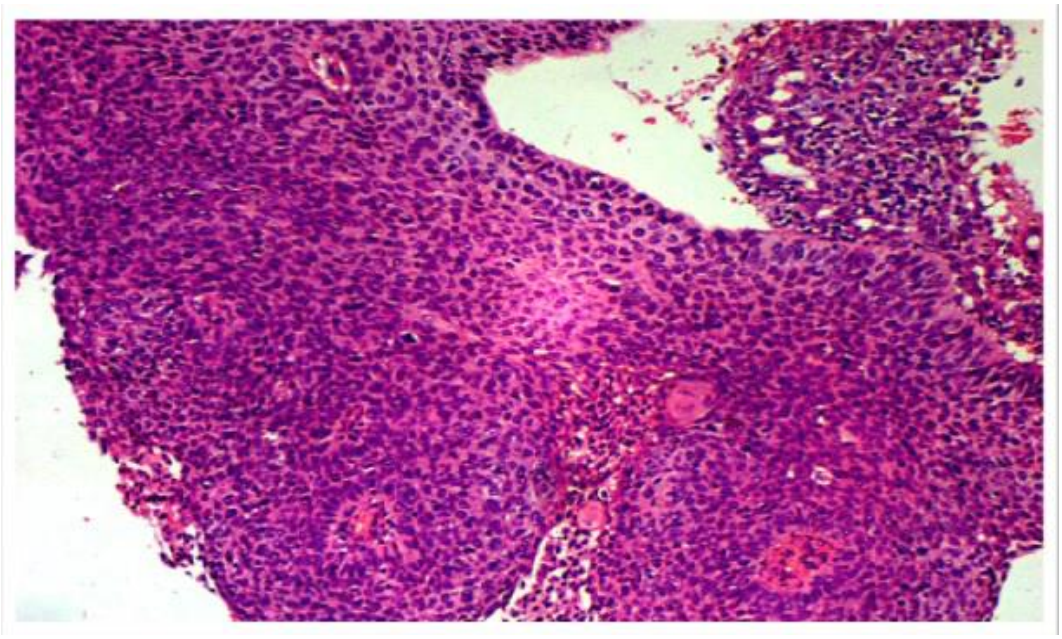
p value	>.005	>.005	>.005	>.005	**<.005**	**<.005**	>.005	>.005	>.005	>.005	>.005	>.005	>.005	>.005	>.005	>.005	>.005	>.005



Graph 1: Expression of biomarkers in CIN & cervical carcinoma



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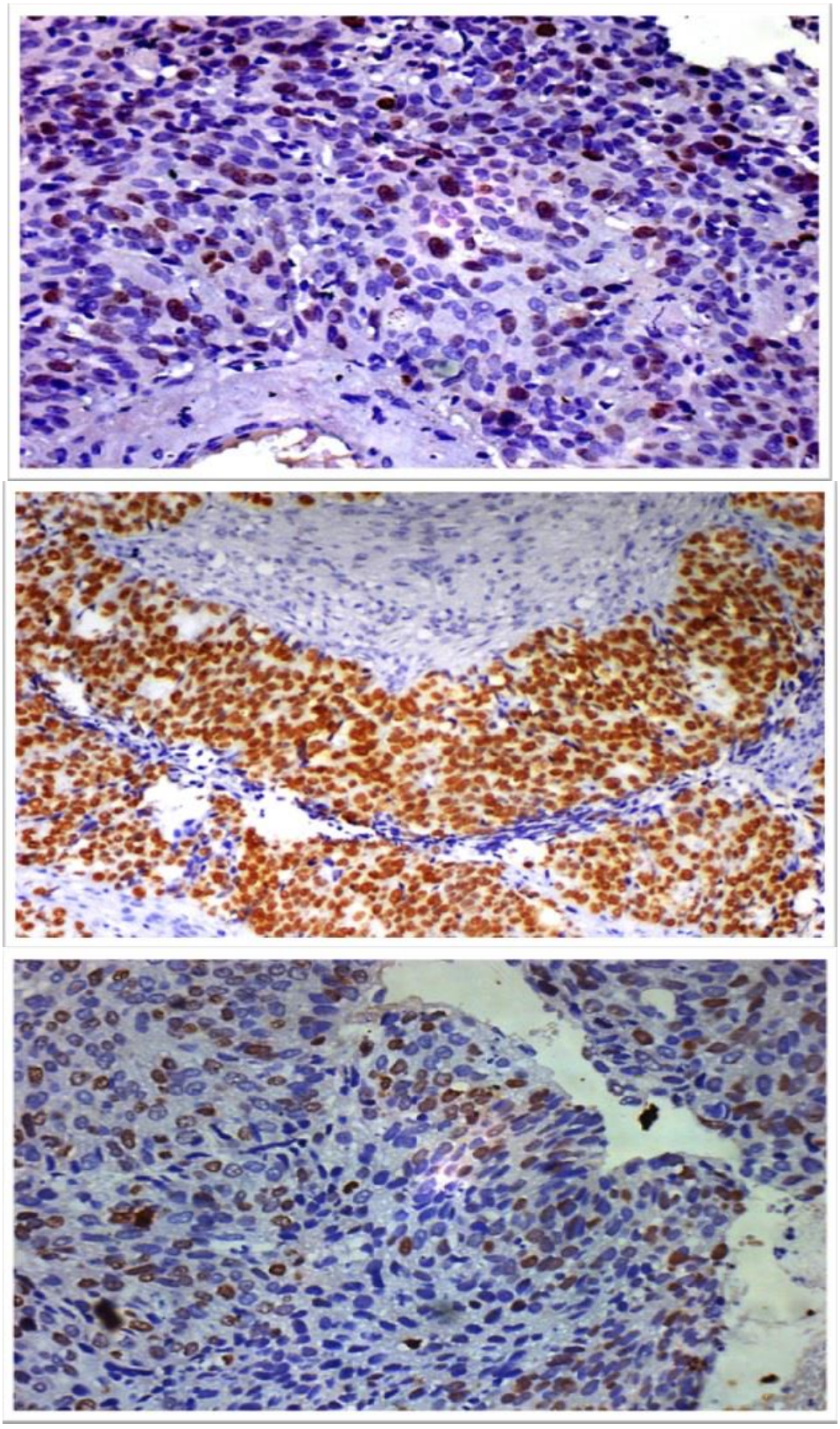

