European Review for Medical and Pharmacological Sciences 2018; 22: 5149-5155

Significance of the Ki-67 proliferation index in the assessment of the therapeutic response to cisplatin-based chemotherapy in patients with advanced cervical cancer

D. KRTINIC[1,2], R. ZIVADINOVIC[3,4], Z. JOVIC[2], S. PESIC[2], D. MIHAILOVIC[5,6], L. RISTIC[7,8], A. CVETANOVIC[1,9], I. TODOROVSKA[1], N. ZIVKOVIC[5,6], G.N. RANKOVIC[2], D. STOKANOVIC[2], B. ZIVADINOVIC[10,11], M. TRANDAFILOVIC[12], M.A. APOSTOLOVIC[13,14], M. GOLUBOVIC[15], A. ZIVADINOVIC[16]

[1]Clinic for Oncology, Department for Pharmacology and Toxicology, Clinical Center, Nis, Serbia
[2]Department for Pharmacology and Toxicology, Faculty of Medicine, University of Nis, Serbia
[3]Clinic for Gynecology and Obstetrics, Clinical Center Nis, Serbia
[4]Department for Gynecology and Obstetrics, Faculty of Medicine, University of Nis, Serbia
[5]Pathology and Pathological Anatomy Center, Clinical Center, Nis, Serbia
[6]Department for Pathology, Faculty of Medicine, University of Nis, Serbia
[7]Clinic for Lung Diseases, Clinical Center, Nis, Serbia
[8]Department for Internal Medicine, Faculty of Medicine, University of Nis, Serbia
[9]Department for Oncology, Faculty of Medicine, University of Nis, Serbia
[10]Clinic for Neurology, Clinical Center, Nis, Serbia
[11]Department for Neurology, Faculty of Medicine, University of Nis, Serbia
[12]Department for Anatomy, Faculty of Medicine, University of Nis, Serbia
[13]Institute for Publich Health, Nis, Serbia
[14]Department for Medical Statistics and Informatics, Faculty of Medicine, University of Nis, Serbia
[15]Clinic for Anesthesiology and Intensive Therapy, Clinical Center Nis, Serbia
[16]Faculty of Medicine, University of Nis, Serbia

Abstract. – OBJECTIVE: The purposes of this study were to examine the therapeutic response of advanced cervical cancer to Ki-67 proliferative index (Ki-67 PI) dependent cisplatin chemotherapy, and to determine Ki-67 PI referential value that is expected to provide a satisfactory therapeutic response of cervical cancer to cisplatin chemotherapy.

PATIENTS AND METHODS: This prospective study enrolled 59 patients treated for cervical cancer at Clinic for Oncology, Clinical Center Nis, Serbia. According to the obtained Ki-67 PI values, patients were divided into three groups, and all the patients received the same cytostatic, cisplatin. Therapeutic response to chemotherapy was evaluated in relation to disease progression presence or absence and progression-free survival after a year follow-up since the first chemotherapy.

RESULTS: Survival rate increases with an increase of Ki-67 PI by Kaplan-Meier survival analysis, meaning that survival rate is statistically significantly shorter in the group of patients with Ki-67 PI < 40% in comparison to patients from other two groups (p=0.010). Mann-Whitney test confirmed a statistically significant increase in survival rate among the groups of patients formed according to Ki-67 PI (p<0.05). Kaplan-Meier survival analysis confirmed that the mean survival rate in the group of patients with Ki-67 PI values over 60% is statistically significantly longer in comparison to patients with Ki-67 PI values below or equal 60% (p<0.001).

CONCLUSIONS: Advanced cervical cancer with a high Ki-67 PI expression responds better to cisplatin-based chemotherapy, thus resulting in a longer survival rate. The values of Ki-67 PI were determined: high Ki-67 PI (≥60%), moderate Ki-67 PI (40-60%), and low Ki-67 PI (≤40%).

Key Words
Cervical cancer, Cisplatin, Ki-67 proliferation index, Survival rate, Therapeutic response.

