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Original Article

Expression of p63 in Cervical Intraepithelial Neoplasia (CIN) and Cervical Cancer

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Key words:
CIN, Cervical Cancer, p63

Abstract:

Background: *Cervical cancer is a major public health problem worldwide. Persistent infection with High-Risk Human Papilloma Virus (HPV) has been the main cause of squamous intraepithelial neoplasia which in turn leads to invasive squamous cell carcinoma. p63 is necessary for the activation of HPV, epithelial proliferation and differentiation. It also regulates the expression of certain cell cycle regulators. It has been reported that, from CIN I to CIN III, p63 expression increases progressively from basal layer to surface. In squamous cell carcinoma, it is expressed throughout the entire thickness of the tumor. Thereby it plays a significant role in diagnosing cervical premalignant and malignant lesions.*

Objective: *To evaluate the relationship of p63 expression with different grades of CIN & invasive SCC.*

Method: *Total 86 paraffin embedded tissue blocks of histopathologically diagnosed cases of CIN and cervical cancer were evaluated by immunohistochemical staining for p63 expression. The study was performed in Sir Salimullah Medical College, Dhaka (from March, 2018 to February, 2020). Statistical analyses were carried out by using SPSS version 22 for Windows. A descriptive analysis was performed for all data. Observations were indicated by frequencies and percentages. Statistical significance was set at "p" value <0.05.*

Results: *Present study showed progressive increase in p63 expression from CIN I to CIN III from basal layer to surface. In invasive squamous cell carcinoma, higher expression of p63 was noted throughout the entire thickness of the tumor. No expression was seen in cervical adenocarcinoma and small cell carcinoma. In adenosquamous carcinoma only the area showing squamous differentiation revealed positive p63 expression. Statistically significant association of p63 expression was found with parity of patients and among grades of CIN .*

Conclusion: *The results of this current study revealed that, p63 has significant association among different grades of CIN. It is also a useful marker in confirming a poorly differentiated squamous cell carcinoma & predicting the progression of a squamous neoplastic lesion from cervical intraepithelial neoplasia to invasive squamous cell carcinoma. Moreover, it is useful to differentiate invasive squamous cell carcinoma from cervical adenocarcinoma.*

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Introduction

Cervical cancer is the fourth most common cancer in women worldwide, which accounts for 6.6 % of all female cancers.[1] 90% of deaths from cervical cancer occur in low and middle-income countries.[2] Unfortunately, it is the second leading cause of cancer deaths among women in Bangladesh. About 12,000 new cases are diagnosed and approximately 6,000 of them die annually.[3]

Infection with oncogenic types of Human Papilloma Virus (HPV-16 & 18) is the most important factor in the development of cervical cancer. Viral oncoprotein E6 and E7 specifically bind and inactivate p53 and retinoblastoma (RB) tumor suppressor proteins. This causes disruption of cell cycle leading to increased cell proliferation.[4]

Invasive cervical cancers, mostly which are squamous cell carcinomas are preceded by precancerous lesions designated as cervical intraepithelial neoplasia (CIN/SIL). About 10% of CIN I usually progress to CIN III, and from CIN III, only 12% progress to invasive cancer. CIN III cases diagnosed in biopsy may also have invasive foci elsewhere.[5] CIN-I is treated as benign & kept under observation. CIN-II & CIN-III are considered precursor of invasive cancer & managed by surgical procedure.[4] So, the exact diagnosis is important.

Though considered as 'gold standard', diagnosis based on histology is complicated by inter-observer variability. For example, truly neoplastic lesions are sometimes misclassified as 'negative for dysplasia' due to various reactive and metaplastic changes. This emphasizes the need for the specific biomarkers for identification of truly dysplastic cells.[6] P16 identifies high risk HPV infected cells and not necessarily all HPV-16 infected cells undergo neoplastic transformation. High expression of Ki-67 indicates a neoplastic progression. In spite of that, more sensitive marker to distinguish the progression of a lesion towards dysplasia and malignancy in cervix is appreciable.[7]

