Significance of hypoxia in uterine cervical cancer. Multicentre study

J. Markowska[1], J.P. Grabowski[1], K. Tomaszewska[1], Z. Kojs[2], J. Pudelek[2], M. Skrzypczak[3], J. Sobotkowski[4], J. Emerich[5], A. Olejek[6], V. Filas[7]

[1]Gynecological Oncology Division, University of Medical Sciences, Poznan;
[2]Gynecological Oncology Department, Oncological Centre, M. Curie-Sklodowska Institute, Krakow;
[3]Gynecological Clinic II, University of Medical Sciences, Lublin; [4]Gynecological Radiotherapy Department Regional Hospital of M. Kopernik, Lodz; [5]Gynecological Department, University of Medical Sciences, Gdansk; [6]Obstetrical and Gynecological Department, University of Medical Sciences, Bytom, Poland; [7]Patomorphology Department, University of Medical Sciences, Poznan (Poland)

Summary

Purpose: The aim of the study was to evaluate hypoxia markers (VEGF, GLUT-1, and HIF-1alpha) in cervical cancer tissue depending on staging (FIGO) and grading. We also analyzed the adverse effects of radiotherapy according to expression levels of hypoxic markers in the studied tissue. *Material and Methods:* Expression of hypoxia-inducible factor-1α (HIF-1alpha), glucose transporter 1 (GLUT-1) and vascular endothelial growth factor (VEGF, also known as proangiogenic factor) were estimated in biopsy or surgical specimens from 106 patients diagnosed with uterine cervical cancer. Immunohistochemical methods with EbVision+ complex using monoclonal antibodies anti-VEGF and anti-HIF-1alpha and polyclonal antibody anti-GLUT-1 were applied. *Results and Conclusions:* Hypoxia features measured by percentage of cells undergoing reaction with antibodies anti-HIF-1alpha, anti-GLUT-1 and anti-VEGF were similar in all clinical stages; however the biggest hypoxia features were shown in low differentiated cancers G2 and G3. The 5-year survival for FIGO Stage III patients was shorter in cases with a high expression of hypoxic markers. We observed adverse effects in 45.3% of patients, which occurred more often in patients with higher expression of the studied factors. The presence of hypoxic cells is established as one of the most important factors affecting resistance against tumor radiotherapy and patient prognosis.

Key words: Hypoxia, Cervical cancer.

Introduction

Hypoxia is the reduction of oxygen in tissues below levels which are considered to be normal. In solid tumors cell proliferation is greater than the rate of blood vessel formation and supplementation. This is the reason hypoxia easily develops within solid tumors. Tumor hypoxia is associated with poor response to therapy. We studied three tumour hypoxia markers: glucose transporter 1 (GLUT-1) [1], vascular endothelial growth factor (VEGF) [2] and hypoxia-inducible factor 1 alpha (HIF-1alpha) [3] in uterine cervical cancer. The expression of biological hypoxia markers and tumor vascularity are critical factors in both disease progression and treatment outcome in cervical cancer. It has been observed that increased expression of these factors is associated with poor prognosis in a variety of tumors including uterine cervical cancer [4-6]. HIF-1alpha can be a candidate for a prognostic indicator as an angiogenic mediator in uterine cervical cancer [4]. Expression of GLUT-1 may also serve as an indicator for the induction of the transcriptional response to hypoxia, which has been linked to enhanced proliferation, resistance to therapy, and metastatic propagation of cancer cells [7].

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Material and Methods

One hundred and six women with uterine cervical cancer were included in our study. All patients had histopathologically diagnosed squamous cell carcinoma and underwent radiotherapy. We studied three hypoxia factors. GLUT-1, VEGF and HIF1-alpha data were analyzed using Statistica 6 - Statsoft software. For the purpose of the study, tissues fixed in 10% neutral buffered formalin were processed by standard histopathological methods to paraffin wax blocks. The study used an immunohistochemistry method with the EbVision+ visualization system (DAKO). For the purpose of labeling tumor markers, the following monoclonal antibodies were used: anti-VEGF, anti-HIF-1-alpha. A polyclonal antibody, anti-GLUT-1 (CHEMICON) was also used. Immunoperoxidase staining was performed on an automated staining machine, the DAKO Autostainer.