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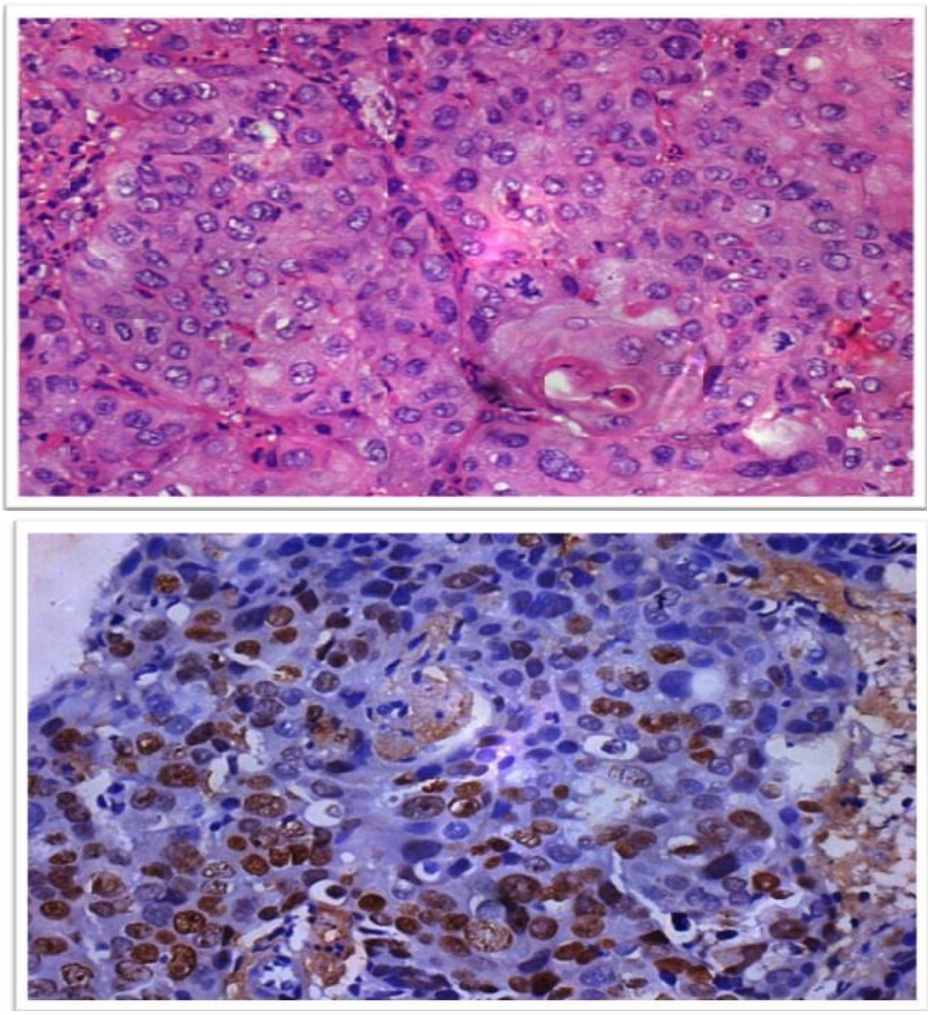

