Original Article

ROC-Analysis Derived Immunohistochemical P53 Cut-Off Scores as an Adjunct to Routine Histopathology for Better Diagnostic Compartmentalisation of Cervical Lesions

Abstract

Objective: The aim of this study was to evaluate the predictive value of Immunohistochemical p53 cut-off scores as an adjunct to routine histopathology for better diagnosis of cervical lesions. **Materials and Methods:** Prospective study carried out for 1 year. After ethical approval and informed consent, a total of 100 cervical tissue samples were analyzed; chronic cervicitis (CC)-15, cervical intraepithelial neoplasia (CIN)-40, and squamous cell carcinoma cervix (SCC)-45 (FIGO 2018 clinical staging). After routine processing of tissue specimen, hematoxylin and eosin (HE) staining was done. Grading of cervical precancerous lesions (CIN) was done as per World Health Organisation criteria as CIN 1,2 or 3. Broder's grading was assigned for every SCC sample. **Results:** Mean p53 scores of CC, CIN, and SCC cases were 0.0, 1.70, and 4.38, respectively, CIN 1, 2, and 3 were 1.07, 1.63, and 2.22, respectively. SCC was differentiated from CIN3 with p53 ≥4.5 as predictor for SCC, sensitivity and specificity were 57.8% and 88.9%, respectively. Overall diagnostic accuracy of the proposed scoring system for differentiating CC, CIN, and SCC was 61%, while the accuracy of previous methods of interpreting p53 immunoreactivity as immunoscore >2 or arbitrary cut-off of >10% cells with nuclear positivity was only 48%. **Conclusion:** ROC-derived immunoscore cut-offs can provide the much-needed objectivity and optimal decision thresholds to immunohistochemistry interpretation.

Keywords: *Cervical cancer, cervical intraepithelial neoplasia I, II, III, immunohistochemistry, scoring methods*

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Introduction

Cervical cancer is the second-leading cause of new cancer cases and related deaths among women in India.[1] Being one of the few preventable cancers, its disproportionate global distribution aptly reflects the success of time-tested screening strategies in developed countries. Failure to establish and sustain resource-intensive cytology-based screening programs coupled with inherent limitations of cytology like low sensitivity have prevented the success of such programs in developing nations.[2]

Cancer cervix has a long preinvasive phase. Cervical Intraepithelial Neoplasia (CIN) is a premalignant lesion that is histologically graded as CIN 1, 2, or 3. Three different grades of CIN give the faulty static impression of the disease whereas CIN is a dynamic lesion that can persist, progress or regress with time.[3] Histopathological interpretations are subjective and can have significant observer-related variability.[4] Moreover, treatment and follow-up of CIN depend solely on its grading and therefore can suffer due to the aforementioned drawbacks of histopathology.

The increasing importance of the role of apoptotic pathways in cancer development combined with limitations of screening modalities and histology grading systems have paved the way for search of biomarkers. In any resource-based setting, incorporating biomarkers with risk-based approach screening can help achieve optimal risk stratifications for every patient.[2]

P53, a tumor suppressor gene, has proven itself a reliable diagnostic adjunct for histotyping of various gynecological cancers.[5] P53 has a significant role in cervical cancer pathogenesis and therefore has been a marker of interest since decades for possible diagnostic and therapeutic targeting.[6]

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Technical advances and widespread availability have made p53 immunohistochemistry (IHC) the single most useful stain. IHC is easier to apply and less expensive compared to mRNA *in situ* hybridization.[7] Nuclear positivity of p53 by IHC is a rapid preliminary indicator of p53 status in tumors.

Tumor marker positivity in IHC is frequently judged using predetermined cut-offs such as 10% cell positivity with the idea that a more detailed analysis of expression will not add any relevant information.[8] On the contrary, the extent of tumor positivity for a biomarker needs to be clearly defined in order to have justifiable clinical and biological relevance. This uniformity allows reproducibility of biomarker expression and reduces observer-associated variability. P53 immunoscore evaluation as percentage of abnormal accumulation of p53 has been shown to be a useful and reproducible predictive factor in colorectal cancer.[8] Objective scoring of IHC improved the accuracy and reproducibility of grading CIN lesions in one study.[9] Similarly in another study, additive and multiplicative Quick scores were shown to be time-saving and simpler while having a meaningful statistically significant correlation with the H score.[10] Quantifying immunoreactivity enables the use of statistical tools like ROC curves to identify threshold values for diagnostic tests with optimum sensitivity and specificity. ROC analysis has been used in the selection and validation of cut-off scores for biomarkers in a variety of human cancers to provide interpretation objectivity against the arbitrarily set thresholds.[8,11,12] The aim of this study was to evaluate the predictive value of Immunohistochemical p53 cut-off scores as an adjunct to routine histopathology for better diagnosis of cervical lesions.

