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The Comparison of Cytology and Biomarker (P53 and Bcl2) Analysis in Cervical Neoplasia: A Prospective Study

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ABSTRACT

Background and aim: Cervical cancer is females' fourth most common cancer. A Pap smear is most commonly used for cervical cancer screening but has a low sensitivity. So, biomarkers can improve the diagnostic efficiency of screening programs. The aim of this study is to evaluate the sensitivity and specificity of cytology and biomarkers p53 and Bcl2 in detecting cervical neoplasia considering histopathological examination as the gold standard.

Material and methods: It was a prospective hospital-based observational study. All cervical biopsies and surgically resected specimens received in the Histopathology laboratory of patients between 30-65 years were included.

Results: One hundred and sixty patients who visited the Gynae-Oncology Outpatient department during the study period were enrolled. Out of these 10 cases were premalignant lesions and 97 cases of invasive carcinoma. Squamous cell carcinoma was the most common histological subtype of invasive cancer. Keratinizing Squamous cell carcinoma was the most common histological subtype of squamous cell carcinoma, and also Endocervical Adenocarcinoma was the most common histological subtype of Adenocarcinoma. Cytology had high specificity of 96.2% and low sensitivity of 59.8% in detecting cervical neoplasia. p53 staining on immunohistochemistry had a sensitivity of 77.6% and specificity of 90.6%. Bcl2 staining on IHC had the highest specificity of 100% and sensitivity of 48%.

Conclusions: Combined use of cytology and biomarkers analysis increases the chances of detecting cervical cancer at early stages than using any single screening test. It will help in reducing patient morbidity and mortality.

1. Introduction

As per World Health Organization (WHO), Cervical cancer is a global issue.[1] It is the fourth most common cancer in women. In 2018, 570000 cases of cervical cancer and 311,000 deaths occurred globally.[1] Currently, cervical cancer screening is done by cytological examination based on a PAP smear examination, but it has some limitations like inadequate sampling, contamination, and false-negative results due to clumping of cells. It has a low sensitivity of 50 to 75% in detecting High grade squamous intraepithelial lesions with high discrepancies between laboratories.[2-4] About 10% of Pap smears classified as LGSIL or ASCUS/AGUS turn out to be high-grade lesions histopathologically.[3] Recently, many prognostic biomarkers such as p53, p63, and BCL2 in cervical cancer have been identified, which show differential expression in various sub-types of cervical cancer.[5] TP53 is a cytological examination was performed on a conventional PAP smear and reported using the Bethesda classification system.[6] The biopsy specimens tumor suppressor gene whose inactivation and malfunctioning are associated with the development and progression of many human cancers, including cervical cancer.[1] BCL2 is an anti-apoptotic intracellular membrane protein. Its overexpression blocks p53-mediated G1 arrest.[5] Therefore, this study evaluated the diagnostic accuracy of cytology and biomarker analysis (P53 and BCL2), considering histopathological examination as the gold standard.

2. Material and methods

It was a prospective hospital-based observational study. All cervical biopsies and surgically resected specimens received in the Histopathology laboratory of patients between the age group of 30-65 years were included. The slides received for review from outside, and specimens from patients with previously diagnosed/treated cases of carcinoma cervix were excluded. The received were processed, embedded in paraffin wax, and made blocks. The sections were cut at 4 µm and stained with hematoxylin and eosin for light

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microscopy. For immunohistochemical examination, the sections were deparaffinized and incubated overnight. Heat-mediated antigen retrieval was done in TRIS buffer in a microwave oven. Endogenous peroxidase was blocked with a peroxidase blocking agent. The section was incubated with primary monoclonal antibody p53 and BCL2 and then with secondary monoclonal antibody. Both primary and secondary antibodies were from Biogenex. The sections were counterstained with H&E and mounted. The examination was done using a compound microscope at 40X. P53 staining showed nuclear positivity, which was considered positive if nuclei of >10% tumour cells were stained. BCL2 staining showed cytoplasmic positivity, which was considered positive if the cytoplasm of >10% tumour cells were stained. All data collected were analyzed using SPSS 21 and Microsoft Excel 2013. The percentage was calculated for the categorical variable. Mean, and standard deviation was used for continuous variable. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for cytological examination and biomarkers (P53 and BCL2). P-value was calculated using Chi-Squared and Fischer's exact test. A p-value of <0.05 was considered significant.