Introduction

With more than half a million new cases each year, uterine cervical cancer (cervical cancer) is the third most common malignant cancer affecting women globally, accounting for 8.8% of all carcinomas in female population. Staging of cer-

Corresponding Author: Dane Krtinic MD; e-mail: kdane86@gmail.com, dane.krtinic@medfak.ni.ac.rs

vical cancer is based on the FIGO classification staging system upon clinical and rectovaginal examination. Incidence and mortality rates of this disease are twice higher in less developed countries than in developed ones[1]. The incidence rate in Serbia in 2002 was 27.3 in 100,000 respondents, which was the highest incidence of cervical cancer in Europe. Currently, it is the second most common cancer regarding new cases in female population in Serbia, after breast cancer[2].

New studies[3,4] marked that protein kinase B (AKT) pathway plays important roles in tumor pathogenesis. This pathway has possible molecular targets for preventing and treating cervical carcinoma, which can provide a new treatment strategy.

Proliferative activity may be determined by counting active cells that are not in the G0 phase of the cell cycle in relation to the total number of cells. The Ki-67 antigen is expressed in all active phases of the cell cycle (G1, S, G2, mitotic cell division), except in G0 phase[5]. Ki-67 immunoreactivity is a highly relevant prognostic marker in various tumors, including head and neck carcinomas, and salivary glands[6,7]. Back in 1989 Haapasalo et al[8] introduced an approach of determining mitotic activity as the number of mitotic figures for the area of tumor tissue in percentage, thus yielding the mitoses per volume (MV) index. This method has been applied in counting Ki-67 positive cells (Ki-67 proliferative index). Estimation of Ki-67 proliferation index is necessary for the standard determination of therapeutic protocols in some types of cancer, such as breast cancer, but it is still a novel approach regarding cervical cancer, lacking literature data on clearly defined cut-off points of Ki-67 proliferation index (Ki-67 PI) for this type of cancer[9].

Cisplatin (cis-diammine-dichloro-platinum) is an inorganic compound and water-soluble platinum complex containing a central platinum atom surrounded by two chloride atoms and two ammonia molecules. As an inhibitor of cellular proliferation, cisplatin may be given either as monotherapy or in combination with other antiproliferative drugs[10,11]. Cisplatin has significant therapeutic effect in numerous malignancies and is mainly used in combination chemotherapy protocols for the treatment of numerous solid tumors, such as: testicular tumors, ovarian cancer, small-cell and non-small-cell lung cancers, head and neck squamous carcinomas, and so on. It has also been shown that cisplatin possesses antitumor activity in cervical cancer, bladder cancer, osteosarcoma, melanoma, neuroblastoma, and esophageal cancer.

Cisplatin is the most commonly used chemotherapeutic agent in cervical cancer treatment. Nowadays it is considered to be a drug of choice for locally advanced, metastatic and recurrent cervical cancer since it has demonstrated the most consistent therapeutic activity[12].

The aims of this study were to examine the therapeutic response of cervical cancer advanced stages (FIGO stages IIb and III) to Ki-67 PI dependent cisplatin chemotherapy, and to determine Ki-67 index referential value that is expected to provide a satisfactory therapeutic response of cervical cancer to cisplatin chemotherapy.

Patients and Methods

Patients

This was a prospective study that enrolled 59 patients treated for cervical cancer at Clinic for Oncology, Clinical Center Nis, Serbia. Only the patients indicated for this type of treatment according to official treatment protocols were included in the study. Exclusions criteria encompassed the patients having Ia, Ib, and IIa stages and undergoing surgery, as well as patients with stage IV due to spreading metastases. This research was approved by the Ethics Committee of Faculty of Medicine (No. 12-1250/7), University of Nis, Serbia.

According to obtained Ki-67 PI values, patients were divided into three groups. The first group comprised 17 patients with a Ki-67 PI value ≤40%, the second group included 19 patients with a Ki-67 PI value from 41 to 60%, and the third group of 23 patients was with a Ki-67 PI value ≥60%. All the patients in these three groups received the same cytostatic, cisplatin (Cisplatin "Ebewe", EBEWE Pharma, Ges.m.b.H. Nfg.KG, Unterach, Austria), for six therapeutic cycles at a dose of 40 mg/m^2 body surface area once a week. Therapeutic response to chemotherapy was evaluated in all three groups of patients (classified on the basis of positivity for the Ki-67 marker) in relation to disease progression presence or absence and progression-free survival after a year follow-up since the first chemotherapy.

