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Prognostic Value of IMP3 Expression in Squamous Cervical Cancer

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Abstract: Cervical cancer remains one of the leading causes of death from malignant diseases in women worldwide. Primary and secondary prevention have led to better outcomes in developed countries, whereas in developing countries, cervical cancer continues to be responsible for an unjustifiably high number of fatalities. The discovery of new tumor biomarkers can lead to earlier diagnosis, better therapeutic decisions, and improved treatment methods. IMP3 is a protein responsible for invasiveness and other aggressive characteristics of tumor processes. Its highly specific expression has been proven in various malignant processes. The level of IMP3 expression in cervical cancer cells could be used as a prognostic factor for a worse disease course. In this study, IMP3 expression was examined in 80 patients who underwent surgery for squamous cell cervical cancer in the first FIGO stage of the disease, and its association with disease-free period and overall survival was investigated. Data analysis did not show a statistically significant association between IMP3 expression and the mentioned primary outcomes, despite its association with clinical-pathological indicators of advanced disease. In conclusion, the analysis of IMP3 protein expression in patients with early-stage cervical cancer is of limited utility.

Key Words: squamous cell, cervical cancer, biomarkers, IMP3, survival analysis

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Cervical cancer can be almost entirely prevented due to the high effectiveness of primary (HPV vaccine) and secondary (screening) prevention. However, due to insufficient implementation of preventive methods in developing countries, it remains the fourth most common cancer among women worldwide.[1] Despite significant progress in the prevention and treatment of cervical cancer, there were approximately 600,000 new cases and 340,000 deaths in 2020.[2] A better understanding of the etiopathogenesis of the tumor process at all its biological levels can lead to better therapeutic decisions and reduce these devastating consequences. Although prevention and early diagnosis are key to better outcomes, the choice of the optimal therapeutic modality for established disease remains controversial.[3]

Treatment modalities for cervical cancer include surgery, radiation, chemotherapy, and their combinations. The optimal treatment modality maximizes overall survival and disease-free survival while minimizing the unwanted side effects of treatment. In the early stages of the disease, surgical intervention is the method of choice, so the choice of the type of surgical procedure is crucial.

Types of surgical procedures include conization, radical trachelectomy, simple and radical hysterectomies with or without lymphadenectomy. Surgical procedures differ in terms of their potential for complete cure and the unwanted consequences of treatment. Conization and radical trachelectomy preserve fertility, while radical hysterectomy involves the resection of the uterus, cervix, parametria, and the upper part of the vagina. Every third patient who undergoes radical hysterectomy experiences some permanent consequences such as urinary incontinence, vesicovaginal fistula, lymphocele, obturator, and genitofemoral neuropraxia.[4] Every second patient who undergoes pelvic lymphadenectomy develops lower limb lymphedema.[5] Given that aggressive surgical procedures are associated with significant comorbidities, it is desirable to identify subpopulations of patients who would benefit from less aggressive surgeries, without an increased risk of recurrence or death from the underlying disease.

In addition to staging, there are numerous prognostic factors that influence the choice of treatment modality, such as histological type, tumor differentiation grade, and lymphovascular invasion. The scientific community is constantly searching for new prognostic factors to help select the optimal surgical procedure.[6] Some of

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these factors fall within the domain of tumor biomarkers, which have gained popularity in recent years.[7]

Tumor biomarkers are substances that indicate the presence and characteristics of the tumor process. The discovery of tumor biomarkers such as genes, proteins, and other cellular molecules enables so-called "precision medicine," which optimizes disease outcomes through individualized approaches.[8] They are categorized into diagnostic and prognostic biomarkers. Prognostic biomarkers predict overall survival and the time to disease recurrence.[9] Therefore, the discovery of new biomarkers for cervical cancer could lead to better selection of patients suitable for less aggressive surgical procedures. One potential biomarker is the insulin-like growth factor II mRNA-binding protein 3 (IMP3).

