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IMMUNOHISTOCHEMICAL EXPRESSION OF KI-67 AND P53 IN CERVICAL CARCINOMA: CORRELATION WITH PROGNOSTIC CLINICOPATHOLOGICAL FEATURES

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ABSTRACT

Cervical carcinoma remains a critical public health issue, especially in developing regions despite advances in HPV vaccination and screening programs. This study evaluates the prognostic significance of Ki-67 and p53 immunohistochemical markers in cervical carcinoma and explores their correlation with FIGO stage, histological grade, and lymphovascular space invasion (LVSI). Fifty cases of cervical carcinoma were analyzed retrospectively using WHO 2020 classification and FIGO 2018 staging. The expression patterns of both Ki-67 and p53 were assessed for all cases and significant associations were found between high Ki-67 index and older age, poor differentiated tumors and LVSI. Similarly, mutated p53 patterns correlated strongly with advanced clinical stage, poor differentiation andlympho-vascular positivity. The presence of a high proliferation rate of expression of Ki-67 alongside a mutated pattern or p53 immunostaining indicated a synergistic molecular phenotype linked with aggressive disease biology. These findings support the integration of Ki-67 and p53 biomarkers into diagnostic workflows and highlight their potential role for guiding personalized treatment strategies. Future large-scale studies with follow-up data are needed to substantiate their utility as routine prognostic biomarkers.

Keywords: cervical carcinoma, Ki-67, p53,

Introduction

According to GLOBOCAN, cervical cancer is still in the top 10 of cancers worldwide when it comes to incidence and mortality, most cases being reported in Asia and Africa (1, 2). Although HPV vaccination was introduced in Romania in 2010, cervical cancer remains the 3rd leading cancer reported in the female population (1, 2). This calls for the early identification of patients with poor prognosis and speedy initiation of therapeutically procedures. Even though progress has been made regarding preventative care (HPV vaccination) and early detection of intraepithelial lesions (Pap-smear and subsequent colposcopy), death toll remains high in developing and underdeveloped countries where most of the patients have a low socioeconomic background and limited resources. Death in these patients is attributed to invasion and metastases resulting from the aggressive tumor (3).To detect high-risk patients with poor prognosis that require immediate treatment, new prognostic markers that are readily available and cost-effective are necessary.

Ki-67, known as Kiel 67 or MKI6,is a well-established nuclear protein that serves as a marker for cellular proliferation (4). During the cellular division cycle, Ki67 is absent in the resting phase, but present in all the other phase – G1, S,

G2 and mitosis (5). The Ki-67 proliferation index is a standard prognostic marker in malignant tumors and reflects tumor aggressiveness (6-8). Ki-67 has been generally used in the evaluation of cervical premalignant lesions (10, 11), but studies in cervical carcinoma are few, with various results and its role in invasive carcinoma is still evolving (12, 13).

P53, also known as "guardian of the genome" represents any isoform of the protein encoded by various organisms including TP53 in humans and Trp53 in mice (14). P53 protein plays an important role in cell division cycle control and apoptosis, and its mutations occur in almost all types of cancer, with frequencies ranging from 5% to 50% (15). Normal cells have a low level of p53. DNA damage can trigger a p53 level increase, which has three major functions: cell growth arrest, DNA repair and apoptosis. Also, the gene's activity can be altered by other mutagens and in such cases protein activity becomes diminished. Few studies elaborate on p53 expression in cervical cancer and its correlation with clinicopathological prognostic factors.

The present study aims to investigate the immunohistochemical expression of Ki-67 and p53 in cervical carcinoma and correlate these findings with clinical and pathological parameters, including FIGO stage, histological grade and lymphovascular space invasion. By identifying meaningful correlations, we aim to propose Ki-67 and p53 as accessible prognostic markers that can enhance clinical decision-making in cervical cancer management.

Materials and Methods

This retrospective study included 50 randomly selected cases of cervical carcinoma diagnosed during the period of January 2019 and December 2021 in the Department of Pathology of "Saint Andrew" County Emergency Clinical Hospital Constanta, Romania without neoadjuvant therapy. We applied the following exclusion criteria: cases that were diagnosed in another medical unit, cases that did not have sufficient tissue for immunohistochemical analysis or showed extensive necrosis and cases with secondary cervical tumors (cervical metastasis). Clinical information was retrieved from patient records and staging was done according to FIGO (International Federation of Gynecology and Obstetrics) staging system of cancer of the cervix uteri (2018) (16). Paraffin blocks and slides of these cases were collected from the archives of the department. Tissue sections 4 micron thick were stained with hematoxylin and eosin (H&E) and evaluated under light microscopy. In all cases included in the study, the histological pattern was identified and documented based on the 2020 WHO classification of tumors of the uterine cervix (17). Histological grade was assessed and subdivided in well differentiated (G1), moderately differentiated (G2) and poorly differentiated (G3).