3. Results

A total of 160 patients who visited Gynaec Oncology OPD during the study period were included. Of 160 patients, 53 were histopathologically diagnosed with benign lesions, while 107 were diagnosed with premalignant and malignant lesions, i.e., cervical neoplasm. Patients with benign lesions were included in the control group and considered negative for malignancy, while patients with cervical neoplasm were considered positive for malignancy. The mean age of patients with malignancy was 54.56±10.98 years. The mean age of patients negative for malignancy was 53.48±9.70 years. The maximum number of patients in both groups was between 41-50 years, followed by 51-60 years. Out of 107 cases of cervical neoplasm, 1.9%(2 cases) were histopathologically diagnosed with low-grade dysplasia, 7.5% (8 patients) with high-grade dysplasia, and 86.9% (93 cases) with Squamous cell carcinoma(SCC) and 3.7% (4 cases) with adenocarcinoma. Squamous cell carcinoma comprised 95.9% of cases (93/107) of invasive carcinoma and was the most common histological subtype, while adenocarcinoma comprised 4.1% of cases (4/107) of total invasive carcinoma cases. Keratinizing SCC comprised 84.9% of cases (79/93) and was the most common histological subtype of SCC. 75.9% of patients (60/79) with keratinizing SCC had grade II cervical cancer. Endocervical adenocarcinoma, the usual type, was the most common histological subtype of adenocarcinoma, accounting for 75% (3 cases), while 1 case was of clear cell adenocarcinoma (25%). The 1 case of clear cell adenocarcinoma was stained with Periodic Acid Stain to rule out metastatic carcinoma from the renal primary. NILM was the most common cytological diagnosis.

Table 1. Correlation of histopathology and cytology.

Histopathology		Cytology							
		NILM	ASCUS	ASC-H	LSIL	HSIL	SCC	Unsatisfactory	Total
Benign	N	51	0	0	2	0	0	0	53
	%	96.2%	0%	0%	3.8%	0%	0%	0%	100%
Low-grade dysplasia	N	2	0	0	0	0	0	0	2
	%	100%	0%	0%	0%	0%	0%	0%	100%
High-grade dysplasia	N	3	0	0	1	1	2	1	8
	%	37.5%	0%	0%	12.5%	12.5%	25%	12.5%	100%
SCC	N	16	4	4	1	31	19	18	93
	%	18.3%	4.3%	4.3%	1.08%	32.26%	20.4%	19.4%	100%
AdenoCa	N	1	0	0	0	1	0	2	4
	%	25%	0%	0%	0%	25%	0%	50%	100%
Total	N	73	4	4	4	33	21	21	160
	%	45.6%	2.5%	2.5%	2.5%	20.6%	13.1%	13.1%	100%

Table 2. Screening efficiency of cytology in comparison to histopathology.

Cytology	Benign	Cervical neoplasm	Total (%)	P-value

Concordant diagnosis	51 (96.2%)	64 (59.8%)	115 (71.9%)	0.00001
Discordant diagnosis	2(3.8%)	43(40.2%)	45 (28.1%)	
Total	53 (100%)	107 (100%)	160 (100%)	

71.9% (115/160) of the cervix showed concordance between cytological and histopathological diagnosis, while 28.1% (45/160) cases were discordant. The difference in results was statistically highly significant, with a P-value of 0.00001. Smear was mainly unsatisfactory due to excessive hemorrhage and drying artifact. Cytology showed a sensitivity of 59.8%, specificity of 96.2%, a positive predictive value of 97%, a negative predictive value of 54.3%, and an accuracy rate of 71.9%. On immunohistochemistry, the biomarker p53 was positive in 55% of cases and negative in 45% of cases.

Table 3. Correlation of p53 staining on IHC and histopathology.