Materials and Methods

This was a prospective study carried out for 1 year. Recruitment of cases was done from women presenting in Gynaecology OPD, Department of Obstetrics and Gynaecology, King Georges Medical University, Lucknow, with symptoms such as discharge per vaginum, postcoital bleeding, postmenopausal bleeding, and menstrual abnormalities or having abnormal cytology reports. Relevant demographic and clinical data were collected. FIGO 2018 clinical staging of cervical cancer cases was done. After ethical approval (Reference code: 90thECM II B-IMR-R/P8) and informed consent, women having obvious growth on the cervix underwent biopsy directly while women with abnormal cytology underwent colposcopy and directed biopsy. LEEP was performed where indicated as per biopsy reports (CIN2 or 3) or high-grade colposcopy and in these women, LEEP tissue histology was included instead of biopsy tissue. A total of 100 cervical tissue samples were obtained: chronic cervicitis (CC)-15, CIN-40, and squamous cell carcinoma cervix (SCC)-45.

Specimen in our study were obtained from symptomatic women undergoing cervical biopsy or LEEP while awaiting further management based on HPE reports. After routine processing of tissue specimen, hematoxylin and eosin (HE) staining was done. Grading of cervical precancerous lesions (CIN) was done as per World Health Organization criteria as CIN 1, 2, or 3. Broder's grading was assigned for every SCC sample.

p53 Immunohistochemistry

IHC was completed following standard procedures. 4-µ paraffin sections cut on silane-coated microscopy slides were first deparaffinized and rehydrated in graded alcohols. Antigen retrieval for p53 was done in Tris EDTA buffer, pH-9 at 98°Cfor 25 min in a microwave oven, followed by tris-buffered saline washing and peroxidase blocking. Sections were incubated with primary antibody (Flex Monoclonal Mouse Anti-human p53 protein, Clone DO-7, Ready-to-Use) for 90 min at room temperature, followed by incubation with secondary antibody (Dako Real Envision Detection System, Peroxidase/Diaminobenzidine [DAB]+Rabbit/Mouse) for 30 min at room temperature. Expressions were localized by incubation with DAB. Finally, slides were stained with hematoxylin. Negative controls were similarly processed by omitting primary antibodies while tonsillar tissue was used as the positive control.

Scoring of immunohistochemistry

Nuclear staining for p53 either as coarse or fine granular brown dots was considered positive. The intensity of the staining pattern and grading of stained tumor cells was done. p53 score was calculated by adding intensity and grade values. Basal layer positivity was found in few cases of CC as well as few CIN cases [Table 1 and Figures 1-3].

Statistical analysis

The results were analyzed using descriptive statistics and making comparisons among various groups. Categorical data were summarized as proportions and percentages while discrete as mean (standard deviation [SD]). All the associations were tested using the Chi-square test. Kruskal Wallis test was used for making comparisons of mean p53 scores between various groups of cervical

Table 1: p53 immunohistochemistry scoring

p53 staining pattern	Intensity
Absent	0
Mild	1
Moderate	2
Severe	3
Percentage tumor cells in 10 HPF	**p53 grade**
Absent	0
1-5	1
6-25	2
26-50	3
51-75	4
>75	5

HPF: High power fields

lesions. ROC curves, area under the curve (AUC), sensitivity, and specificity were calculated for obtaining immunohistochemical p53 cut-off scores for various groups. Statistical analysis was performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). A value of $P < 0.05$ was considered statistically significant.