IMP3 is a protein that regulates the cell cycle by binding to various RNA molecules. Its expression promotes cell growth and migration during embryogenesis and stimulates the proliferation and invasiveness of tumor cells in vitro.[10] In recent years, numerous studies have indicated a connection between elevated IMP3 expression and poorer disease outcomes in various types of tumors.[11] However, the results are not universal, and there is significant heterogeneity in IMP3 expression among different tumor types.[12] IMP3 expression is associated with poorer survival in patients with cervical cancer.[13] However, its connection with treatment outcomes in patients with early-stage disease who are candidates for less aggressive surgical procedures has been insufficiently explored.

RESEARCH OBJECTIVE

The research objective is to determine the association of IMP3 expression with disease-free survival and overall survival, that is, to establish its prognostic value. The study involved patients diagnosed with squamous cell cervical cancer in the first FIGO stage of the disease.

RESEARCH PURPOSE

The purpose of the research is to improve the treatment outcomes of patients with operable cervical cancer by using immunohistochemical analysis of IMP3 expression.

INITIAL ASSUMPTION

The initial assumption is that the level of IMP3 expression in the tumor cells of squamous cell cervical carcinoma is not associated with major disease outcomes, that is, disease-free survival and overall survival.

METHODS AND MATERIALS

Ethics

The research was approved by the Ethics Committee of the Medical School of Mostar and was registered in the clinical trials registry (ClinicalTrials.gov Identifier: NCT05151159).

Study Participants

The study included 80 patients who were surgically treated for squamous cell carcinoma of the cervix at the Clinical Hospital Center "Rijeka" in the period from 2011 to 2018. Patients who had undergone preoperative chemotherapy and/or radiotherapy, those with concomitant gynecological or other malignant diseases, and those with other histological types of tumors (adenocarcinoma, adenosquamous carcinoma, and neuroendocrine tumor) were excluded from the study.

Data Collection

Clinical data were collected during the initial hospitalization and subsequent follow-up visits by filling out specialized forms. Dependent variables in the forms included age, type of surgery, tumor type, grade, tumor size, depth of invasion of surrounding tissue, lymphovascular invasion, FIGO stage, lymph node involvement, oncological treatment performed, time without disease, and time until death or end of follow-up. Data on IMP3 expression were obtained through immunohistochemical analysis of tumor tissue samples. Tumor samples in which more than 10% of cells were stained were considered to have positive IMP3 expression.

Immunohistochemical Analysis of Samples

Monoclonal antibodies to IMP3 (DAKO) were used for the immunohistochemical analysis at a dilution of 1:100. Tumor samples were obtained by cutting archived paraffin blocks to a thickness of 4 μm. The tissues were deparaffinized in xylene and rehydrated in gradually decreasing concentrations of alcohol. Endogenous peroxidase was inactivated by a 30-minute incubation in a 0.1% hydrogen peroxide solution at room temperature. To detect antigenic sites, the sections were boiled in citrate buffer (pH 9) in a microwave oven at 95°C for 10 minutes, then rinsed in phosphate buffer solution (PBS). After cooling, the sections were incubated for 60 minutes with primary antibodies, followed by secondary detection with diaminobenzidine (DAB) for 10 minutes at room temperature, using an antibody-independent EnVision Detection System Peroxidase (DAB, Rabbit/Mouse K 5007). Subsequently, the sections were rinsed with distilled water and stained with hematoxylin. The sections were then mounted in an appropriate medium and covered with glass coverslips, and the preparations were examined under a light microscope (Olympus BX40, Olympus, Tokyo, Japan).