Immunohistochemical Analysis

Squamous cell carcinoma (planocellular) as a histological type of cervical cancer has been confirmed in all the patients in experimental groups.

Table I. Distribution of patients in groups and according to the stage of the disease.

Disease stage	Patients numbers	I group	II group	III group
IIb	24	7	8	9
III	35	10	11	14
Total	59	17	19	23

The analyses of all histopathological samples and additional immunohistochemical staining on Ki-67 proliferative marker that was used in this study were performed at the Pathology and Pathological Anatomy Center, Clinical Center Nis, Serbia.

While reviewing the hematoxylin-eosin (HE) stained slides of available paraffin blocks, a slide with a representative tumor, certain diagnosis and adequate cellular tissue was selected from each case for immunohistochemical staining.

Immunohistochemical staining was applied to formalin-fixed and paraffin-embedded tissue using Dako-Autostainer Link 48 (Dako, Burlington, Ontario, Canada). Developing of color was made by EnVision Flex Target Retrieval Solutions (Dako) using diaminobenzidine (DAB) as the chromogen. In accordance with manufacturer's instructions, detection Kit (Dako) was used. Anti-Ki67 (MiB-1, ready to use; Dako, Glostrup, Denmark) antibody was applied in this analysis.

Ki-67 Proliferation Index

Ki-67 proliferation index was calculated from the ratio of the number of cervical tumor cells stained by Ki-67 to the total number of cervical tumor cells counted per microscopic field.

Statistical Analysis

A software package SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical data analysis. The non-parametric Mann-Whitney U test was used to compare numerical variables, since the data were not normally distributed. Univariate and multivariate regression analysis was used to determine predictive factors with Tukey post hoc test, whereas the Kaplan-Meier curve was used for estimation of survival time. The results for $p<0.05$ were considered statistically significant.

Results

As previously mentioned, aiming at the possibility of significant survival rate, the patients were divided into three groups (the first group with Ki-67 PI value <40%; the second group with Ki-67 PI value 40-60%; the third group with Ki-67 PI value >60%). The distribution of patients in relation to the stage of the disease and formed groups is shown in Table I.

Kaplan-Meier analysis showed that the mean survival time in the group IIb did not statistically significantly differ in relation to stage III cervical cancer (Log-rank=0.382; $p=0.537$).

Figure 2 shows that survival rate increases with an increase of Ki-67 PI by Kaplan-Meier survival analysis, meaning that survival rate is statistically significantly shorter in the group of patients with Ki-67 PI < 40% in comparison to patients from other two groups, as can be seen in Table III (Log-rank=9.189; $p=0.010$).

Figure 1 shows Kaplan-Meier survival curve in relation to the stage of the disease. Table II demonstrates Ki-67 PI values according to the stages of the disease.

As illustrated in Figure 3, Mann-Whitney test confirmed a statistically significant increase in survival rate among the groups of patients formed according to Ki-67 PI ($p<0.05$).

Additionally, the patients may be divided into two groups according to ROC curve analysis that established 60% as the cut-off value of Ki-67 PI (the first group ≤60% and the second group >60%). Kaplan-Meier survival analysis confirmed that the mean survival rate in the group of patients with Ki-67 PI values over 60% is statistically significantly longer in comparison to patients with Ki-67 PI values below or equal 60% (Log-rank=35.349; $p<0.001$).

Table IV shows the mean survival rate in comparison to the groups defined on the basis of Ki-67 PI value of 60%.

Table II. Ki67 PI values according to the stage of the disease.