Results

Fifteen cases of CC, 40 CIN (CIN 1-14, CIN 2-8, CIN 3-18), and 45 cases of squamous cell cancer cervix were evaluated for p53 expression immunohistochemically and scored. The mean age of women in the CIN group was 44.33 years (SD 11.98) while in SCC was 51.56 years (SD 9.57). This difference was statistically significant ($P < 0.001$). Majority of the SCC cases were observed at a higher age, mostly after 40 years, while premalignant cases were observed mostly below the age of 40 years. Mean parity was 3.43 in CIN and 3.96 in the SCC group with no significant difference. Cancer cervix was more common in postmenopausal females (60%) ($P = 0.003$). Contraception prevalence was low in all three groups. Tubal ligation (13.3%) was the most preferred method of contraception in cancer group. Majority of women in all three groups belonged to the upper lower class of socioeconomic status as per the modified Kuppuswamy scale [Table 2].

Discharge per vaginuum prevailed as the most common symptom in CC/CIN cases, while postmenopausal bleeding was significantly more in SCC cases ($P < 0.001$). The most common examination findings in cancer cervix group were growth on the cervix (82.8%). Majority of the women with cancer cervix belonged to FIGO stage IIB (42.2%) and no significant difference was found in average p53 scores among various stages ($P = 0.710$). As per Broder's classification, majority of the cervical cancer samples were moderately differentiated (MD, n = 35). The median p53 scores of PD, MD, and WD grades of cervical cancer samples were 5.0 (4.0–6.0), 5.00 (0.0–7.0), and 5.0 (0.0–6.0), respectively. Significant difference was found in average p53 scores among the three SCC grades ($P < 0.001$).

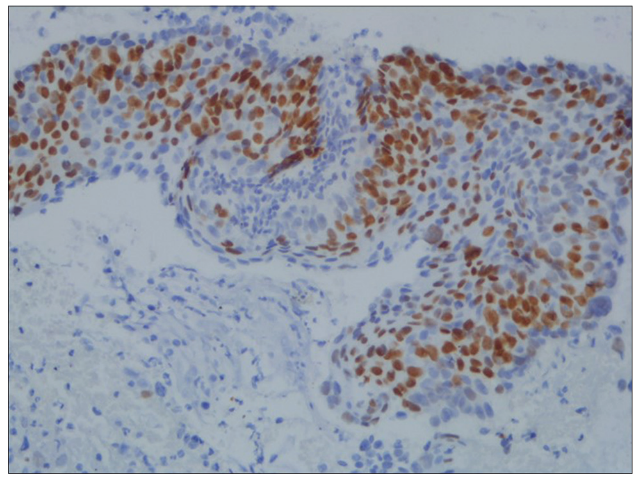

Figure 1: P53 strongly positive: Tumor cells showing high intensity +++ and more than 50% tumor cells positivity of P53 in high-grade cervical intraepithelial neoplasia–III ×200

Table 2: Demographic characteristics of women among various groups of cervical lesions

Demographic parameter	CC, *n* (%)	CIN, *n* (%)	SCC, *n* (%)	χ^2	*P*
Age (years)					
20-29	4 (26.7)	1 (2.5)	0	37.95	<0.001
30-39	6 (40.0)	17 (42.5)	4 (8.9)		
40-49	2 (13.3)	10 (25.0)	15 (33.3)		
50-59	3 (20.0)	3 (7.5)	13 (28.9)		
60-69	0	9 (22.5)	13 (28.9)		
Mean±SD	37.53±10.05	44.33±11.98	51.56±9.57		
Parity, mean±SD	3.73±2.09	3.43±1.85	3.96±1.55	6.77	0.149
Menopausal status					
No	12 (80.0)	28 (70.0)	18 (40.0)	11.33	0.003
Yes	3 (20.0)	12 (30.0)	27 (60.0)		
Contraception					
Tubal ligation	2 (13.3)	4 (10.0)	6 (13.3)	3.13	0.792
IUCD	1 (6.7)	1 (2.5)	0		
Barrier	1 (6.7)	5 (12.5)	5 (11.1)		
None	11 (73.3)	30 (75.0)	34 (75.6)		
Socioeconomic status					
Upper middle	3 (20.0)	4 (10.0)	5 (11.1)	4.02	0.674
Lower middle	3 (20.0)	13 (32.5)	11 (24.4)		
Upper lower	7 (46.7)	16 (40.0)	16 (35.6)		
Lower	2 (13.3)	7 (17.5)	13 (28.9)		

