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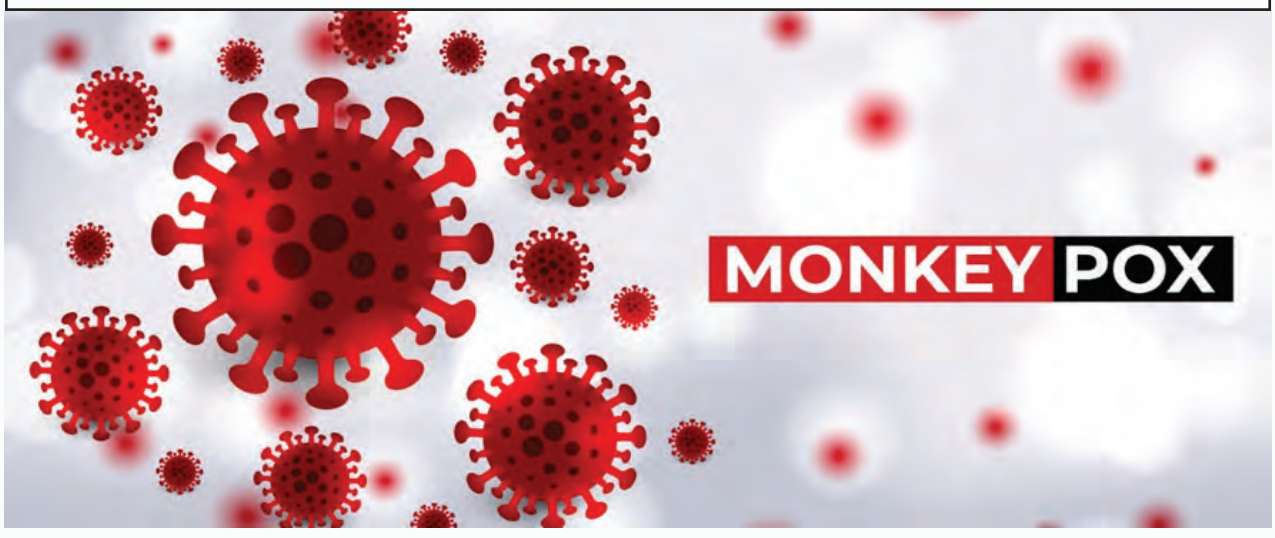


The International Tobacco Control Conference on

The Scourge of Smoking and Conflicts of Interest

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Original Article

Immunohistochemical Study of Cyclin D1 and HER2/neu in Pre–neoplastic and Neoplastic Epithelial Lesions of Cervix

Neha D. Newadkar, Siddhi Gaurish Sinai Khandeparkar, Bageshri P. Gogate, Aishawarya Gotekar, Priyanka Bhave, Rashmi Shinde, Aanchal Goyal

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Abstract

Background: Among cancers affecting women worldwide, cervical cancer is positioned at the fourth rank.

Aim: The present study was undertaken to evaluate the immunoexpression and interrelationship of cyclin D1 (CD1) and HER2/neu in histopathologically diagnosed cases of cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma (ICC) and its association with other clinicopathological parameters.

Materials and Methods: This study included 120 cases of cervical lesions; out of which 10 cases were of negative for malignancy/dysplasia (NED), 15 cases of CIN I, 9 cases of CIN II and 21 cases of CIN III and 65 cases of invasive cervical carcinoma (ICC). A technique of manual tissue array was employed to study the expression pattern of CD1 and HER2/neu in all the cases. Results were subjected to statistical analysis.

Results: CD1 immunoexpression was statistically significantly higher in normal + LSIL (low grade squamous intraepithelial lesion) (CIN I) cases than HSIL (high grade squamous intraepithelial lesion) (CIN II+III) + ICC. As the severity of the cervical lesions increased, CD1 immunoexpression statistically significantly shifted from nucleus to cytoplasm. Statistically significant inverse relationship was noted between CD1 expression and grade of ICC cases. CD1 IHC marker showed 63.64% sensitivity, 0% specificity, 87.5% PPV and 0% NPV. The overall agreement of CD1 staining with H&E diagnosis was 58.33% (κ=0.1538 i.e. poor). HER2/neu immunoexpression increased statistically significantly with increasing severity of lesion from CIN–I to CIN–III to ICC. None of the NED cases in present study showed HER2/neu positivity. With increasing stage and grade of ICC, the percentage of cases expressing HER2/neu statistically significantly increased. Her2/neu IHC marker showed 56.63% sensitivity, 100% specificity, 100% PPV and 17.24% NPV. The overall agreement of HER2/neu staining with H&E diagnosis was 60% (κ=0.1771 i.e. poor). The agreement between HER2/neu and CD1 immunostaining was 40%.

Conclusion: Cyclin D1 and HER2/neu IHC marker panel could be used as prognostic markers and could be routinely incorporated into the surgical pathology report which could aid in understanding the biology of the tumor and better patient management. ICC expressing CD1 and HER2/neu could benefit from specific targeted therapy.