Statistical Analysis

Categorical variables are presented as frequency and percentage. Numerical variables, depending on the distribution of data, are shown as the arithmetic mean and standard deviation or as the median and interquartile range. Differences in outcome variables (disease-free period and overall survival) between subgroups of cancer categorized by IMP3 expression were analyzed using the Kaplan-Meier method and the log-rank test. Univariate and multivariate Cox regression analysis was performed to

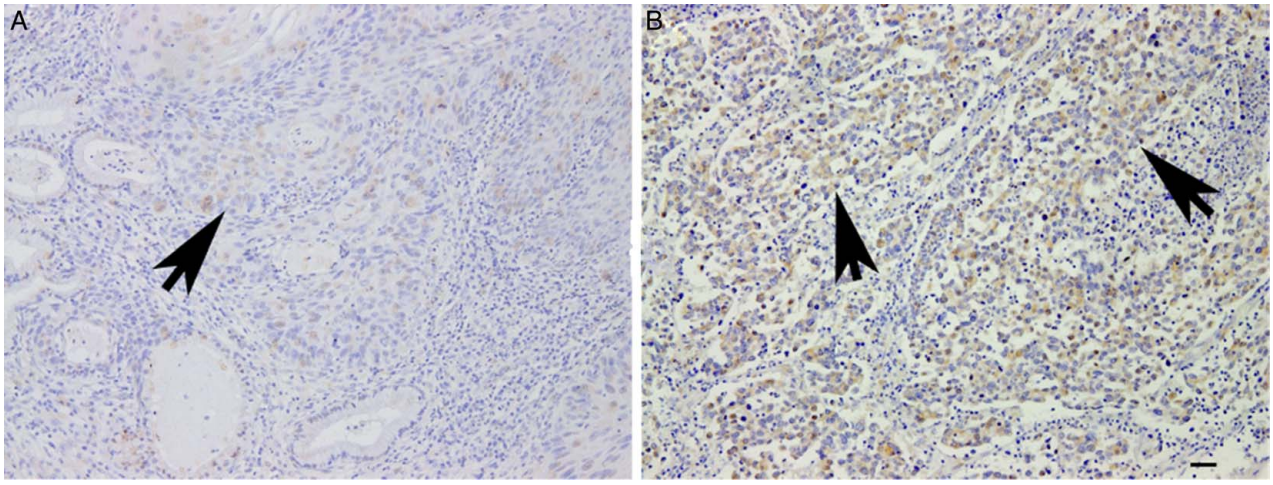

FIGURE 1. (A, B) Immunohistochemical staining of IMP3 in squamous cervical cancer tissue. The images depict a progression from focal IMP3 expression in early-stage squamous cervical cancer to more diffuse and stronger expression in later stages. These findings illustrate the variability in IMP3 expression across different stages of squamous cervical cancer.

assess the association of IMP3 status, FIGO classification, age, and tumor differentiation grade with overall survival and disease-free survival.

RESULTS

Immunohistochemical IMP3 staining revealed distinct patterns in squamous cervical cancer tissues (Fig. 1). In early stages of squmaous cervical cancer, IMP3 staining is mostly focal, whereas, in later stages, it becomes stronger and more diffuse. These findings highlight the variability in IMP3 expression across different stages of squamous cervical cancer progression.

Descriptive Statistics

One-third of the tumor samples exhibit positive IMP3 expression (26/80). The distribution of clinical-pathological factors between groups with positive and negative IMP3 expression is shown for categorical (Table 1) and numerical variables (Table 2).

TABLE 1. Distribution of Clinical-pathological Factors Between Groups With Positive and Negative IMP3 Expression (Categorical Variables)

Count (% within IMP3 group)	IMP3 −	IMP3 +
	54	26
FIGO		
IA1	32 (60)	4 (15)
IA2	5 (9)	1 (4)
IB1	8 (15)	6 (23)
IB2	9 (16)	15 (58)
Lymphovascular space invasion		
No	38 (70)	9 (32)
Yes	16 (30)	17 (58)
Histologic grade		
Well	26 (48)	3 (11)
Moderate	24 (44)	14 (54)
Poor	4 (8)	9 (35)
Oncologic therapy		
Radiotherapy	5 (9)	7 (27)
Chemotherapy	0	0
Combination	1 (2)	3 (11)

Survival Analysis

The median follow-up of the 80 patients was 29 months, with an interquartile range of 16 to 54 months. During this period, 8 patients experienced disease relapse, and 3 patients passed away.