Results

Our analysis was performed to evaluate the relationship between stage, grade of the cancer or therapy adverse effects and hypoxia markers. The intensity of staining of GLUT-1, VEGF and HIF-1 alpha was considered as weak to strong (+ to +++) and the percentage of stained cells as: $< 10\%$, $< 50\%$ and $> 50\%$. In all cases of studied hypoxia factors: GLUT-1, VEGF and HIF-1 alpha we observed a correlation between staining intensity and grade of the cancer ($p < 0.05$). In grade 2 and 3 we noticed a higher intensity of staining of hypoxia markers in comparison with grade 1 (Table 1). We did not observe

Table 1. — *GLUT-1, VEGF and HIF-1 alpha intensity of staining according to grade ($p < 0.05$).*

Grade	GLUT-1			VEGF			HIF-1 alpha		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
"+"	13	8	4	10	11	4	13	9	3
"++"	11	21	10	12	19	9	9	16	10
"+++"	5	17	17	7	16	18	7	21	18

GLUT-1: glucose transporter 1; VEGF: vascular endothelial growth factor; HIF-1 alpha: hypoxia inducible factor 1 alpha.

any correlation between stage (FIGO) of the disease and intensity of staining. Percentage of cells stained was not correlated either with stage or grade of disease. An overall 5-year survival rate of 62.3% was observed which does not differ from data of other authors [8, 9, 10]. There was a significant difference in 5-year survival rate in Stage III (FIGO) according to intensity of staining of studied hypoxia factors. The 5-survival rate of the patients with higher intensity of staining in Stage III (FIGO) (Figure 1) was worse than for patients with lower staining intensity in cases of all studied factors. We did not observe this situation in Stage I or II (FIGO) of the disease (Figures 2 and 3). Additionally we analyzed the radiotherapy toxic effects which appeared during treatment in 44 patients (41.5%). Most common reactions were in order: proctitis, cystitis, ulceration of vaginal epithelium, skin irritations, diarrhea, vertigo, postradiation colitis and cystitis. Although the above data concerning radiotherapy toxicity were not enough to perform statistical analyses we did observe a correlation between them and the staining intensity of hypoxia markers (Table 2). Toxic reactions after radiotherapy were observed in patients with higher staining intensity of hypoxia markers. In four cases we also noticed metastases to the abdominal/paraaortal lymph nodes or to the lungs. All four patients were diagnosed with FIGO Stage IIIB and with high staining intensity of "++" or "+++" in cases of all three markers.

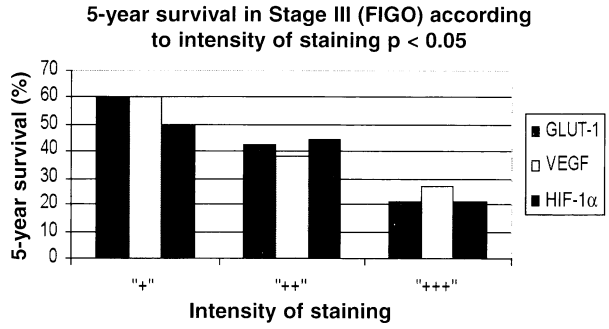

Figure 1.

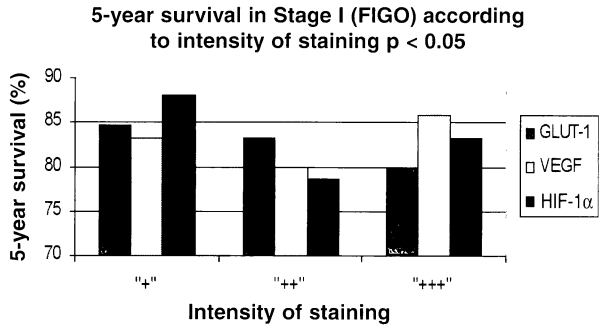

Figure 2.