The immunohistochemistry (IHC) was performed, according to the instructions provided by the manufacturer, using Ki67 and p53 antibodies from Biocare Medical (Table 1).

For Ki67 antibody, a positive reaction was considered when a nuclear brown immunostaining of the tumor cells was identified. Based on the immunostaining of the tumor cell, the proliferation nuclear rate (Ki67 index) was calculated as the proportion of tumor cells with positive nuclear immunoreactivity relative to all tumor cells evaluated× 100%. Some studies choose to divide the proliferation index in 3 groups using the cut-off value of 30% and respectively 50% (<30% - low; 30-50% - medium, > 50% - high proliferation group) (18).

Immunohistochemical assessment of p53 antibody was performed, taken into consideration only nuclear positive immunoreaction and both the intensity and the percentage of labeled tumor cells were evaluated. Two types of immunostaining patterns were recorded: non-mutant pattern, known as "wild-type" pattern with a focal and variable nuclear stain of the tumor cells; mutant pattern or aberrant pattern with two subtypes: "null-type" with no nuclear immunolabeling in all tumor cells or significantly reduce p53 staining and "overexpressed type" when it was observed a diffuse, high or moderate intensity positive nuclear immunostaining in more than 80% of the tumor cells (19, 20).

Table 1 Antibodies used for immunohistochemical analysis of cervicalcarcinoma s

Antibody	Clone	Type of antibody	Dilution	Normal staining	Positive external control tissue
KI67	SP6	Rabbit monoclonal antibody	"ready to use"	Nuclear	Tonsil
P53	DO-7	Mousse monoclonal antibody	"ready to use"	Nuclear	Breast carcinoma or colorectal adenocarcinoma

Statistical analysis was performed to study the association of Ki-67 and p53 with clinical stage, histological grade and LVSI. The tools used were IBM SPSS Statistics V26; IBM Corp., Armonk, New York, USA using Chi-square test and Spearman's correlation test. Continuous variables (e.g., age) were summarized as mean ± SD and median after assessing normality with the Shapiro–Wilk test; categorical variables were presented as counts and percentages; p-value < 0.05 was considered statistically significant. The study was conducted after approval from the Institutional Ethical Committee of "Saint Andrew" County Emergency Clinical Hospital Constanta, Romania (42006/02.09.2020).

Results

Out of the 50 cases, only 2 cases were hysterectomy specimens, the rest 48 (96%) cases were biopsy specimens. The mean age of the patients was 55,54±12,61 years old with a median of 54,5 years old, and the highest incidence (66%) was noted in the 45-69 years age group, frequently from urban region (52%). Most of the patients complained about abnormal vaginal bleeding occasionally associated with lower abdominal discomfort. Regarding the histological type, 48 of these cases were invasive squamous cell carcinoma (SCC) and 2 adenocarcinomas (AC), and the most common morphological subtype recorded in the present study was the invasive SCC -keratinizing recorded in 26 cases (Table 2) and non-keratinizing subtype (Figure 1A).

Regarding FIGO staging system, at the time of diagnosis the most frequent was stage III with 18 (36%), followed by stage II with 17 (34%) cases (Table 2). Most of the cases in our study were poorly differentiated 70% followed by those with a moderate degree of differentiation (26%).

Table 2: The main clinical and morphological features of the selected cases.

Characteristics	Numbers (n)	Percentage (%)
Clinical FIGO Stage		
Stage I	7	14%
Stage II	17	34%
Stage III	18	36%
Stage IV	8	16%
Histological Type		
Invasive squamous cell carcinoma	48	96%
Adenocarcinoma	2	4%
Histological Pattern for SCC		
Keratinizing	26	52%
Nonkeratinizing	16	32%
Basaloid	1	2%
Papillary	4	8%
Warty	1	2%
Histological Grade		
Well differentiated (G1)	2	4%
Moderately differentiated (G2)	13	26%
Poorly differentiated (G3)	35	70%

Both AC cases included in the study were moderately differentiated (G2), usual type, non-mucinous with a destructive invasion pattern – pattern C according to the Silva classification system