CC: Chronic cervicitis, CIN: Cervical intraepithelial neoplasia, SCC: Squamous cell carcinoma cervix, IUCD: Intrauterine contraceptive device, SD: Standard deviation

P53 expression

Mean p53 scores of CC, CIN, and SCC cases were 0.0, 1.70, and 4.38, respectively. A significant difference was found in average p53 scores between the three groups ($P < 0.001$). Within the CIN group, the mean p53 scores also showed a statistically significant graded increment with increasing severity of the lesion. Mean p53 scores for CIN 1, 2, and 3 were 1.07, 1.63, and 2.22, respectively. When considering immunoscore cut-off 2 for positive p53 expression to compare with existing studies, p53 positivity in various groups of lesions was as follows: CC-0%, CIN1-16.7%, CIN2-37.5%, CIN3-55.6%, and SCC-77.8% [Table 3 and Figure 4].

CIN1 was differentiated from CC with 1.0 ≤p53 <2.5 as predictor for CIN1. The sensitivity, specificity, and AUROC were 42.9%, 100%, and 71.4%, respectively. p53 score calculated on CIN1 histopathology when falling between this range correctly identified only 42.9% of cases of CIN1 while a p53 score <1.0 on a histopathology sample of CIN1 ruled out CIN1 with 100% specificity. Hence as per this system, the sample could safely be adjudged biologically as CC. CIN2 will be differentiated from CIN1 and CC with 2.5 ≤p53 <3.5 as predictor for CIN2. The sensitivity, specificity, and AUROC are 37.5%, 85.7%, and 62.1%, respectively. CIN3 was differentiated from lower lesions with 3.5 ≤p53 <4.5 as predictor for CIN3. The sensitivity, specificity, and AUROC were 33.3%, 100%, and 61.5%, respectively. A CIN3 sample with a p53 score falling in this range had 33.3% chance of being CIN3, while a p53 score <3.5 had 100% chance of it being CIN2/CIN1/CC. SCC was differentiated from CIN3 with p53 ≥4.5 as predictor for SCC. The sensitivity and specificity were 57.8% and 88.9%, respectively, coupled with a maximum AUROC of 78.1%.

As per this IHC score categorization, SCC was differentiated from CIN3 with maximum validity. Nonmalignant lesion (CC) was differentiated from premalignant lesion (CIN1) with 100% specificity. Furthermore, high-grade preinvasive lesion (CIN3) was differentiated from lower-grade lesions with 100% specificity [Table 4]. Overall diagnostic accuracy of the proposed scoring system for differentiating CC, CIN, and SCC was 61%, while the accuracy of previous methods of interpreting p53 immunoreactivity as immunoscore >2[10,13,14] or arbitrary cut-off of >10%[15,16] cells with nuclear positivity was only 48% [Table 5].

Discussion

Cervical cancer has a slow progression from preinvasive CIN to invasive carcinoma, thereby providing ample opportunity to detect and successfully treat the precursor lesions. Classification of cervical cancer precursors should accurately reflect the natural history of disease progression and optimally aid in clinical decision making

Table 3: Comparison of p53 score among various groups of cervical lesions

Case HPE	p53 score (I+G)			Kruskal-Wallis test	
	Mean±SD	Minimum	Maximum	χ^2	*P*
CC	0.00±0.00	0.0	0.0	45.15	<0.001
CIN	1.70±1.71	0.0	5.0		
SCC	4.38±2.22	0.0	7.0		

CC: Chronic cervicitis, CIN: Cervical intraepithelial neoplasia, SCC: Squamous cell carcinoma cervix, SD: Standard deviation, HPE: Histopathological Examination

Table 4: Receiver operating characteristic analysis for defining immunohistochemical p53 cut-off scores for various categories of cervical lesions

Cervical lesion	Cut off	Sensitivity	Specificity	AUROC (%)
CIN1	1.0≤p53<2.5	42.9	100.0	71.4
CIN2	2.5≤p53<3.5	37.5	85.7	62.1
CIN3	3.5≤p53<4.5	33.3	100.0	61.5
SCC	p53≥4.5	57.8	88.9	78.1