Cervical cancer is classified histomorphologically into Squamous cell carcinoma, Adenocarcinoma, Adenosquamous carcinoma, Neuroendocrine carcinoma and others. Squamous cell carcinoma is most common & have a better prognosis than others. Increasing tumor grade correlates with tumor aggressiveness.[4] Response to different treatment modalities varies among different morphological types. Adenocarcinoma is more radio and chemo-resistant. Targeted therapy wih bevacizumab (against VEGF) is significantly effective in SCC but not in ADC.[8] These reveal the importance of detecting exact histological type, which is of great problem in case of poorly differentiated carcinoma. Moreover, squamous cell carcinoma & neuroendocrine carcinoma both have small cell morphology. In that cases immuno-histochemistry can help over routine histo-pathology.[9]

p63 is a member of the p53 family of transcription factors. It is expressed in the basal layer of stratified epithelium (eg. skin, esophagus, exocervix, tonsil and bladder) and certain glandular structures(prostate, breast). In squamous epithelium it promotes proliferation of basal layer stem cells, at suprabasal layers p63 levels are down regulated, allowing cells to undergo differentiation.[10] The interaction of HPV oncoproteins E6 and E7 with cell cycle mechanism causes altered expression of p63.[11] In mild dysplasia (CIN-I) it is expressed in basal and parabasal layers, extending into the middle and upper layers in moderate and severe dysplasia (CIN-II and III). It is therefore helpful for confirmation of the diagnosis in equivocal cases.[12]

p63 expression has also been found in SCC of the oral cavity, lung, uterine cervix, head- neck, skin and esophagus but not in adenocarcinomas including those of breast and prostate.[13] In head-neck and oral squamous cell carcinoma p63 expression increased significantly with increasing grade of SCC.[14,15] In esophageal and skin SCC no association was noted with grade.[16,17] Very few studies were performed incorporating p63 and cervical neoplastic lesions, none of these commented on association with grade.

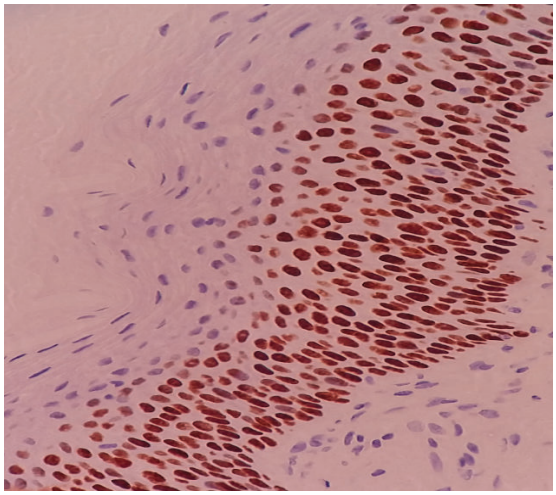
Overexpressed p63 inhibit p53 and mediate survival in SCC cells by inhibiting p73 dependent apoptosis.[18] It is supposed that small inhibitory RNA (si-RNA) targeted against specific p63 isoform induce apoptosis in SCC cells with no impact on normal keratinocytes.[19] All these things signify the importance of more studies on CIN & cervical carcinoma incorporating p63 expression.

Methods

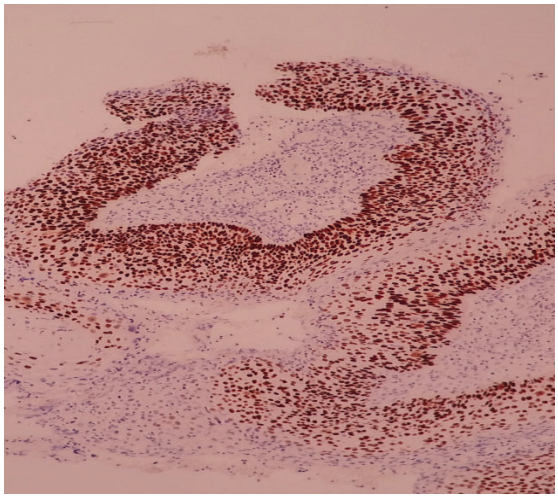
This study was conducted in Department of Pathology, Sir Salimullah Medical College and Mitford Hospital, Dhaka during the period from March, 2018 to February, 2020. After approval from the institutional ethics committee and obtaining informed written consent, 86 adult female patients with CIN & cervical cancer diagnosed histopathologically were included. Tissue blocks with extensive necrosis or hemorrhage, patients previously exposed to chemotherapy or radiotherapy, or with metastatic carcinoma of cervix were excluded. Immunohistochemical staining for p63 was evaluated by the number(%) of p63 positive cells and classified on a four point scale as -,1+, 2+, 3+ . Statistical analysis were carried out by using Statistical Package for Social Sciences version 22 for Windows. A descriptive analysis was performed for all data. The mean values were calculated for continuous variables. Quantitative observations were indicated by frequencies and percentages. A "p" value <0.05 was considered as significant.