Histopathology			P53		Total
			Positive	Negative	
Benign		N	5	48	53
		%	9.4%	90.6%	100%
Premalignant	Low-grade dysplasia	N	0	2	2
		%	0%	100%	100%
	High-grade dysplasia	N	4	4	8
		%	50%	50%	100%
SCC	Keratinizing	N	63	16	79
		%	79.7%	20.3%	100%
	Non- Keratinizing	N	13	1	14
		%	92.9%	7.1%	100%
Adenocarcinoma	Endocervical adenocarcinoma, usual type	N	3	0	3
		%	100%	0%	100%
	Clear cell type	N	0	1	1
		%	0%	100%	100%
Total		N	88	72	160
		%	55%	45%	100%

Table 4. Screening efficiency of p53 in diagnosing cervical neoplasm.

	Histopathology	
P53	Positive	Negative
Positive	83 (TP)	5 (FP)
Negative	24 (FN)	48N (TN)

The difference between p53 staining in benign and cervical neoplasm was statistically highly significant, with a p-value of 0.0001. The difference between p53 staining in low and high-grade dysplasia was statistically insignificant, with a p-value of 0.4667. The difference between p53 staining in Squamous cell carcinoma and adenocarcinoma was statistically significant, with a p-value of 0.013. The biomarker p53 showed a sensitivity of 77.6%,

specificity of 90.6%, a positive predictive value of 94.3%, a negative predictive value of 66.7%, and an accuracy rate of 81.9%. The biomarker BCL2 was also analyzed using immunohistochemistry. It was negative in all benign lesions.

Table 5. Correlation of BCL2 staining on IHC and histopathology.

Histopathology			BCL2		Total
			Positive	Negative	
Benign		N	0	53	53
		%	0%	100%	100%
Premalignant	Low-grade dysplasia	N	0	2	2
		%	0%	100%	100%
	High-grade dysplasia	N	4	4	8
		%	50%	50%	100%
SCC	Keratinizing	N	37	42	79
		%	46.8%	53.2%	100%
	Non-keratinizing	N	6	8	14
		%	42.9%	57.1%	100%
Adenocarcinoma	Endocervical adenocarcinoma, usual type	N	2	1	3
		%	66.7%	33.3%	100%
	Clear cell type	N	0	1	1
		%	0%	100%	100%
Total		N	49	111	160
		%	30.6%	69.4%	100%

Table 6. Screening efficiency of BCL2 in cervical neoplasm.

	Histopathology	
BCL2	Positive	Negative
Positive	49 (TP)	0 (FP)
Negative	58 (FN)	53 (TN)

The difference in bcl2 staining in benign and premalignant/ malignant lesions was statistically highly significant, with a p-value of 0.00001. The difference between BCL2 staining in low and high-grade dysplasia was statistically insignificant, with a p-value of 0.466. The difference between BCL2 staining in SCC and adenocarcinoma was statistically insignificant, with a p-value of 0.715. BCL2 staining showed 48% sensitivity,100% specificity,100% positive predictive value,52.3% negative predictive value and accuracy rate of 66.9%.