	$\bar{x}$	SD	Log-rank test	p
IIb (n = 24)	10.71	0.56		
III (n = 35)	12.00	0.00	0.382	0.537

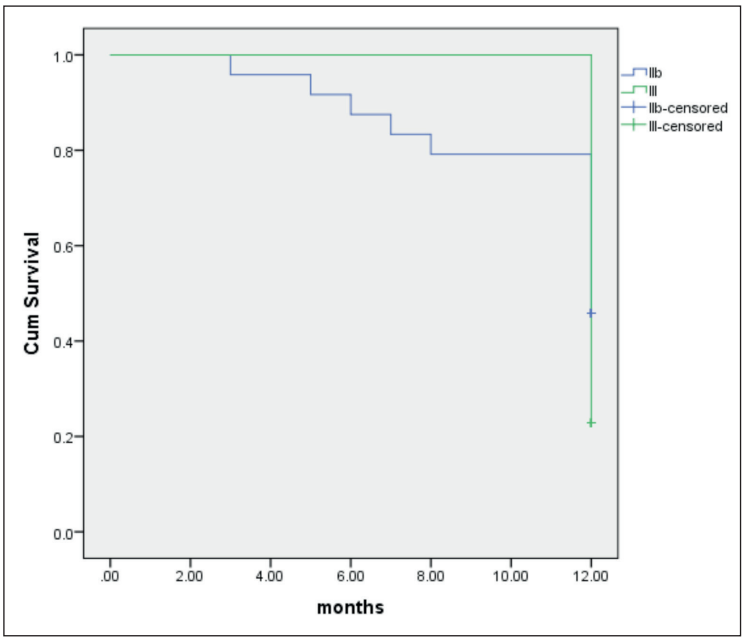

Figure 1. Kaplan-Meier survival curve in relation to the stage of the disease.

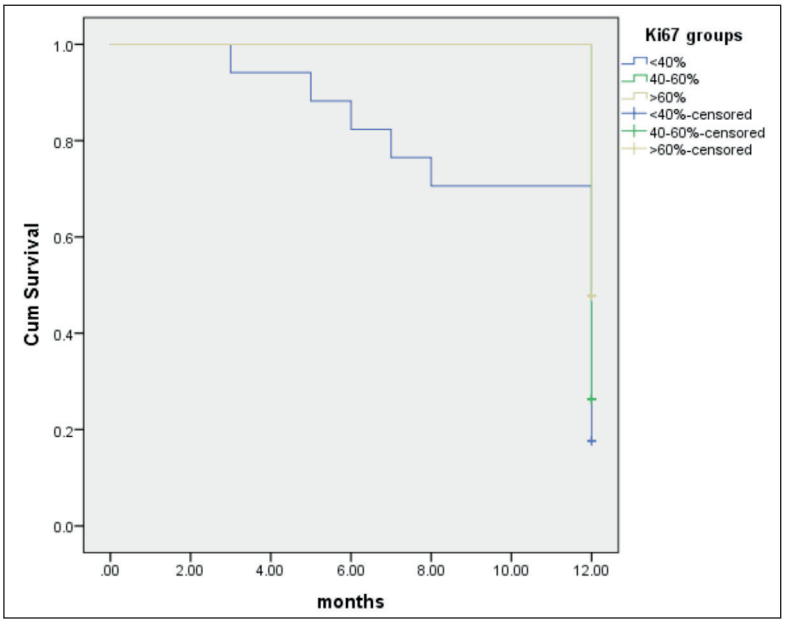

Figure 2. Kaplan-Meier survival curve in relation to experimental groups formed by Ki-67 proliferative index.

Table III. Mean survival time in relation to examined group according to Ki-67 proliferation index.

Groups	$\bar{x}$	SD	95% CI	Log-rank	p
≤ 40 (n = 17)	10.17	0.75	8.709-11.644	9.189	0.010
40-60 (n = 19)	12.00	0.00	12.00-12.00		
> 60 (n = 23)	12.00	0.00	12.00-12.00		

Table IV. Mean survival time in comparison to two groups formed on the basis of Ki-67 proliferation index.