KEYWORDS: Cervical lesions, SCC, IHC

Introduction

Among cancers affecting women worldwide, cervical cancer is positioned at the fourth rank. In 2020, 604,127 new cases of cervical cancer were documented globally and 341,831 deaths were attributed to this malignancy by Globocan 2020. [1] In India, cervical cancer accounted for 9.4% of all cancers and 18.3% (123,907) of new cases in 2020. In 12 Indian states, cervical cancer ranks as the second most common cause of cancer–related deaths among females. [2]

Screening tests such as visual inspection with acetic acid and/or Lugol's Iodine, PAP smear (Papanicolaou test)

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with liquid based cytology technique, high risk human papilloma virus (HR–HPV) DNA (deoxyribose nucleic acid) testing together with prophylactic HPV vaccines are used for early detection and prevention of cervical cancer which has substantially reduced the incidence of cervical neoplasia, especially in developed countries.[3] However, a substantial number of cases of cervical cancer have still been missed due to subjective test criteria in evaluating the PAP smears.[4] The search for new markers with prognostic or predictive value is of vital significance in the development of effective anti–cancer therapies.

Cyclin D1 (CD1), together with cyclin dependent kinases (CDK4 and CDK6), is a key regulator of G1 to S phase transition in various cell types. The CD1 gene maps to 11q13 and regarded as cellular oncogene. During the normal cell cycle, CD1 complexes with CDK4 and promotes the phosphorylation of the tumor suppressor retinoblastoma protein (RBp) and thereby relieves G1 arrest. The transcription factor E2F is then initiated to promote DNA synthesis, allowing the completion of cell division by moving from the G1 phase to the S phase. CD1 can thus be seen as shortening the G1 phase of the cell cycle.[5]

When CD1 is dysregulated and multiple cancer–related genes result in its increased expression, the time cells spend in the G1 phase of the cell cycle is significantly decreased, causing the cells to enter the S phase early, in turn resulting in uncontrolled cell proliferation and transformation, leading to carcinogenesis. [6]

The association between cervical cancer and HR–HPV infection (HPV16/18) has been well established.[7] E6 and E7 protein of HR–HPV degrades tumor suppressor protein p53 by ubiquitin mediated proteolysis interfering with the cell's ability to undergo apoptosis, a normal response to DNA damage, causing accumulation of genetic mutations as the virus infected cell divides. E7 protein of the high–risk HPV interacts with RBp liberating E2F transcription factor which enhances CD1 expression by binding to the promoter region of CD1 gene amplifying the activity of CD1–CDK complex promoting cell cycle progression by phosphorylating RBp. The disruption of p53 and RBp functions result in evasion of normal cell cycle checkpoints and regulatory mechanisms resulting in cervical cancers caused by genomic instability because of viral DNA integration into the cellular DNA disrupting the viral E2 gene which is negative regulator of E6 and E7 viral proteins. When viral DNA integrates into different chromosomal loci in the host genome, it can have significant implications for the functions of nearby oncogenes and tumor suppressor genes.[8] In cervical cancer, expression of CD1 is found to be aberrant. Some studies have found elevated levels of this protein, whereas others have observed it to be under expressed.[5]

HER2/neu maps to 17q12 and encodes a protein with tyrosine–kinase activity. The expression of HER2/neu is believed to be associated with aggressive biological behavior and metastatic potential. In cases of CIN and squamous cell carcinoma (SCC), it has been observed that there is greater c–erbB2 expression as the severity of the lesion increases.[9]

Very few studies documenting CD1 and HER/2neu expression in cervical lesions in Indian population is found in literature. The present study was undertaken to evaluate the immunoexpression and interrelationship of cyclin D1 and HER2/neu in histopathologically diagnosed cases of cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma (ICC) and its association with other clinicopathological parameters like grade and stage of tumor wherever possible.

Materials And Methods

This cross–sectional study was conducted in a tertiary care hospital. Ethical clearance was obtained from institute's ethical committee. This study included 120 cases of cervical lesions; out of which 10 cases were of non–specific chronic cervicitis (NED), 15 cases of CIN I, 9 cases of CIN II and 21 cases of CIN III and 65 cases of invasive cervical carcinoma (ICC). These cases were diagnosed and/or operated upon (biopsy and/ or hysterectomy) during the period from August 2014 to June 2019. The available data such as age, menopausal status, presenting symptoms and type of specimen for all the patients were collected from the records of histopathology section of the Department of Pathology. Cases in which records/slides/blocks were not available were excluded from the study.

All routinely processed paraffin embedded tissue blocks and Hematoxylin and Eosin (H&E) stained slides of these cases were retrieved. The slides were reviewed by two experienced histopathologists. The cases were classified according to histomorphological subtype and pathological staging was done as per guidelines of World Health Organization.[9] FIGO was done in cases received as surgical specimen.[9]

We selected the representative slide and paraffin block for immunohistochemistry (IHC) staining. A technique of tissue microarray array (TMA) was employed along with IHC staining of entire section in some cases.[10] The monoclonal antibodies to CD1 (clone EP12, Dako), and HER2/neu (Clone CB11, Novacastra) were used. Appropriate positive controls were taken for the IHC stains as per literature.[11] Negative control (without adding primary antibody) was included in all batches. Antigen retrieval was done using Citrate Buffer Antigen Retrieval

Protocol. Pressure cooker was used as heating source. The sections stained with monoclonal antibodies using IHC staining procedure were examined alongside H&E–stained specimens, to identify the precise locations of the lesions.