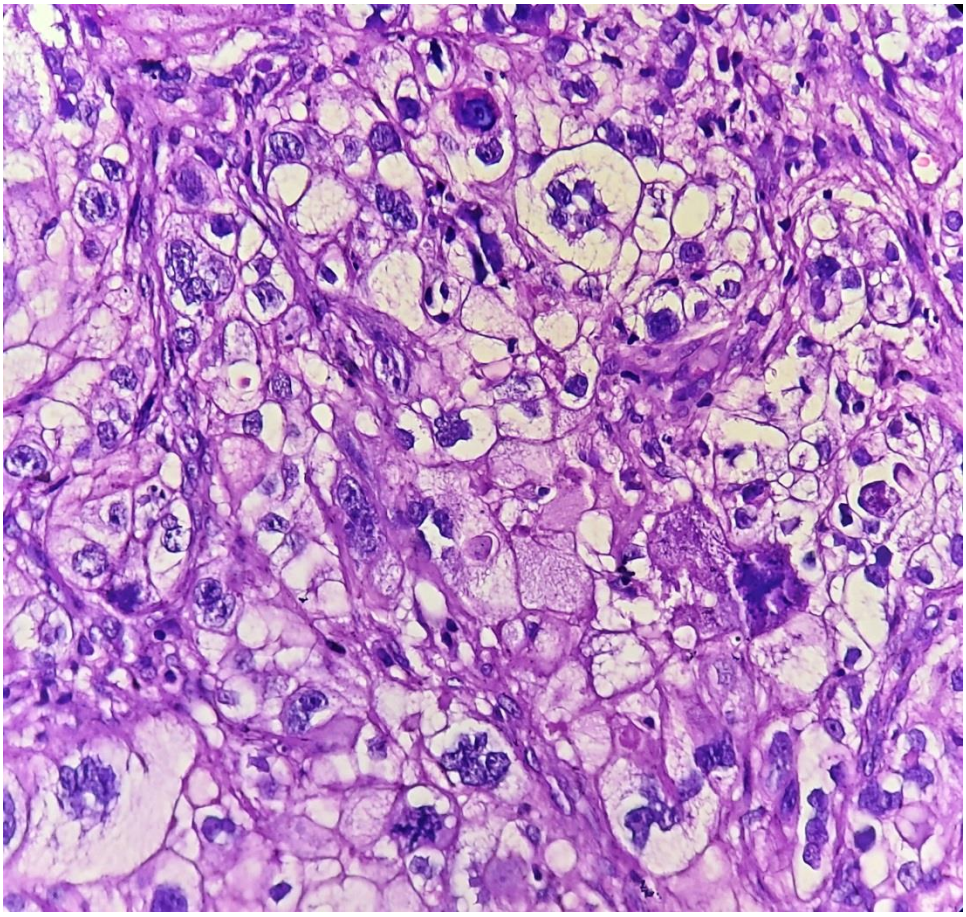

Fig. 1. (H&E, 400X); Clear cell adenocarcinoma-tumour cells arranged in a solid pattern with clear, granular, eosinophilic cytoplasm.

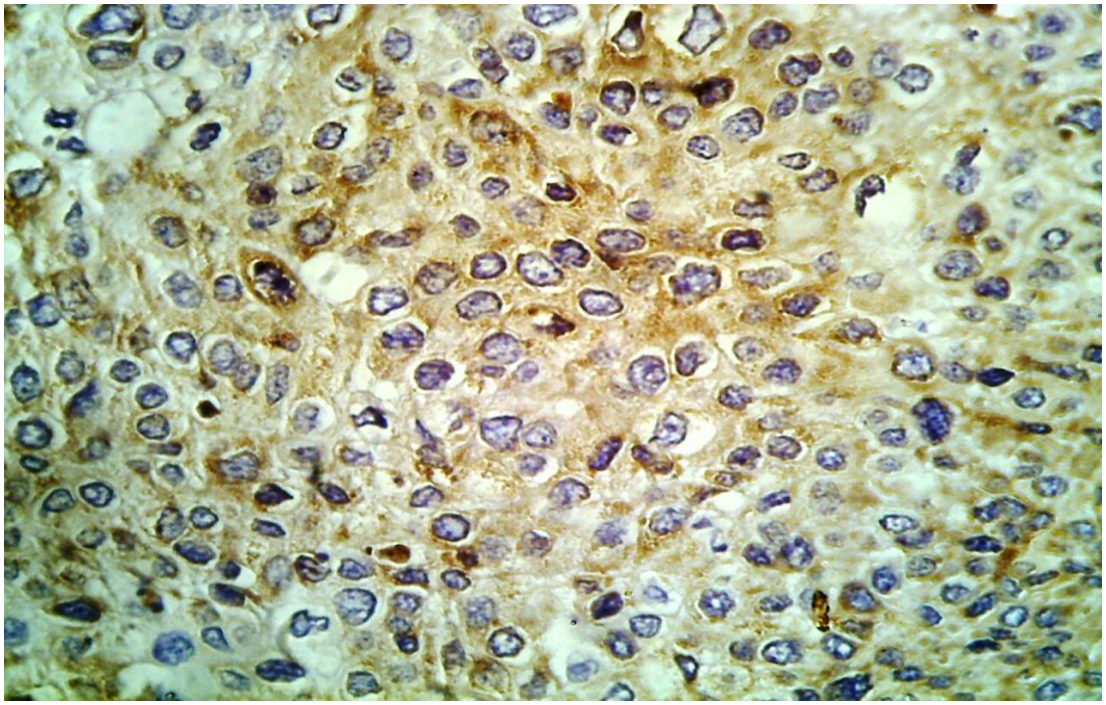

Fig. 2. Bcl2 in SCC(400X)-shows cytoplasmic positivity in tumour cells.