Groups	$\bar{x}$	SD	95% CI	Log-rank	p
≤ 60% (n = 34)	7.18	0.45	6.306-8.062	35.349	<0.001
> 60% (n = 25)	11.61	0.16	11.310-11.928		

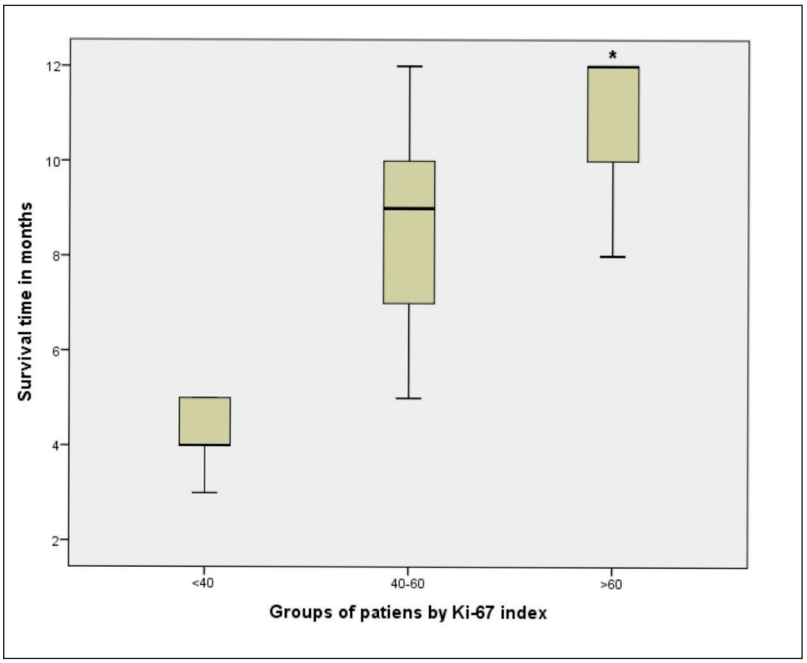

Figure 3. Comparison of survival rates among the experimental groups of patients (*p<0.05).

Discussion

Cisplatin was approved for clinical use in bladder, cervical, esophageal, head and neck, testicular, and ovarian cancers by the U.S. Food and Drug Administration in 1978[13]. The mechanism of its action is inhibition of cell division in all the phases of the cell cycle because it causes DNA breakage during replication by its ability to crosslink with the purine bases, to interfere with DNA repair mechanism, and to induce apoptosis in cancer cells[11]. It was proven that cisplatin can induce autophagy, for example, in HO8910 ovarian cancer cells[14].

To achieve optimal therapeutic outcome while minimizing side effects, cisplatin dose should rely on clinical, renal, and hematologic status of the patient. The usual dose regimens include the range of 50 to 120 mg/m^2 body surface over a period of 6 to 8 hours once every 3-4 weeks depending on the tumor type and patient's status, or 15 to 20 mg/m^2 body surface administered as an intravenous infusion daily for 5 consecutive days every 3-4 weeks. For treatment of cervical cancer a recommended dose of cisplatin is 40 mg/m^2 body surface weekly for 6 therapeutic cycles[15]. The therapeutic approach depends on the stage of the disease, thus surgical management is feasible only in locally advanced invasive cervical cancer, stages Ib and IIa. Patients with these stages of the disease undergo to radical hysterectomy with regional pelvic lymphadenectomy. Treatment recommendation for advanced stages of the disease higher than IIB includes radical or palliative radiotherapy.

Chemotherapy is employed in the treatment of early-stage invasive cervical cancer (Ib, IIa), where pathohistological samples surgically obtained reveal the presence of metastases in local pelvic lymph nodes or the presence of lymphovascular invasion. Patients with advanced stages of the disease (higher than IIb) are most commonly treated with a combination of radiation therapy and chemotherapy. Based on the results of clinical trials, chemotherapy applied concurrently with radiation therapy, chemoradiation, is a new standard of therapy for advanced stages of cervical cancer[16,17]. Our study included the patients treated with cisplatin-based chemotherapy concurrently with radiation therapy.