DISCUSSION

HPV plays a critical role in genesis of cervical cancer. Two oncoproteins E_6 and E_7 encoded by high risk HPV serotypes cause cell cycle deregulation leading to neoplastic transformation. E_6 oncoprotein causes functional inactivation of p53. On the otherhand, E_7 oncoprotein causes functional inactivation of pRb resulting in over-expression of p16 protein and its accumulation in the cells. Thus, p16 can act as a surrogate marker for HPV mediated pRb catabolism.[7] In our study, we found that p16 among the three markers showed the highest positivity (83%) in cervical cancer cases. We also observed that p16 positivity increased with the increase of CIN grade. In CIN category, CIN III showed highest positivity (83%) followed by CIN II (43%) and CIN I (40%). Denton et al[8] and Samarwarawrhana et al[9], also reported similar finding in their study. Keating et al[10] and Kales et al[11] noted that p16 expression was rare in CIN I lesions specially those associated with LR-HPV. Thus, p16 immunostaining may allow precise identification of CIN I lesions associated with HR-HPV types, CIN II, CIN III and cervical cancers in biopsy sections. This will help to reduce false positives and false negatives thereby significantly improving cervical cancer and pre-cancer detection.[12]

p53 is a tumour suppressor protein. In HPV infection E_6 oncoprotein inactivates this protein and as a result it starts accumulating in the cells. Inactivation of p53 is the most common genetic alteration in human carcinogenesis.[13] in our study, p53 positivity was more in CIN cases (89%) as compared to carcinoma. Our observations were in concordance with Delllas et al[14] and Avall et all[15]. We also noted a higher p53 expression in well differentiated carcinoma compared to poorly differentiated carcinoma. However, there are some studies which contradict our findings and some authors have found no significant correlation between the stages of CIN and p53 expression.[16]More studies with large sample size are needed to further evaluate the role of p53 in CIN and cervical carcinoma. To conclude, p53 may emerge as a biomarker for CIN.

Ki-67 is a proliferation marker. Expression of Ki-67 is used to determine cell proliferation status.[17,18] We observed that the Ki-67 expression increased with a grade of the lesion. But we did not find any significant association of Ki-67 expression with CIN and cervical carcinoma. Some studies are in concordance with our finding, but few also differ in this regard.[19,20] This deviation may occur due variability in the intensity of expression of Ki-67 throughout the cycle.[21]

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After analyzing each of the above biomarkers, we also compared all of them. We observed that p16 was most sensitive followed by p53 and Ki-67 in the detection of CIN and cervical carcinoma. We also noted that p53 was most sensitive for the detection of CIN cases as it showed highest positivity in these cases ($p<.005$) and p16 expression in CIN cases increased with increasing grades of CIN. So for detection of CIN lesions, p16 may be more specific whereas p53 is a more sensitive marker. CIN is a reversible process and early diagnosis is always needed to treat this lesion, so p53 may play an important role in early detection. In our study, p16 also emerged as the best biomarker for detection of cervical carcinoma cases. Therefore, p16 has a valuable role for the diagnosis of cervical carcinoma specially when there is diagnostic dilemma due to the presence of dense inflammation or inter observer variability.

Thus, we concluded that p16, p53 and Ki-67 cell cycle proteins are aberrantly expressed in CIN and cervical carcinoma with p16 and p53 demonstrating a significant association with the CIN and cervical carcinoma. Immunohistochemical detection p16 expression can be used as a specific diagnostic marker for CIN and cervical carcinoma. It also, helps in detection of cervical carcinoma which is masked due to the presence of dense inflammation and interobserver variability. p53 emerged as a single sensitive biomarker for detection of CIN. Thus, all the markers specially p16 and p53 can act as adjuncts for detection of CIN and cervical carcinoma at an early stage thus help in preventing this fatal disease.

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