For CD1, nuclear as well as cytoplasmic expression of CD1 was considered in the present study. The intensity of the immunohistochemical reaction was recorded as 0, negative (no staining of any nuclei /cytoplasm even at high magnification); 1, weak (only visible at high magnification); 2, moderate (readily visible at low magnification); or 3, strong (strikingly positive even at low power magnification). The proportion of nuclei/cytoplasm showing positive staining was also recorded as either: 0, no staining; 1, <1%; 2, 1–10%; 3, 11–33%; 4, 34–66%; 5, 67–100% staining. The proportion and intensity scores were subsequently added to obtain a total score, which ranged from 0 to 8. Lesions were categorized into following groups: negative/weak (total scores 0–2), moderate (total scores 3–5) and strong (total scores 6–8).[6]

Intensity of HER2/neu expression was graded according to the 2014 ASCO/CAP guidelines as complete intense circumferential membrane staining within >10% of tumor cells (3+); circumferential staining that is incomplete and/ or weak/moderate within >10% of tumor cells or complete intense circumferential membrane staining within <10% of tum or cells (2+); membrane staining that is incomplete and is faint/barely perceptible and within >10% of tumor cells (1+) and no staining is observed or shows membrane staining that is incomplete and is faint/barely perceptible and within <10% of tumor cells. Her2 overexpression was assessed as negative for scores 0 or +1 and positive for scores of +2 and +3.[9]

The Primer of Biostatistics 7.0 program was used for statistical analysis. Quantitative data was presented with the help of mean. Qualitative data was presented with the help of frequency and percentage table. Sensitivity, specificity, predictive values, and accuracy of biomarkers (cyclin D1 and HER2/neu) were calculated. The results were statistically significant when the p value was < 0.05 and highly statistically significant when p value was < 0.01.

Results

A total of 120 cases were included in the present study. Out of which, 94 cases were received as cervical biopsies (78 punch and 16 cone amputated) and 26 cases were received as surgical specimens (trans abdominal hysterectomy). Out of 120 cases, SCC was maximum (n=50), of which 35 cases were of large cell keratinizing subtype (LCKSCC), 15 cases were of large cell nonkeratinizing subtype (LCNKSCC), 14 cases were of papillary squamo–transitional subtype (PSTC) and one of adenosquamous carcinoma. All cases of SCC and one case of adenosquamous carcinoma (65) were considered together as ICC in further discussion.

The youngest and oldest patient of CIN was 28 and 80 years of age respectively with a mean age of 48.15 years. The youngest and oldest patient of ICC was 28 and 80 years of age respectively with a mean age of 52.63 years. Out of the 120 cases studied, 101 (84.17%) of them were postmenopausal. The most common presenting symptom in cases of CIN was excessive vaginal bleeding (18/45, 40%) followed by vaginal discharge (16/45, 35.50%). The most common presenting symptom in cases of ICC was post–menopausal bleeding (22/65, 33.84%) followed by vaginal discharge (18/65, 27.69%). Clinical staging was available in 26 cases. As most of the specimen received were biopsies, pathological staging was not possible in them. 50% (13/26) of the cases presented at an advanced stage of the disease (IIIB). No case belonged to stage IV in the present study.

Statistically significant association was seen between histological subtypes of cervical lesions and CD1 staining intensity ($\chi^2=37.822$, $p<0.01$) (Table 1), proportion of CD1 staining ($\chi^2=49.464$, $p<0.01$) (Table 2) and CD1 score ($\chi^2=42.233$, $p<0.01$) (Table 3). Percentage of CD1 positive cases was higher in normal (9/10, 90%) and LSIL (low grade squamous intraepithelial lesion) (CIN I) (14/15, 93.33%) cases (9+14) than HSIL (High grade squamous intraepithelial lesion) [CIN II (6/9. 66.67%) and III (10/21, 47.61%)] and ICC (38/65, 58.46%) cases. This was found to be statistically significant. ($\chi^2=11.898$, $p<0.05$) (Table 4) As the severity of the lesion increased from CIN I to CIN III to ICC immunoexpression of cyclin D1expression shifted from nucleus to cytoplasm. ($\chi^2=39.816$, $p<0.01$) (Table 5) Maximum percent of cases which were CD1 negative belonged to poorly differentiated ICC cases (PDICC) (22/65, 33.85%). This was found to be statistically significant. ($\chi^2=7.135$, $p<0.05$) (Table 6) Association between CD1 expression and clinicopathological features of ICC is shown in Table 7. The sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy of CD1 immunoexpression in comparison to histopathological diagnosis was 61.82%, 10%, 88.31%, 2.33% and 57.50% respectively. The overall agreement of CD1 expression with histopathological diagnosis was 57.50% ($\kappa=0.1127$ i.e., poor). (Table 8)