Proliferation marker Ki-67 has already been recognized and validated as a specific and sensitive marker in cervical intraepithelial neoplasia[18,19]. The Ki-67 protein may be a biomarker of the proliferative activity and progressive potential of normal, dysplastic and neoplastic cervical changes, with certain therapeutic implications[20,21]. Also, Ki-67 may be a sensitive biological indicator of progression, independent of age and menopausal status[22]. In spite of advancement in understanding the role of Ki-67 in the assessment of dysfunctional cervical le-

sion, its prognostic value in cervical cancer is still controversial. Although some authors have not shown any associations regarding Ki-67 and cervical cancer prognosis, others have advocated the importance of Ki-67 for the evaluation of cell kinetics in response to the therapy[23].

Available literature data have not revealed cut-off values of the Ki-67 proliferation marker for cervical cancer. Our patients were divided into three groups with the values of the Ki-67 proliferation index differing for 20% in each group and statistical analysis showed that an increase of Ki-67 proliferation index of 20% caused statistically significant survival rate increase for $p<0.001$ in relation to the previously observed group. In our study the cut-off value of Ki-67 proliferation marker was determined to be 60% and it affected the statistically significant survival of patients with cervical cancer.

Since the study included patients with advanced stage of the malignant disease when surgical treatment is not indicated, the survival rate in these patients depended on the cervical cancer sensitivity to chemotherapy administered. The result of the study showed the statistically significant positive correlation between the Ki-67 PI values and therapeutic response to cisplatin-based chemotherapy. The higher Ki-67 PI, the higher the cell count in active phase in cell division, thus being the most sensitive to chemotherapy.

Conclusions

We proved that advanced cervical cancer with a high Ki-67 PI expression responds better to cisplatin-based chemotherapy, thus resulting in longer survival rate. The values of Ki-67 PI were determined as high Ki-67 PI (≥ 60%), moderate Ki-67 PI (40-60%), and low Ki-67 PI (≤ 40%) according to statistically significant differences in the survival rate of patients with cervical cancer. Patients with high Ki-67 PI (≥ 60%) demonstrated the best response to cisplatin-based chemotherapy and consequently had the longest survival rate. Since cisplatin shows toxicity as a chemotherapeutic agent, determination of Ki-67 PI value before further therapeutic decisions are made may help in selecting the patients who are believed to be associated with a favorable treatment response. In this way, consistently with modern principles of oncology patients management, determination of Ki-67 PI in advanced cervical cancer may allow an individualized treatment approach for each patient.

Acknowledgements:

This study was funded by Ministry of Education, Science and Technological Development of the Republic of Serbia grant number III 41018 and Internal Scientific Research Project of the Faculty of Medicine University of Nis No. 34.

Conflict of Interest:

The Authors declare that they have no conflict of interests.

References

1) Small W Jr, Bacon MA, Bajaj A, Chuang LT, Fisher BJ, Harkenrider MM, Jhingran A, Kitchener HC, Mileshkin LR, Viswanathan AN, Gaffney DK. Cervical cancer: a global health crisis. Cancer 2017; 123: 2404-2412.
2) Simic V, Jovanovic V, Obradovic V, Stevanovic Lj. Epidemiološke karakteristike karcinoma grlića materice. Zdravstvena zaštita 2013; 42: 71-76.
3) Li B, Wu N, Zhang XJ, Wei ZL, Shang LX. MicroRNA-409 inhibits the proliferative ability of cervical carcinoma cells by regulating AKT. Eur Rev Med Pharmacol Sci 2018; 22: 936-942.
4) Wang F, Tan WH, Liu W, Jin YX, Dong DD, Zhao XJ, Liu Q. Effects of miR-214 on cervical cancer cell proliferation, apoptosis and invasion via modulating PI3K/AKT/mTOR signal pathway. Eur Rev Med Pharmacol Sci 2018; 22: 1891-1898.
5) Pich A, Chiusa L, Navone R. Prognostic relevance of cell proliferation in head and neck tumors. Ann Oncol 2004; 15: 1319-1329.
6) Skalova A, Lehtonen H, von Boguslawsky K, Leivo I. Prognostic significance of cell proliferation in mucoepidermoid carcinomas of the salivary gland: clinicopathological study using MIB1 antibody in paraffin sections. Hum Pathol 1994; 25: 929-935.
7) Dodd RL, Slevin NJ. Salivary gland adenoid cystic carcinoma: a review of chemotherapy and molecular therapies. Oral Oncol 2006; 42: 759-769.
8) Haapasalo H, Pesonen E, Collan Y. Volume corrected mitotic index (M/V-INDEX). The standard of mitotic activity in neoplasms. Pathol Res Pract 1989; 185: 551-554.
9) Kanthiya K, Khunnarong J, Tangjitgamol S, Puripat N, Tanvanich S. Expression of the p16 and Ki67 in cervical squamous intraepithelial lesions and cancer. Asian Pac J Cancer Prev 2016; 17: 3201-3206.
10) Falcetta FS, Medeiros LR, Edelweiss MI, Pohlmann PR, Stein AT, Rosa DD. Adjuvant platinum-based chemotherapy for early stage cervical cancer. Cochrane Database Syst Rev 2016; 11: CD005342.
11) Eljack ND, Ma HY, Drucker J, Shen C, Hambley TW, New EJ, Friedrich T, Clarke RJ. Mechanisms of cell uptake and toxicity of the anticancer drug cisplatin. Metallomics 2014; 6: 2126-2133.
12) Moore DH, Tian C, Monk BJ, Long HJ, Omura GA, Bloss JD. Prognostic factors for response to cisplatin-based chemotherapy in advanced cervical carcinoma: a Gynecologic Oncology Group Study. Gynecol Oncol 2010; 116: 44-49.