Kaplan-Meier survival curves for the groups with positive and negative IMP3 expression are displayed for disease-free survival (Fig. 2) and overall survival (Fig. 3). The log-rank comparison of the two groups did not show statistically significant differences in the duration of disease-free survival ($\chi 2 = 1.37$, $P = 0.24$), nor in overall survival ($\chi 2 = 0.01$, $P = 0.91$).

Univariate and Multivariate Analysis

In the univariate analysis based on the Cox regression model, IMP3 expression did not prove to be a statistically significant prognostic factor for disease-free or overall survival (Table 3).

In the multivariate analysis, the FIGO classification was found to be an independent prognostic factor for disease-free survival but not for overall survival. IMP3 status, age, and tumor grade were not statistically significant prognostic factors for both of the studied outcomes (Tables 4 and 5). Other factors were excluded from the model due to their high correlation with the FIGO disease stage.

TABLE 2. Distribution of Clinical-pathological Factors Between Groups With Positive and Negative IMP3 Expression (Numerical Variables)

Mean (± SD)	IMP3 −	IMP3 +
Age, y	50 (± 12)	50 (± 14)
Tumor size, mm	9 (± 4)	19 (± 7)
Stromal invasion depth, mm	4 (± 2)	7 (± 2)

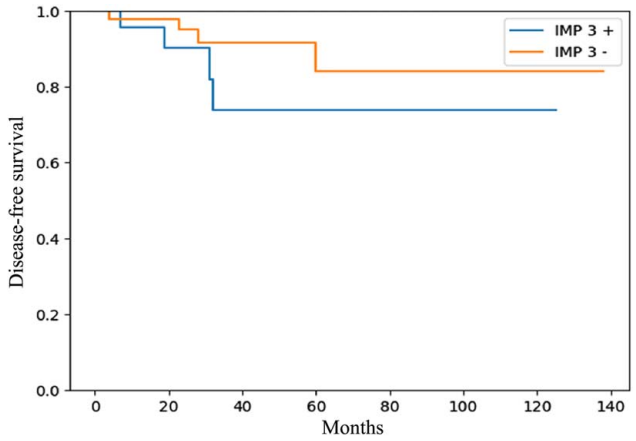

FIGURE 2. Kaplan-Meier survival curves for disease-free survival based on IMP3 expression status in patients with squamous cervical cancer. The curves compare the duration of disease-free survival between patients with positive and negative IMP3 expression, showing no statistically significant difference ($\chi 2 = 1.37$, $P = 0.24$). full color online

DISCUSSION

Cervical cancer is one of the leading causes of cancer-related mortality in women, and scientific research is directed toward optimizing early disease detection and treatment outcomes. One of the main areas of research is focused on new genes, proteins, and other mediators of cellular processes that could enable earlier disease detection, inform about prognosis, or facilitate new treatment methods. IMP3 has shown potential as a prognostic factor in patients with cervical cancer, but its true clinical utility is yet to be established.

The clinical application of IMP3 expression analysis as a prognostic marker for more severe forms of malignant diseases remains uncertain. Although it exhibits high specificity and sensitivity for advanced disease in some malignancies, its heterogeneity in various malignant processes limits its universal application.[12] Investigating IMP3 analysis in specific clinical scenarios can expand its clinical utility and optimize treatment outcomes for specific types of malignant diseases.