All the NED cases showed negative HER2/neu immunoexpression. HER2/neu was positive in 20% (3/15) cases of CIN I, 33.33% (3/9) cases of CIN II, 42.85% (9/21) cases of CIN III and 69.23% (45/65) cases of ICC. Percentage of cases showing positive HER2/neu immunoexpression statistically significantly increased

Histopathological subtype	Total cases	Cyclin D1 staining intensity					
		0	1	2	3	chi square value	p value
NED	10	0 (0%)	1 (10%)	1 (10%)	8 (80%)	37.822	<0.01
CIN I	15	1 (6.66%)	3 (20%)	2 (13.33%)	9 (60%)		
CIN II	9	3 (33.33%)	5 (55.55%)	1 (11.11%)	0 (0%)		
CIN III	21	11 (52.38%)	2 (9.52%)	2 (9.52%)	6 (28.57%)		
IC	65	25 (38.46%)	10 (15.38%)	16 (45.71%)	14 (21.54%)		

Table 1: Cyclin D1 staining intensity according to histopathological subtypes

NED– negative for malignancy/dysplasia, CIN – cervical intraepithelial neoplasia, IC– invasive carcinoma

Histopathological subtype	Total cases	Cyclin D1 staining proportion							
		0	1	2	3	4	5	chi square value	p value
NED	10	0 (0%)	1 (10%)	8 (80%)	1 (10%)	0 (0%)	0 (0%)	49.464	<0.01
CIN I	15	1 (6.67%)	0 (0%)	3 (20%)	10 (66.66%)	1 (6.67%)	0 (0%)		
CIN II	9	3 (33.33%)	0 (0%)	6 (66.67%)	0 (0%)	0 (0%)	0 (0%)		
CIN III	21	11 (52.38%)	0 (0%)	4 (19.05%)	4 (19.05%)	1 (4.76%)	1 (4.76%)		
IC	65	25 (38.46%)	2 (3.08%)	18 (27.69%)	10 (15.38%)	9 (13.85%)	1 (1.54%)		

Table 2: Proportion of Cyclin D1 staining according to histopathological subtypes

NED– negative for malignancy/dysplasia, CIN – cervical intraepithelial neoplasia, IC– invasive carcinoma

Histopathological subtype	Total cases	Cyclin D1 score				
		0–2 (Weak)	3–5 v(moderate)	6–8 (strong)	chi square value	p value
NED	10	1 (10%)	0 (0%)	9 (90%)	42.233	<0.01
CIN I	15	1 (6.66%)	4 (26.66%)	10 (66.66%)		
CIN II	9	3 (33.33%)	6 (66.67%)	0 (0%)		
CIN III	21	11 (52.38%)	6 (28.57%)	4 (19.05%)		
IC	65	27 (41.54%)	27 (41.54%)	11 (16.92 %)		

Table 3: Cyclin D1 expression score according to histopathological subtypes

NED– negative for malignancy/dysplasia, CIN – cervical intraepithelial neoplasia, IC– invasive carcinoma

Histopathological subtype	Total cases	Cyclin D1 expression			
		negative	positive	chi square value	p value
NED	10	1 (10%)	9 (90%)	11.898	<0.05
CIN I	15	1 (6.66%)	14 (93.33%)		
CIN II	9	3 (33.33%)	6 (66.67%)		
CIN III	21	11 (52.38%)	10 (47.62%)		
IC	65	27 (41.54%)	38 (58.46%)		

Table 4: Cyclin D1 expression score according to histopathological subtypes

NED– negative for malignancy/dysplasia, CIN – cervical intraepithelial neoplasia, IC– invasive carcinoma

Histopathological subtype	Total cases expressing Cyclin D1	Cyclin D1 IHC staining pattern				
		Nuclear	Nuclear + cytoplasmic	Cytoplasmic	chi square value	p value
NED	9	9 (100%)	0 (0%)	0 (0%)	39.816	<o.o1
CIN I	14	14 (100%%)	0 (0%)	0 (0%)		
CIN II	6	5 (83.33%)	0 (0%)	1 (16.67%)		
CIN III	10	7 (70%)	1 (10%)	2 (20%)		
IC	38	8 (21.05%)	12 (31.58%)	18 (47.37%)		

Table 5: Cyclin D1 expression score according to histopathological subtypes

NED– negative for malignancy/dysplasia, CIN – cervical intraepithelial neoplasia, IC– invasive carcinoma, IHC– immunohistochemistry

Grade of Invasive carcinoma	Total cases	Cyclin D1 score				
		0–2 (Weak)	3–5 (moderate)	6–8 (strong)	chi square value	p value
Well differentiated	11	1 (9.09%)	5 (45.45%)	5 (45.45%)	11.809	<o.o5
Moderately differentiated	12	4 (33.33%)	7 (58.33%)	1 (8.33%)		
Poorly differentiated	42	22 (52.38%)	15 (35.71%)	5 (11.9%)		