Results

This cross sectional study was conducted on 86 histopathologically diagnosed CIN(28) & cervical cancer(58) patients. Histopathology was done by H & E followed by immunohistochemistry for p63 to observe the usefulness of this immune marker in patients with cervical neoplasia. The following observations were found.



A (x 200)



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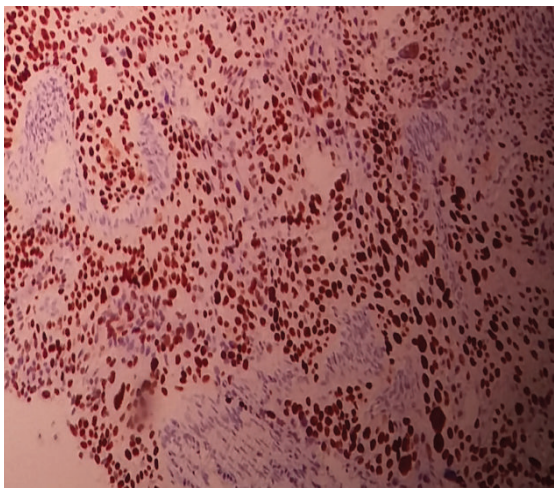

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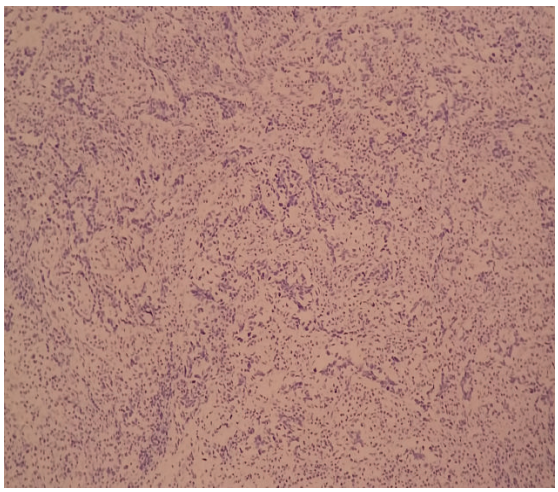

D (x100)

Fig.-1: *Photomicrograph of p63 expression in A) CIN II B) CINIII C) Poorly differentiated squamous cell carcinoma (SCC) and D) Poorly differentiated adenocarcinoma.*

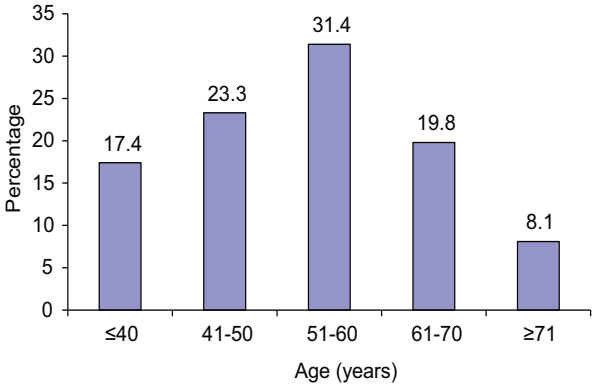

Fig.-2: *Bar diagram showing age distribution of the study patients(n=86)*

Table I
Association between CIN grades with P63 expression (n= 28)

CIN	p63 expression			P
	n	Mean±SD	Min-max	value
CIN I (A)	12	28.50±11.41	8-50	
CIN II (B)	9	49.33±17.55	8-70	
CIN III (C)	7	69.14±13.55	50-84	
Statistical analysis				
A vs B vs C				0.001[s]
A vs B				0.004[s]
A vs C				0.001[s]
B vs C				0.027[s]

s= significant
ANOVA followed by Bonferroni test was performed to compare between groups.