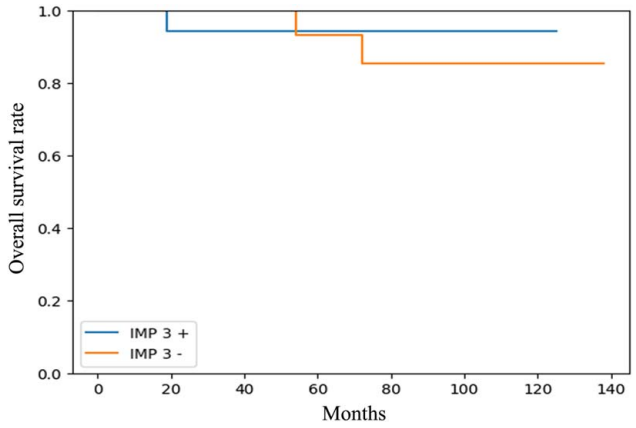

FIGURE 3. Kaplan-Meier survival curves for overall survival based on IMP3 expression status in patients with squamous cervical cancer. The comparison of survival times between patients with positive and negative IMP3 expression shows no statistically significant difference ($\chi 2 = 0.01$, $P = 0.9$). full color online

TABLE 3. Univariate Analysis of the Association Between IMP3 Status and Primary Outcomes

	Hazard Ratio (95% CI)	*P*
Disease-free survival	2.24 (0.56–8.98)	0.25
Overall survival	1.15 (0.10–12.75)	0.91

In the context of cervical cancer, IMP3 expression predicts the progression of cervical intraepithelial neoplasia to the invasive form of the disease[14] and is associated with shorter overall survival.[13] Although these studies have significantly contributed to a better understanding of the value of IMP3 expression analysis in patients with cervical cancer, numerous questions remain unanswered. Previous research has included a heterogeneous group of patients with cervical cancers at various stages of the disease. Furthermore, the value of IMP3 expression in predicting recurrences, that is, disease-free periods, has not been investigated. This study aimed to address these limitations and shed new light on the potential clinical benefit of IMP3 expression analysis.

Contrary to previous studies, these results indicate that patients with IMP3-positive cervical cancers did not exhibit statistically significant differences in disease-free survival or overall survival, despite having a higher proportion of clinical-pathological indicators of advanced disease. For example, cervical tumors with positive IMP3 status belonged to a higher FIGO stage, were less differentiated, more frequently invaded surrounding tissues, and more frequently underwent oncological treatment, which is consistent with earlier research.[11]

Given that the prognosis of patients with squamous cervical carcinoma in FIGO stage I is actually excellent, our study probably does not have enough power to detect prognostic differences based on IMP3 staining. Namely, in our sample, of 80 patients, there were only 3 deaths and 8 disease recurrences. Therefore, although the prognostic utility of IMP3 staining has not been demonstrated, this research once again proves the importance of early disease detection in the favorable prognosis of patients with squamous cell cervical cancer.

TABLE 4. Multivariate Analysis of the Association Between IMP3 Status and Other Clinical-pathological Factors With Disease-free Survival

	Hazard ratio (95% CI)	*P*
Age	0.98 (0.91–1.06)	0.63
FIGO stage	2.71 (1.07–7.07)	0.04*
Hystologic grade	1.42 (0.46–4.38)	0.54
IMP3 status	0.76 (0.15–3.91)	0.74

*FIGO stage is the only statistically significant factor associated with disease-free survival (P=0.04), while age, histologic grade, and IMP3 status did not show signifivant associations.

TABLE 5. Multivariate Analysis of the Association Between IMP3 Status and Other Clinical-pathological Factors With Overall Survival

	Hazard Ratio (95% CI)	*P*
Age	1.16 (0.98–1.36)	0.08
FIGO stage	2.67 (0.57–12.51)	0.21
Hystologic grade	0.62 (0.08–4.56)	0.63
IMP3 status	2.26 (0.12–42.55)	0.59

CONCLUSIONS

In patients with early-stage squamous cell cervical carcinoma, the analysis of IMP3 expression is a prognostic factor of limited utility. Further research is needed to determine clinical scenarios where IMP3 expression analysis can be beneficial in making more informed therapeutic decisions.

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