Table 6: Cyclin D1 expression score according to histopathological subtypes

Clinical parameters		Total no. of cases (n, %)	CyclinD1 expression		c2	p value
			Positive (%) (38/65, 58.46%)	Negative (%) (27/65, 41.54%)		
			Total			
Age (yrs)	<45	20 (30.77)	13 (20)	7 (10.77)	0.194	0.66
	>45	45 (69.23)	25 (38.46)	20 (30.77)		
Tumor grade	WDSCC	11 (16.92)	10 (15.38)	1 (1.54)	7.135	<0.05
	MDSCC	12 (18.46)	8 (12.31)	4 (6.15)		
	PDSCCC	42 (64.62)	20 (30.77)	22 (33.85)		
FIGO stage	I	9 (34.62)	5 (19.23)	4 (12.5)	0.686	0.71
	II	4 (15.38)	2 (7.69)	2 (7.69)		
	III	13 (50)	9 (34.62)	4 (11.1)		
Tumor Size (cm)	<2	17 (65.38)	12 (46.15)	5 (19.23)	0.774	0.379
	≥2	9 (34.62)	4 (15.38)	5 (19.23)		
Lymph node metastasis	Present	13 (50)	9 (34.62)	4 (15.38)	0.162	0.687
	Absent	13 (50)	7 (26.92)	6 (23.08)		

Table 7: Cyclin D1 expression and clinicopathological parameters in Invasive cervical carcinoma

SCC–squamous cell carcinoma, WDSCC– well differentiated SCC, MDSCC– moderately differentiated SCC, PDSCC– poorly differentiated SCC

with increasing severity of lesion from CIN I to CIN III to ICC. (χ^2=15.664, p<0.01) There was no association between percentage of ICC cases showing HER2/neu immunoexpression and age of the patient. (Table 9) 33.33% (3/9) of stage I, 75% (3/4) of stage II and 84.8% (11/13) of stage III ICC cases expressed HER2/neu. Thus, with increasing stage, the percentage of cases expressing HER2/neu statistically significantly increased (χ^2=6.711, p<0.05). 45.45% (5/11) of well differentiated (WD) ICC, 58.83% (7/12) of moderately differentiated (MD) ICC and 83.33% (35/42) of PDICC showed HER2/neu immunoexpression. As the grade of ICC increased the percentage of cases expressing HER/neu also statistically significantly increased. (χ^2=11.121, p<0.05) Highest number of cases showing LN metastasis (11/26, 42.31%) showed Her2/neu expression though not statistically significant (χ^2=2.719, p=0.099). The sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy of HER2/neu immunoexpression in comparison to histopathological diagnosis was 54.55%, 100%, 100%, 16.67% and 58.33% respectively. The overall agreement of HER2/neu staining with histopathological diagnosis was 58.33% (κ=0.1667 i.e., poor). (Table 10)

33 HER2/neu positive cases were also positive for Cyclin D1 and 16 HER2/neu negative cases were negative for CD1 staining. The agreement between HER2/neu and CD1 immunostaining was 40.83%. (κ= 0.1833 i.e., fair) (Table 11)

Cyclin D1 expression	Haematoxylin & Eosin diagnosis	
	CIN+IC (positive)	NED (Negative)
Positive	68	9
Negative	42	1

Table 8: Agreement of Cyclin D1 expression with Haematoxylin & Eosin diagnosis

CIN– cervical intraepithelial neoplasia, IC invasive cervical carcinoma, NED– negative for malignancy/dysplasia

Clinical parameters		Total no. of cases (n, %)	HER2/neu expression		c2	p value
			Positive (%) (45/65, 69.23%)	Negative (%) (20/65, 30.77%)		
			Total			
Age (yrs)	<50	20 (30.77)	10 (15.38)	10 (15.38)	2.405	0.66
	>50	45 (69.23)	12 (18.46)	33 (50.77)		
Tumor grade	WDSCC	11 (16.92)	5 (7.69)	6 (9.23)	11.121	<0.05
	MDSCC	12 (18.46)	5 (7.69)	7 (10.77)		
	PDSCCC	42 (64.62)	35 (53.85)	7 (10.77)		
FIGO stage	I	9 (34.62)	3 (11.54)	6 (23.08)	6.711	<0.05
	II	4 (15.38)	3 (11.54)	4 (15.38)		
	III	13 (50)	11 (42.31)	2 (7.69)		
Lymph node metastasis	Present	13 (50)	11 (42.31)	2 (7.69)	2.719	0.099
	Absent	13 (50)	6 (23.08)	7 (26.92)		

Table 9: HER2/neu expression and clinicopathological parameters in Invasive cervical carcinoma

SCC–squamous cell carcinoma, WDSCC– well differentiated SCC, MDSCC– moderately differentiated SCC, PDSCC– poorly differentiated SCC

Cyclin D1 expression	Haematoxylin & Eosin diagnosis	
	(CIN+IC (positive	(NED (Negative
Positive	60	0
Negative	50	10