Analysis of the Ki-67 IHC staining showed that the high proliferation index was the most common and was encountered in 21 (42%) of cases (Figure 1B), followed by those cases with a moderate proliferation index recorded in 20 (40%) of cases. Among the cases included in the study, a high Ki67 index was observed mostly in patients who were at least 56,5 years old (66.7%), while patients younger than that showed mostly a low or moderate Ki67 index. This association was found to be statistically significant (p=0.025). Cases with a high proliferation index were more frequently observed within clinical FIGO III stage but we did not record statistically significant differences (Table 3) between KI67 index (low and moderate / high proliferation nuclear rate) and clinical FIGO stage(early FIGO I+II/ advance FIGO III+IV). Among the histological grades, a high proliferation index was observed mostly in poorly differentiated (G3) carcinoma group and less so in well (G1) and moderately (G2) differentiated group. This association between Ki-67 index and histological grade was found to be statistically significant

(p=0.039) (Table 3). Lympho- vascular invasion was frequently observed in cases with a high Ki-67 index (81%), this association was found to be statistically significant (p = 0.037) (Table 3).

When it comes to p53 immunohistochemical assessment, 18 (36%) of cases showed a non-mutated pattern and 32 (64%) cases showed a mutated pattern. "Null type" pattern was recorded with a 44.0% rate and "overexpression" pattern with 22,0% (Figure 1C) from all cases with a mutated pattern. Analysis of p53 staining pattern revealed no association with age, but there is a statistically significant difference between p53 staining pattern and clinical FIGO stage (early versus advanced stage) (p = 0.002). Most cases of mutated pattern were observed in stage III with a 15 frequency, while cases of non-mutated pattern were recorded in stage II (10 cases) (Table 3).Most cases that showed mutated pattern of p53 stain were poorly differentiated (84,4%) and 55,6% of non-mutated pattern of P53 with well or moderate degree of differentiation, these results being statistically significant (p = 0.003) (Table 3). Also, most cases that presented LVSI showed a mutated p53 stain pattern(25 cases), this it was found to be also statistically significant (p=0.002) (Table 3). In the present study, cases with p53 mutational pattern were associated frequently (53.1%) with a high Ki-67 proliferation index (>50%) and non-mutational pattern had been mostly with low or moderate nuclear proliferation rate (77.8%), these associations being statistically significant (p=0.009) (Figure 2). The Spearman correlation (ρ=0.30; p=0.034) indicates a moderate and positive relationship between p53 status and proliferation rate.

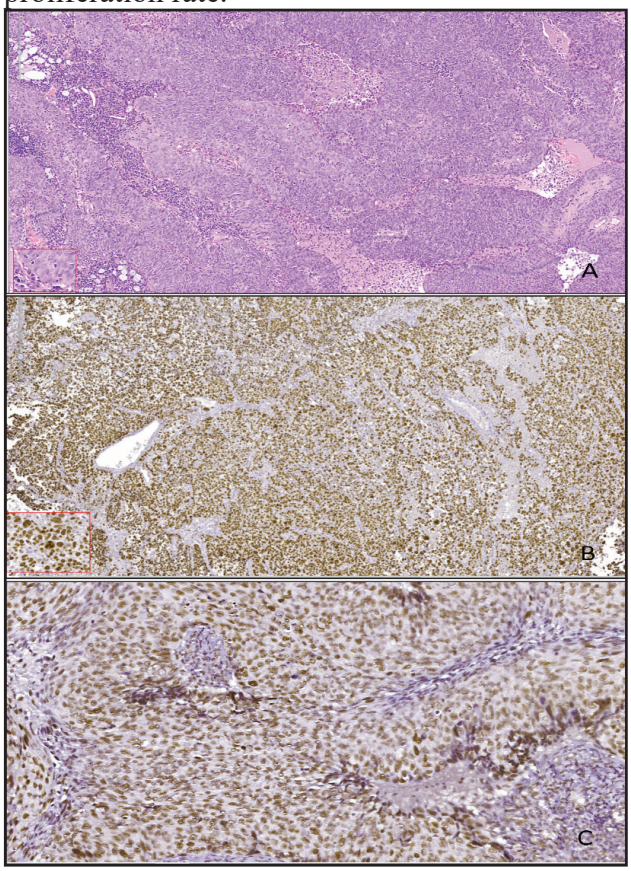

Figure 1 Invasive squamous cell carcinoma poorly differentiation G3 (Fig 1A, HE stains, Ob 40x; in case Ob 200x), with a high nuclear proliferation rate (Fig 1B, IHC KI67, Ob 40x, in case Ob 200x) and "over-expression" pattern of P53 (Fig 1C, IHC P53, Ob 100x)

Table 3 Correlations between KI 67 index and p53 pattern with the main clinical-morphological features