CIN: Cervical intraepithelial neoplasia, SCC: Squamous cell carcinoma cervix, AUROC: Area under the receiver operating characteristic curve

Table 5: Validity of proposed cut-off p53 scores against previous thresholds of p53 positivity for various groups of cervical lesions

Cervical lesion	p53 <1	1.0 ≤p53 <2.5 (CIN1)	2.5 ≤p53 <3.5 (CIN2)	3.5 ≤p53 <4.5 (CIN3)	p53≥4.5 (SCC)
CC	15	0	0	0	0
CIN1	8	4	1	1	0
CIN2	3	2	3	0	0
CIN3	7	1	4	4	2
SCC	6	4	1	8	26
Cervical lesion HPE	**Total cases**	**0-2 (negative p53 expression) (*n*)**	**3-8 (positive p53 expression), *n* (%)**		
CC	15	15	0		
CIN1	14	12	2 (16.7)		
CIN2	8	5	3 (37.5)		
CIN3	18	8	10 (55.6)		
SCC	45	10	35 (77.8)		

p53 additive cut-off score>2: Reference Ayatollahi *et al.*[10]. This cut-off was chosen to compare with qualitative studies. CIN: Cervical intraepithelial neoplasia, SCC: Squamous cell carcinoma cervix, HPE: Histopathological Examination

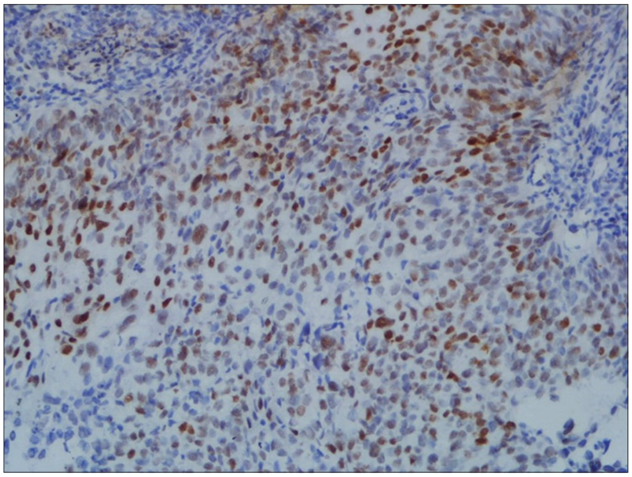

Figure 2: P53 strongly positive: Tumor cells showing moderate intensity ++ and more than 75% tumor cells positivity of P53 in squamous cell carcinoma of uterine cervix ×200

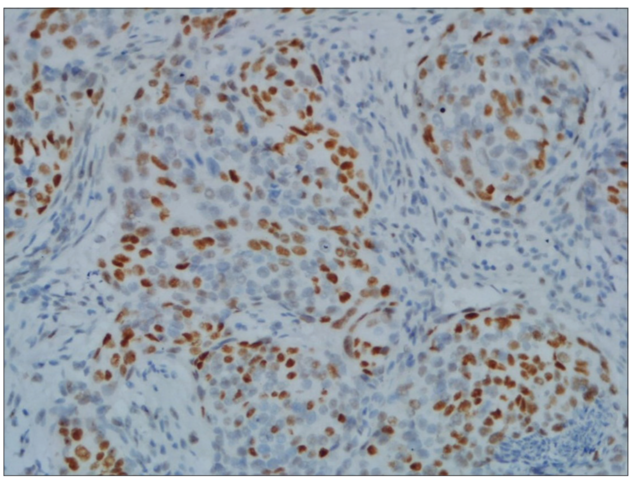

Figure 3: P53 strongly positive: Tumor cells showing high intensity +++ and <50% tumor cells positivity of P53 in squamous cell carcinoma of uterine cervix ×200