Table 10: Agreement of HER2/neu expression with Haematoxylin & Eosin diagnosis

CIN– cervical intraepithelial neoplasia, IC invasive cervical carcinoma, NED– negative for malignancy/dysplasia

HER2/neu	Cyclin D1	
	Positive	Negative
Positive	33	27
Negative	44	16

Table 11: Agreement between HER2/neu and Cyclin D1 expression in invasive cervical carcinoma

Study name	Histopathological subtypes				
	(%,NED (n	(%,CIN I (n	(%,CIN II (n	(%,CIN III (n	(%,ICC (n
Chowdhary et al	ND	(0) 0/3	(0) 0/4	(100) 3/3	(67.50) 27/40
Bajpai et al	ND	(10.77) 7	(22.22) 2/9	(25) 2/8	(48.84) 21/43
Joseph et al	ND	0	(100) 2/2	(100) 1/1	(90.90) 20/22
Carerras et al	0	(11) 1	ND	(33) 3	(50) 5
Present Study	0	(20) 3/15	(33.33) 3/9	(42.85) 9/21	(69.23) 45/65

Table 12: Percentage positivity of HER2/neu expression in other studies

NED– negative for malignancy/dysplasia, CIN – cervical intraepithelial neoplasia, ICC– invasive carcinoma, ND– not done

Study name	WD ICC	MD ICC	PD ICC
Chowdhary et al	2 (50)	12 (50)	6 (85)
Bajpai et al	3 (14.2)	2 (13.3)	4 (57)
Gupta et al	0	(55)	(80)
Sarwade et al	ND	6 (37.5)	6 (66.6)
Joseph et al	3 (100)	5 (100)	12 (100)
Present Study	5/11 (45.45)	5/12 (41.67)	35/42 (83.33)

Table 13: Percentage positivity of HER2/neu expression in other studies according to grade of differentiation in ICC

NED– negative for malignancy/dysplasia, ICC– invasive carcinoma, ND– not done, WD– well differentiated, MD– moderately differentiated, PD– poorly differentiated

Discussion

CD1 is a key regulatory protein in the cell cycle and is over–expressed in many tumors, including endometrial, thyroid, urothelial, breast, brain gliomas, and esophageal cancers.[12] Varying expressions of CD1 has been documented in literature in CIN I, II, III and ICC. The time lag between HPV infection, a major etiological factor in cervical carcinogenesis and the diagnosis of cancer indicates that multiple steps, as well as multiple factors, may be necessary for the development of cervical cancer.[5]

In India, about %6.6 of women in the general population are estimated to harbor cervical HPV infection. HPV serotypes 16 and 18 account for nearly %76.7 of cervical cancer in India.[7] A study by Sauvaget et al documents high risk HPV infection prevalence of 10.3% in Maharashtrian middle aged women.[13] In our institute, HPV prevalence was found to be 10% and prevalence of serotype 16 was 50% and that of serotype 18, 31 and 66 was 16.66% each, done in select women with abnormal pap smear and normal pap smear with suspicious unhealthy cervix after application of 5% acetic acid and/or Lugol's iodine after undergoing HPV DNA polymerase chain reaction (PCR) testing and further subtyping.[14]

In this study, statistically significant association was seen between histological subtypes of cervical lesions and CD1 score calculated from CD1 staining intensity and proportion of CD1 staining scores. A more objective and inclusive method as Gu et al in calculating CD1 expression score was performed in this study. Percentage of cyclin D1 positive cases was statistically significantly higher in normal + LSIL (CIN I) than HSIL (CIN II + III) and ICC cases. Similar finding is documented in literature by Carreras et al[11] and Cho et al.[15] In contrast to this, Bae et al has documented that although cyclin D1 was under expressed in cervical neoplasia, it was more frequently expressed in malignant lesions. The study further mentions that under expression of CD1 was regulated at the level of transcription in cervical cancer and that positive CD1 immunostaining was associated with decreased disease–free survival and a lower overall survival.[16]

As the severity of the lesion increased from CIN I to CIN III to ICC immunoexpression of CD1 shifted from nucleus