Morpho-pathological features		KI67 Index		P value*	P53 pattern		P value*
		Low/Moderate N (%)	High N (%)		Non-mutated N (%)	Mutated N (%)	
Age (years old)	< 56.5	19 (65.5)	7 (33.3)	0.025	10 (55.6)	16 (50.0)	0.706
	≥ 56.5	10 (34.5)	14 (66.7)		8 (44.4)	16 (50.0)	
FIGO stage	I+II	17 (58.6)	7 (33.3)	0.077	14 (77.8)	10 (31.1)	**0.002**
	III+IV	12 (41.4)	14 (66.7)		4 (22.2)	22 (68.8)	
Grade	G1+G2	12 (41.4)	3 (14.3)	0.039	10 (55.6)	5 (15.6)	**0.003**
	G3	17 (58.6)	18 (85.7)		8 (44.4)	27 (84.4)	
ILV	No	15 (51.7)	4 (19.0)	0.037	12 (66.7)	7 (21.9)	**0.002**
	Yes	14 (48.3)	17 (81.0)		6 (33.3)	25 (78.1)	

* Chi square test; bold values are statistically significant

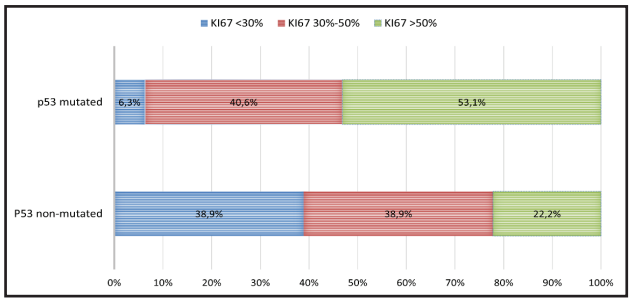

Figure 2 Distribution of cases based on p53 pattern of immunostaining and nuclear proliferation rate (Ki67 index)

Discussion

Worldwide, cervical carcinoma was ranked 8th in the incidence top of all cancers with 662.301 cases and 9th in the mortality top of all cancers with 348.874 in 2022, according to GLOBOCAN (1, 2). In Romania, in 2022, the incidence of cervical cancer was of 3368 new cases -ranked 3rd frequent cancer in the female population with a mortality of 1793 cases and a 5-year prevalence of 11.278 cases (1, 2). At least two studies have reported an increasing incidence of this malignancy in younger patients (21, 22). One study reported the survival rate for stage IB1 cervical carcinoma of more than 90% (23). This highlights the importance of finding easily available and viable prognostic markers for cervical carcinoma and identifying aggressiveness markers that could modify the therapeutical and post therapeutical approach.

As a cell proliferation marker, Ki-67 immunohistochemical assessment was mostly studied in intraepithelial cervical lesions and less so in cervical cancer, where studies have shown mixed results. Studies like that of Gogoi PK et al (2016),which reported a higher expression of Ki-67 (24) and Zhong P. et al (2015), which reported that 19 out of the 22 cases studied showed high expression of Ki-67 (25), are aligned with the present study results with a 42% rate of high Ki-67 index. Also, we note that the proliferative activity is age-dependent, with older patients demonstrating a significantly higher proliferation index.

Clinical stage is considered a poor prognostic factor. In our study, most cases that presented a high expression of Ki-67 were diagnosed in an advanced stage. This finding between index Ki-67 and clinical stage was not statistically significant in the current study. Several studies have attested worse progression of disease with higher clinical stage (23, 26). There have been studies that showed a statistically significant correlation between high Ki-67 proliferation index and clinical stage (27-30), but other studies contradict this correlation (31, 34).

Another independent prognostic factor is histological grade, and several studies have shown shortened survival (overall survival and disease free) in poorly differentiated cervical carcinoma (35-37). In our study the high Ki-67 proliferation index was frequently associated with poorly differentiated compared with low Ki-67 proliferation index, mostly present in well and moderately differentiated cases. These associations were statistically significant. Other studies offered conflicting results, some authors reporting similar associations (24, 38), while others did not (19, 34, 39).

LVSI is another prognostic factor in cervical cancer and is defined as the presence of tumoral cells in spaces lined by endothelium and is currently recognized as one of the most important risk factors for pelvic lymph nodes metastasis (40, 41). In our study 31 cases presented LSVI while most of them (17 cases) also presented a high Ki-67 proliferation index. On the other hand, most cases that did not show LVSI were more frequently associated with a low Ki-67 proliferation index. This association, between Ki-67 index and LVSI, was statistically significant in our research. This result is in discordance with a study performed by Tatari M et al (2022) that did not report an association between Ki-67 expression and LVSI (40).