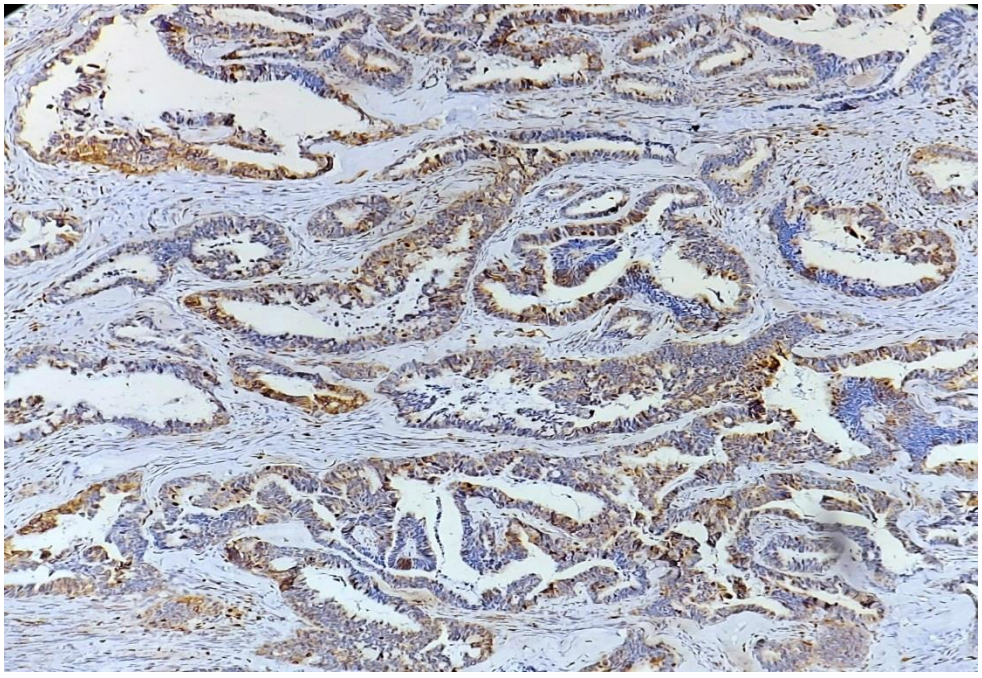

Fig. 3. BCL2 in Adenocarcinoma (100X)- cytoplasmic positivity in tumour cells.