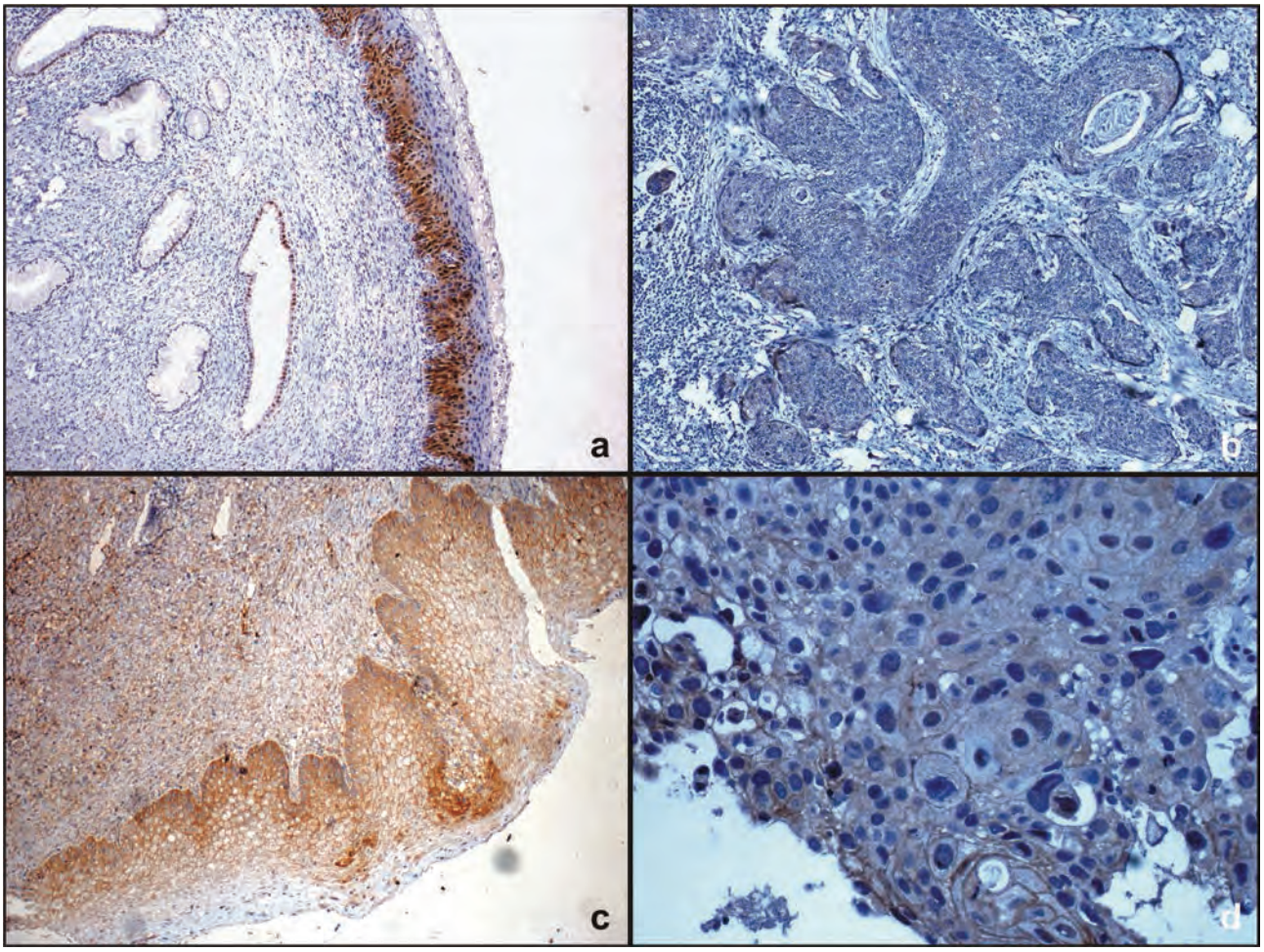


Figure 1: Photomicrograph of a case of a) CIN II showing nuclear and cytoplasmic Cyclin D1 immunostaining, score of 4 (2+2), (x100) b) SCC showing diffuse cytoplasmic Cyclin D1 immunostaining, score of 7 (2+5), (x400) c) CIN II showing +3 HER2/neu immunoreactivity, (x100) d) SCC showing +3 HER2/neu immunoreactivity, (x400)

to cytoplasm in this study. Similar finding is documented in literature by Carreras et al, Bae et al and Nichols et al.[11,16,17] Carraras et al documents nuclear cyclin D1 expression in 81.81% cases of LSIL, 11.11% of CIN III and 30% of SCC cases. Cytoplasmic CD1 expression was seen in 18.18% cases of CIN I, 88.88% cases of CIN III and 70 % of SCC cases. It is reasoned that CD1, which is found normally in the nucleus in the G1 phase, is phosphorylated, ubiquitinized, and carried to the cytoplasm in the S phase via nuclear pores.[11] Neoplastic cervical tissue is bound to have a greater number of cells that too in S phase causing cytoplasmic expression of CD1 in more severe cervical lesions. Birgul et al documents nuclear staining of CD1 in LSIL and that of cytoplasmic staining in HSIL and SCC cases.[18]

CD1 negative expression was statistically significantly seen in PDICC cases (22/65, 33.85%) in this study as documented by Jiahui Gu et al.[6] The reverse relationship observed between cyclin D1 overexpression and tumor grade suggests that higher expression of cyclin D1 may directly or indirectly result in maturation of tumor cells. Maximum cases of ICC with size less than 2cm showed statistically insignificant CD1 immunoexpression in this study. Statistically significant association between lesion size and CD1a isoform immunoexpression was observed by Jiahui Gu et al.[6] Maximum ICC cases showing lymph node metastasis expressed statistically insignificant CD1. Jiahui Gu et al documents statistically significant association between lymph node metastasis and CD1b isoform expression.[6] In this study maximum cases of FIGO stage III showed CD1 immunoexpression though statistically insignificant as documented in literature by Jiahu et al.[6]

The sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy of cyclin D1 immunoexpression in comparison to histopathological diagnosis was 61.82%, 10%, 88.31%, 2.33% and 57.50% respectively. The overall agreement of CD1 expression with histopathological diagnosis was 57.50% (κ=0.1127 i.e., poor). This is not documented in literature.