P53 is a nuclear protein and plays an important role in cell division cycle control and apoptosis and its mutations occur in almost all types of cancer (15). As carcinogenesis entails loss of regulation of the cell cycle and apoptosis, in this study, we demonstrated the association of p53 with established poor clinic pathological factors of cervical carcinoma as clinical stage, histological grade and LVSI.

IHC stain pattern of p53 are divided in non-mutated and mutated (42); the non-mutated

one is interchangeably named “wild type” and represents a normal protein, while the mutated one indicates anomalies of p53 and is further categorized in overexpression (42, 43), null-type (42, 43) and newer described cytoplasmatic type (44-45) and “mid-epithelial” type (46). The cases in the current study stained for p53 showed a mutated pattern of immunostaining in 64% of patients, mostly “null pattern”. The reported results are concordant to Freier CP et al (2016) and Tan G et al (2007) who reported results of mutated p53 stains of up to 66% and respectively 84,8% in their cohorts (47, 48).

As we mentioned before, clinical stage, histological grade and LVSI are considered poor prognostic factors in cervical cancer. Following evaluation of p53 stain pattern, we concluded that there is a statistically significant association between mutated and non-mutated pattern of p53 immunostaining in concordance with some studies which attest that expression of p53 increases with the gravity of the lesion (49) and is associated with cervical cancer stage in a statistically significant manner (50, 51).But there are other studies which did not report statistically differences regarding p53 and disease stage (52, 53).

Contradictory results are also reported when p53 stain patterns were evaluated with the degree of tumor differentiation. Our study concluded that most cases with mutated p53 stain patterns were present in poorly differentiated carcinoma while those tumors which preserve a degree of differentiation (well or moderate) were frequently associated with a non-mutated p53 stain pattern. This association was statistically significantly similar with some studies like those of Stoenescu TM et al (2011) or of the Vemula S et al (2021) (50-51), but other studies did not substantiate this finding (53, 54)

Studies that evaluated expression of both p53 and Ki-67 in cervical cancer are limited and offer contradicting results. A recent study by Gahine R et al (2025) demonstrated that the pattern of expression of Ki-67 and p53 increases with the severity of the disease (55); other studies showed a statistically significant association between Ki-67 and p53 (50, 56).In this study the association between Ki-67 proliferation index and p53 was statistically significant (p=0.009). Of the 32 cases of mutational p53 stain pattern, 53,1% presented a high Ki-67 proliferation index while non-mutational p53 stain pattern was mostly found in the low and moderate proliferation Ki67 group. Contrary to our study, Vasilescu et al (2009) and Horikoshi et al (2005) found no association between Ki-67 and p53 expression (57, 58).Clinically, the occurrence of both p53 mutation and high Ki67 delineates a higherrisk phenotype likely to be in concordance with other adverse features (e.g., grade, LVSI, advanced stage) and may justify treatment intensification and closer surveillance.

Cervical cancer treatment hasn’t changed considerably in the past two decades, surgical treatment, adjuvant and neo-adjuvant chemotherapy and radiotherapy (including brachytherapy) remain the main management options. Newer approaches include sentinel lymph node excision in selected cases (59) and the use of modern drugs as anti-angiogenic agents such as Bevacizumab (59) and immunotherapeutic ones such as the newly marketed one, pembrolizumab (60). Post-treatment monitoring and recovery care are also important issues in such cases. Extensive surgery, chemotherapy, radiotherapy and brachytherapy can induce major changes inside the pelvis and induce pelvic discomfort and urinary issues. Also, the effects of sudden onset surgically induced menopause can be an additional factor for the issues previously mentioned. Management of such complaints can be done in multiple ways, but Kegel exercises or a combination of intravaginal estriol therapy and Kegel exercises could be helpful in such cases (61, 62).

Better stratification of patients that enables personalized treatment is a constant need in the management of cervical cancer. Comprehensive panels that integrate clinicopathological features with relevant biomarkers need to be developed to enhance tumor characterization and thereby facilitate more personalized therapeutic strategies, ultimately improving patient outcomes and quality of life.

Conclusions

In conclusion, the current study demonstrated the important role of Ki-67 and

p53 biomarkers and their immunohistochemical assessment, alongside other established clinic-pathological factors, might lead to a better selection of patients for tailor therapy. Even so, it is worth mentioning that there are some limitations regarding our study- the cohort was small (50 cases) with only 2 of the cases presenting well differentiated grades and 2 cases of cervical adenocarcinoma. Both Ki-67 and p53 biomarkers have the potential to be used as routine prognostic markers as in breast carcinoma, but larger scale studies with follow-up of the patients are needed to confirm these findings.

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