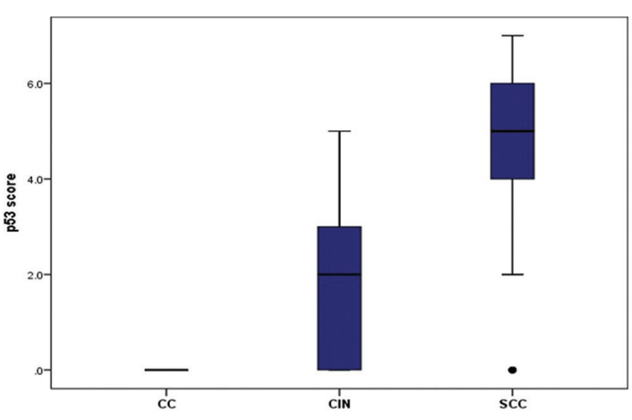

Figure 4: Showing comparison of p53 score among various groups of cervical lesions

while minimizing over or under treatment and associated financial burden. The Bethesda system of cytology and the WHO histological diagnosis of CIN are relevant for the management of disease. The severity of CIN is expressed by its microscopic grade and becomes the basis for the treatment of the patient. The drawbacks of the histopathological classification system are faulty static impression of a dynamic lesion that can persist, progress, or regress with time, intra- and interobserver variability, difficulty in distinguishing CIN reliably from nonneoplastic lesions and difficulty in reliably designating CIN 2.[4,17] In a multicenter randomized study, the maximum number of diagnostic disagreements were between normal cervical tissue and CIN 1.[4] CIN 2 diagnosis had the lowest class-specific agreement in the same study. Biomarkers can serve as useful adjuncts in such situations. IHC expression of p53 can be combined with routine screening modalities to detect precancerous lesions of the cervix, identify patients at greater risk of progression, accordingly provide treatment and frame follow-up protocol. Further, biomarkers can also be used in prognostication and targeted therapy by defining the biological behavior of the tumor.[18]

p53 is a tumor suppressor gene located on chromosome 17pl3 and regulates cell proliferation. HPV oncoprotein E6 binds to p53 and disrupts its normal function.[19] Positive staining for p53 protein by IHC is considered to be abnormal because normal protein has a short half-life while the mutated/inactivated protein is stable, accumulates intranuclearly, and hence is detected on IHC.[20] The proportional increase in p53 expression with advancing severity of cervical neoplastic lesions has been suggested.[21-23] An association between p53 expression and the overall survival of cervical cancer patients has been observed.[24]

van Zummeren *et al*.[9] demonstrated higher accuracy and reproducibility of immunoscoring IHC expression of biomarkers in grading CIN lesions rather than choosing arbitrary thresholds for defining positivity. By the use of immunoscores they could divide classical CIN2 into more accurate grades of CIN 1 or 3. This can standardize the definition of CIN grading and allow more accurate comparison of CIN-based management strategies. Further, Ayatollahi *et al*.[10] showed the meaningful correlation between time-consuming H score and additive quick scores for interpreting p53 expression in cervical cancer. Hence simpler quick scores can aptly quantify p53 expression.

The present study was aimed at assessing p53 expression in various grades of cervical lesions and quantifying the same using quick additive immunoscores. Further using ROC analysis p53 cut-off scores were determined for distinguishing each class of cervical lesion (nonmalignant CC, premalignant CIN, and invasive cervical cancer SCC). Individual cut-off scores for each grade of CIN (1, 2, and 3) were also determined.

In this study, all the benign cases (CC) cases were negative for p53 expression, similar to the study conducted by Mitildzans *et al*.,[21] Feng *et al*.,[20] Grace *et al*.,[25] and

Sandhu *et al*.[26] In studies done by Singh and Bannur[13] and Raju *et al*.,[27] 10% and 20% of cases of the normal epithelium (control), respectively, showed weak p53 positivity that was restricted to the basal layer mainly. Various authors have highlighted the idea of increasing p53 expression with advancing grade of CIN which could establish p53 as a potential biomarker for the progression of lesion.[21-23,25] Our study also observed a progressive increase in p53 expression with an increasing grade of CIN (mean p53 score 1.07 in CIN 1, 1.63 in CIN 2, and 2.22 in CIN 3) with a statistically significant difference ($P < 0.001$). When considering immunoscore cut-off 2 for positive p53 expression to compare with existing studies, a similar graded increment in p53 positivity was noted (CC-0%, CIN1-16.7%, CIN2-37.5%, CIN3-55.6%, and SCC-77.8%).