IHC study of HER2/neu revealed that there was a statistically significant overexpression in different groups as we moved from NED to CIN I to CIN II+III to IC as documented by Bajpai et al and Carerras et al.[9,11] (Table 12). None of the NED cases in present study showed HER2/neu positivity. In this study, HER2/neu positivity showed an incremental trend from well differentiated (54.54%) to poorly differentiated (85.7%) ICC cases as documented in various studies. (Bajpai S et al, Sarwade et al, Chowdhary et al and Gupta et al)[9,19,20,21] (Table 13) With increasing stage, the percentage of cases expressing HER2/neu statistically significantly increased as documented in literature by Chowdhary et al and Gupta et al.[20,21] However, Joseph et al and Sarwade et al did not find any association between HER2/neu expression and stage of IC.[19,22] Highest number of cases showing LN metastasis showed Her2/neu expression though not statistically significant as documented in literature by Chowdhary et al, Sarvade et al and Joseph et al.[19,20,21] However, Mandai et al[23] and Yong et al[24] analyzed HER–2/neu expression in 39 and 74 cases of cervical adenocarcinoma respectively and reported that a significant correlation existed between HER–2/neu expression and lymph node metastasis. The above observations indicate that HER2/neu expression being a late event in cervical carcinogenesis. The sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy of HER2/neu immunoexpression in comparison to histopathological diagnosis was 56.36%, 100%, 100%, 17.24% and 60% respectively. Overall agreement between HER2/neu expression and histopathological diagnosis was 60%. The agreement between HER2/neu and Cyclin D1 immunostaining was 40%. There was no study documented in literature for association between HER2/neu and CD1 to compare with the present study.

Many strategies for cervical cancer therapy are being developed. The CDK 4/6–cyclin D–RBp–E2F pathway dysregulation is regarded as key factor for cervical cancer development. Ribociclib, selective CDK4/6 inhibitor, is a novel therapeutic approach showing promise as documented by few studies. However, more clinical trial data need to be documented to elucidate the matter further.[25, 26] HER2/neu inhibitor–based therapy for HER2/neu amplified cervical cancer refractory to conventional therapy has been recommended in literature.[27]

The following conclusions were drawn from the above study.

1. A statistically highly significant association was observed between CD1 score calculated from intensity and proportion of Cyclin D1 nuclear/cytoplasmic staining and cervical lesions. ($p<0.01$) Cyclin D1 immunoexpression was statistically significantly higher in normal + LSIL (CIN I) cases than HSIL (CIN II and III) + IC. ($p<0.05$)
2. Most cases showing nuclear staining pattern were normal control cases and that with cytoplasmic immunoexpression was seen in ICC cases. As the severity of the cervical lesions increased, CD1 immunoexpression statistically significantly shifted from nucleus to cytoplasm. ($p<0.01$)
3. Statistically significant inverse relationship was noted between CD1 expression and grade of ICC cases. ($p<0.05$)
4. Maximum cases showing CD1 immunoexpression had tumor size of less than 2cm. ($p<0.01$)
5. Cyclin D1 IHC marker showed 63.64% sensitivity, 0% specificity, 87.5% PPV and 0% NPV. The overall agreement of Cyclin D1 staining with H&E diagnosis was 58.33% ($\kappa=0.1538$ i.e. poor)
6. HER2/neu immunoexpression increased statistically significantly with increasing severity of lesion from CIN–I to CIN–III to IC. ($p<0.01$) None of the NED cases in present study showed HER2/neu positivity.
7. With increasing stage and grade of IC, the percentage of cases expressing HER2/neu statistically significantly increased ($p<0.05$)
8. Her2/neu IHC marker showed 56.63% sensitivity, 100% specificity, 100% PPV and 17.24% NPV. The overall agreement of HER2/neu staining with H&E diagnosis was 60% ($\kappa=0.1771$ i.e. poor)
9. The agreement between HER2/neu and Cyclin D1 immunostaining was 40%.

Thus, CD1 and HER2/neu IHC marker panel could be used as prognostic markers and could be routinely incorporated into the surgical pathology report which could aid in understanding the biology of the tumor and better patient management. ICC expressing CD1 and HER2/neu could benefit from specific targeted therapy.

Limitations

TMA technique was used for cyclin D1 and HER2/neu. Whole sections were not used for their IHC evaluation. However, utmost care was taken to sample the most representative area from the original whole section blocks for TMA. HER2 was assessed only by IHC. Evaluation by fluorescence in–situ hybridization was not available, especially for the equivocal cases with HER2 expression 2+. Follow–up time for the patients was limited.

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We have not received any funding. I hereby declare that there is no conflict of interest.

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