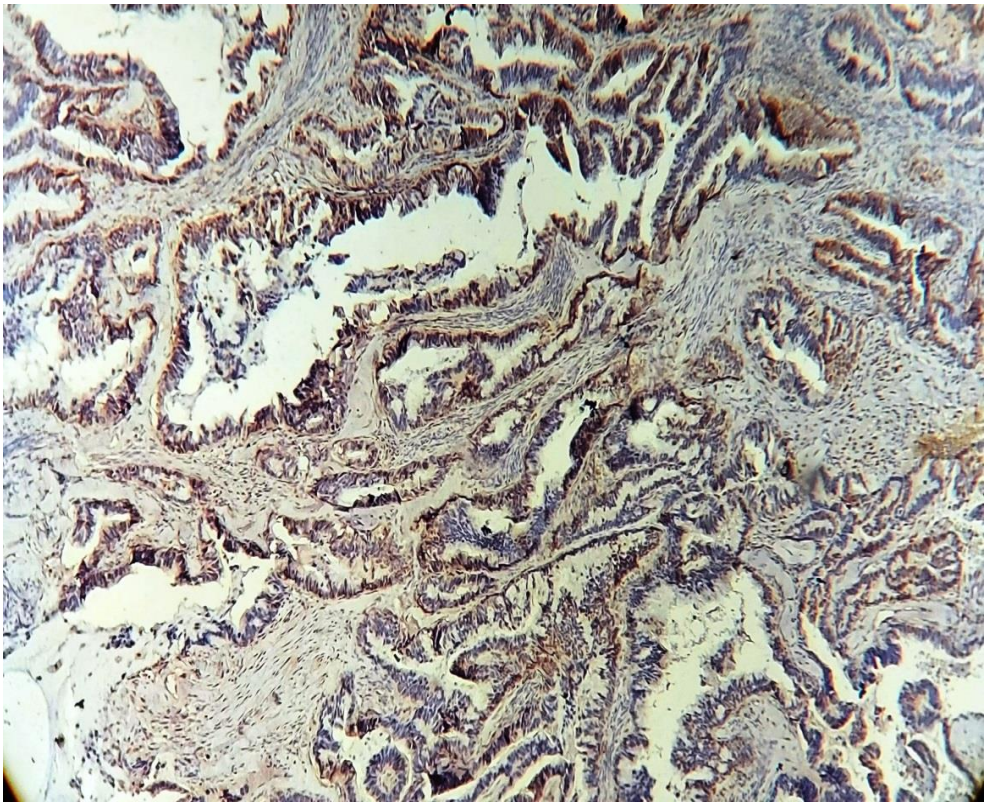

Fig. 4. P53 in adenocarcinoma(100X)-Nuclear positivity in tumour cells.

4. Discussion

The present study had mean age in 5th decade of life similar to studies by Neha Dahiya et al.[7] Aanchal Jain et al.,[8] Khalissa Deffar et al.,[9] Anita Kumari et al.,[10] Manjit Kumar Rana et al.,[11] and Rubal Jain et al.[12] In the present study, it was observed that squamous cell carcinoma was the most common histological subtype, similar to studies by Atul Jain et al.[13] Ranjit Rani R et al.,[14] Aanchal Jain et al.,[8] Anita Kumari et al.,[10] Rubal Jain et al.[12] Cytology showed low sensitivity and high specificity, similar to studies by Anuja Nanda et al.[15] and M Tripura Sundari et al.[16] Moreover, it was in contrast to Maria Adamopoulou et al.[17] also, Saurabh Bodey et al. reported high sensitivity.[18] Krishnan Baskaran et al.[19] p53 was negative in the normal sample but positive in 100% of cases of invasive cancer and 80% cases of

High-grade squamous intraepithelial lesions. Jasneet Kaur Sandhu et al.[20] also observed a high prevalence of p53 in invasive carcinoma, 86.7%, whereas Shailaja Shukla et al.[21] observed that p53 was positive in only 42.1% cases of CIN and 50% cases of invasive cancer. The present study had decreased prevalence of p53 in invasive cancer and High grade squamous intraepithelial lesion than the studies by Krishnan Bhaskaran et al. and increased positivity than the studies by Shailaja Shukla et al. Shailaja Shukla et al.[21] observed a prevalence of BCL2 in 43.75% of cases similar to our study. Chitrangi Prashant Barpande et al.[22] a prevalence of 19.38% in invasive cancer was lower than that observed in our study. Chitrangi Prashant Barpande et al.[22] A prevalence of 44.4% in premalignant lesions was similar to the present study. Shreedevi Kamraddi et al.[23] and C Dimitrakakis et al.[4] conducted a study to reveal the expression of BCL2 in the premalignant lesion and found that BCL2 positivity increased with increasing grade. Maria Adamopoulou et al.[17] studied the combined efficacy of three biomarkers, p16,p53, and BCL2, in cervical neoplasia. They observed sensitivity of 83.3%, a specificity of 65.4%, a positive predictive value of 76.9%, and a negative predictive value of 73.9%.[17]

5. Conclusion

Cytology as a screening method has the least sensitivity in detecting cervical neoplasm. P53 immunostaining has the best results with high sensitivity, specificity, positive predictive value, and accuracy rate. Bcl2 staining has the highest specificity. So, a combination of cytology and the above biomarkers can improve the efficiency of screening programs, which will help reduce cervical cancer-related morbidity and mortality.

Conflict of Interest

The authors declared that there is no conflict of interest.

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