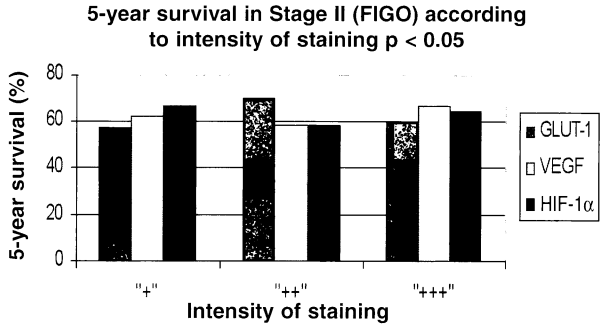

Figure 3.

Discussion

In many previous studies authors have indicated the role of HIF-1alpha, VEGF and GLUT-1 as hypoxia markers in different cancers [1, 2, 11]. Our analysis took into consideration the above-mentioned factors as hypoxia markers but also their tumor tissue appearance in patient outcome evaluations. Most of the women included in our study were in Stage I and II (FIGO). Treatment success in this group depends mostly on surgery and in second-line on radiation or chemoradiation. Among these women we did not notice any correlation between HIF-1alpha, VEGF and GLUT-1 expression level and stage. Since hypoxia plays an important role in radiation resistance, we noted a correlation between expression and outcome in patients diagnosed with Stage III (FIGO), hence in the group where primary therapy is radiation or chemoradiation. Similar conclusions concerning HIF-1 alpha only were noted by Burri *et al.* [12]. The observed correlations may suggest that hypoxia is a

Table 2. — *Toxic effects observed after radiotherapy according to hypoxia factors and staining intensity.*

Staining intensity	GLUT-1			VEGF			HIF-1 alpha		
	"+"	"++"	"+++"	"+"	"++"	"+++"	"+"	"++"	"+++"
Proctitis	2	4	5	1	4	6	2	3	6
Cystitis	1	3	5	2	2	3	1	4	4
Ulceration of vaginal epithelium	1	1	4		2	4	1	2	3
Skin irritations	1	2	2	1	1	3	1	1	3
Diarrhea	1	1	3	1	2	2	2	1	2
Vertigo	1	1	1		1	2	1	2	
Nausea		1	1			2			2
Postradiative cystitis			2		1	1			2
Postradiative colitis			1			1			1

GLUT-1: glucose transporter; VEGF: vascular endothelial growth factor; HIF-1alpha: hypoxia inducible factor 1 alpha.

very important prognostic factor for patients with Stage IIA who cannot profit from surgery, but may benefit from therapies that use levels of cancer cell oxygenation to limit cancer invasion and its metastatic potential [13-16]. Especially high expression of markers occurred in low differentiated cancer which worsens prognosis.

Ishikawa *et al.* [17] investigated HIF-1 alpha in a group of patients with Stage IIIB cervical squamous carcinoma according to metastasis free-survival. They suggest an important role of HIF-1alpha as a metastasis prognostic factor. Our data are insufficient to confirm this thesis, but we observed that recurrence occurred only in the Stage III patients analyzed in our study. Moreover these patients presented high levels of all hypoxia markers ("++", "+++").

Toxic effects after radiotherapy were observed in 41.5% of patients. Proctitis, cystitis and colon ulceration were the most common complications. Of interest is that these reactions occurred more often in patients with a staining intensity of "++" or "+++". Hypoxia marker evaluation combined with the new treatment methods mentioned before give us a chance to modify the radiation rate and therefore to help avoid the toxic effects concerned with this therapy.

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Address reprint requests to:
J. MARKOWSKA, M.D., Ph.D.
Department of Oncology
Division of Gynecologic Oncology
University of Medical Sciences in
Poznan Ul. Lakowa 1/2
61-878 Poznan (Poland)