13) Lebwohl D, Canetta R. Clinical development of platinum complexes in cancer therapy: an historical perspective and an update. Eur J Cancer 1998; 34: 1522-1534.
14) Zou SH, Du X, Sun FD, Wang PC, Li M. Cisplatin supresses tumor proliferation by inhibiting autophagy in ovarian cancer via long non-coding RNA RP11-135L22.1. Eur Rev Med Pharmacol Sci 2018; 22: 928-935.
15) Marth C, Landoni F, Mahner S, McCormack M, Gonzalez-Martin A, Colombo N; ESMO Guidelines Committee. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2017; 28: iv72-iv83.
16) Datta NR, Stutz E, Liu M, Rogers S, Klingbiel D, Siebenhüner A, Singh S, Bodis S. Concurrent chemoradiotherapy vs. radiotherapy alone in locally advanced cervix cancer: A systematic review and meta-analysis. Gynecol Oncol 2017; 145: 374-385.
17) Petrelli F, De Stefani A, Raspagliesi F, Lorusso D, Barni S. Radiotherapy with concurrent cisplatin-based doublet or weekly cisplatin for cervical cancer: a systematic review and meta-analysis. Gynecol Oncol 2014; 134: 166-171.
18) Kruse AJ, Baak JP, de Bruin PC, Jiwa M, Snijders WP, Boodt PJ, Fons G, Houben PW, The HS. Ki-67 immunoquantitation in cervical intraepithelial neoplasia (CIN): a sensitive marker for grading. J Pathol 2001; 193: 48-54.
19) Maeda MY, Simões M, Wakamatsu A, Longatto Filho AL, Oyafuso M, de Mello ES, Otta MM, Alves VA. Relevance of the rates of PCNA, Ki-67 and p53 expression according to the epithelial compartment in cervical lesions. Pathologica 2001; 93: 189-195.
20) Yim EK, Park JS. Biomarkers in cervical cancer. Biomarker Insights 2006; 2: 215-225.
21) Calil LN, Edelweiss MI, Meurer L, Igansi CN, Bozzetti MC. p16 INK4a and Ki67 expression in normal, dysplastic and neoplastic uterine cervical epithelium and human papillomavirus (HPV) infection. Pathol Res Pract 2014; 210: 482-487.
22) Anju M, Mati GM. Assessment of monoclonal antibody MIB-1 labeling indices in cervical intraepithelial lesion of the uterine cervix in paraffin section. J Obst Gyn India 2008; 58: 327-332.
23) Ross W, Hall PA. Ki67: from antibody to molecule to understanding? Clin Mol Pathol 1995; 48: M113-M117.