Discussion

In this study the mean age of the patients was 54.22±11.78 ranged from 27 to 78 years. Highest number of cases were in 51-60 years age group (64% were post-menopausal). CIN-I cases were highest in 4th decade and CIN-II were in 5th decade. Most of the CIN-III and cancer cases were observed in 6th decade. Karim S.S. also found gradual increase in mean age with increased CIN grade and malignancy.[20] Most of the cases in this study got married between 15-20 years of age. Nessa et al. also reported that early exposed are more susceptible to HPV infection.[21]

Repeated trauma to the cervix during child birth, hormonal changes & weaker immune system during pregnancy also makes women more susceptible to HPV infection.[22] In this study, nearly all the invasive cancer cases were multipara. Nessa et al. found more than 50% of their studied respondent having >2 children.[21]

Total 69 cases showed positive P63 expression. The positive cases are CIN, SCC and adenosquamous carcinoma. The mean P63 expression was 46.98±28.67 ranging from 0 to 88. Ulziibat et al. found mean p63 expression 36 ± 21.9 in Mongolian women(n=40) ranging from 5-70%.[23] This result is varied from the present study. These can be explained by the influence of geography and difference in sample size.

26 out of 28(92.86%) CIN cases showed positive p63 expression. 1 CIN-I and 1 CIN-II negative cases may be not truely neoplastic, rather reactive. The mean p63 expression was 28.5±11.41 in CIN-I, 49.33±17.55 in CIN-II, 69.14±13.55 in CIN-III ranging from 08-84. Statistically significant increase in mean p63 expression was noted from CIN I to CIN III. From CIN I to CIN III p63 expression increased progressively from basal layer to surface. Mitildzans et al. showed similar finding, supported that P63 immunostain can be useful in diagnosing CIN.[12]

40(93.02%) out of 43 patients of SCC showed positive p63 expression in present study. Among them 50% showed strong positivity. Ilhami et al. reported 73.21% SCC cases with strong positivity.[24] This difference may be due to difference in the threshold level of p63 positivity, HPV strain, technical aspects or other confounding factors. No significant difference in p63 expression was noted among different grades of SCC.

All adenocarcinomas and single small cell carcinoma cases were negative. 3 adeno-squamous cases showed moderate p63 positivity in their squamous portion only. Ulziibat et al. as well as Jacob & Sundaram observed similar findings.[23, 25] All these observations support the significance of p63 immonoexpression in diagnosing cervical SCC and differentiating SCC from non SCC cases, as well as to diagnose adenosquamous carcinoma by marking the squamous differentiated area. From CIN III to SCC no significant difference was noted in mean p63 expression.

Out of total 6 poorly differentiated carcinoma 5 showed moderate to strong reactivity for p63

indicating squamous differentiation. Single case showed 0% p63 positivity, indicating glandular differentiation. Single case with small cell morphology also showed negative p63 expression, supporting the diagnosis for neuroendocrine carcinoma. Positive chromogranin staining confirmed the diagnosis.

To sum up, findings of present study demonstrates that p63 can be a useful immunomarker in diagnosing cervical premalignant and malignant lesions. It is reliable in exact grading of CIN. It is also a powerful marker of squamous differentiaton. It clarifies the spectrum of poorly differentiated carcinoma and differentiate pure squamous or glandular, from adenosquamous carcinoma.

In an over populated country like Bangladesh, where women usually get married in their teens, have a high parity and unaware of the importance of cervical cancer screening system it is important to improve the conventional screening and diagnosing techniques. Therefore, incorporation of p63 will be helpful in early & reliable detection of cervical neoplastic lesions.

Conclusion

In the present study, p63 expression increased progressively from basal layer to surface in CIN I to CIN III. In invasive SCC, it was consistently higher throughout the entire thickness of the tumor and not expressed in adenocarcinoma and small cell carcinoma. In adenosquamous carcinoma only the squamous differentiation areas showed p63 expression. Thus the pattern of overexpression of p63 demonstrates the possibilities of potential use as a diagnostic marker for cervical premalignant and malignant squamous epithelial lesions.

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