In the present study, there was a significant difference in p53 expression (Mean p53 scores) between each group of cervical lesions (CC, CIN and SCC-0, 1.7, 4.38 respectively), within the CIN group also, the mean p53 scores showed a statistically significant graded increment of expression with increasing severity (Mean p53 scores for CIN 1 was 1.07, for CIN 2 was 1.63, and for CIN 3 was 2.22). Using ROC analysis of p53 expression we defined a threshold range for the particular histopathological cervical lesion. Although the sensitivity of such a ranged was low, the specificity was high for each subset.

For example, p53 score calculated for a given CIN 1 specimen when falling between the defined ROC ranges ($1 \leq$ p53 < 2.5) correctly identified only 42.9% of cases of CIN 1, but those identified cases have 100% chance of there being CIN 1.

Similarly, a p53 score between the range of 3.5–4.5 identified only 33% of cases of CIN 3 (low sensitivity) but those identified cases have 100% chance of there being CIN 3 (high specificity), Generally, a screening test should be highly sensitive, whereas a follow-up confirmatory test should be highly specific.

The purpose of our IHC scoring system is to provide a confirmatory diagnosis to aid an already existing histological diagnosis and tests with high specificity serve this idea adequately. Therefore, our proposed ROC-based p53 system can act as an adjunct to diagnosing histological specimen rather than itself being a primary diagnostic system.

Using high specificity of IHC, above defined thresholds, a binary system of classification can be developed to differentiate Nonmalignant lesions from premalignant, premalignant from malignant lesions, high-grade intraepithelial lesions (CIN 3) from low-grade lesions. Hence, using the high specificity of ROC defined thresholds in this study, a particular histological specimen having p53 within its defined ROC range rules in favor of that particular diagnosis strongly. However, due to the low sensitivity of thresholds in the present study, the number of true positive identified for each category will be less.

Grace *et al*.[25] demonstrated a highly significant positive correlation for p53 expression level with different stages from mild dysplasia (CIN 1) to invasive cancer. Similar increment of expression with increasing severity of CIN lesion was shown by Madumati *et al*.[16] Mitildzans *et al*.[21] demonstrated remarkably increased expression of p53 from the control group to the CIN group and within the CIN group also there was a significant increment of expression with increasing severity. Shukla *et al*.[14] also showed increasing positivity of p53 expression with increasing grade of the lesion (CIN1 22.2%, CIN2 50%, and CIN3 100%). An Indian study by Ghosh *et al*.[15] showed 51% positivity in cervical preneoplastic lesions which were significantly linked to histology grades CIN 1 to CIN 2 with a slight reduction in CIN 3. Tan *et al*.[28] showed a significant difference of expression between CIN 3 and SCC and concluded that p53 may serve as a helpful adjunct in differentiating the two groups of lesions in difficult situations. Feng *et al*.[20] also showed p53 overexpression in both cervical preneoplastic and neoplastic lesions while other authors failed to acknowledge any such significant difference in expression of p53 in various grades of cervical lesions.[29-31] The incidence of p53 positivity in (SCC cervix) was 80% in the present study. The range of nuclear p53 positivity in cervical carcinoma has been reported with considerable variation: ranging from 100%,[20,21,31] around 85%[10,13,27] to as low as 63%,[29] 45.5%,[16] and 28.5%.[23] The conflicting results in different studies can be attributed to different scoring systems for judging p53 positivity coupled with different fixation, antigen retrieval methods, and antibody selection.

Although the sample size in our study was small, our findings reflect p53 scores to be a useful adjunct to routine histopathology in differentiating nonmalignant from premalignant lesions (CC from CIN 1), high-grade CIN from low-grade CIN, and premalignant from malignant lesions (CIN 3 from SCC). Objectified p53 expression in cervical lesions can further be utilized for prognostication, assessing response to therapy and possible targeted gene therapy in future.

Conclusion

p53 expression can be utilized as a rapid biomarker for differentiation of various categories of cervical neoplastic lesions and aid histopathology in better diagnostic compartmentalization of CIN group. ROC-derived immunoscore cut-offs can provide the much-needed objectivity and optimal decision thresholds to IHC interpretation.

Ethical clearance

This study was approved in Institutional Ethics Committee (Ref. Code: 90th ECM II B-IMR-R/P8) Dated 21.08.2018), Research Cell, King George's Medical University, Lucknow, Uttar Pradesh, India.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest.

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