GROWTH FRACTION DEFINED BY KI-67 IMMUNOSTAINING AS A SELECTION FOR COMBINED THERAPY OF RADIATION AND MITOMYCIN-C IN LOCALLY ADVANCED CERVICAL CANCERS

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ABSTRACT

OBJECTIVE To study the relationship between low growth fraction and hypoxic condition of the tumor that allows bioreductive agents, such as Mitomycin-C (MMC), to work more effectively in such environment. A prospective study was conducted at our institution between October 1994 and October 1996.

MATERIALS AND METHODS Sixty-one locally advanced cervical cancer patients with a low growth fraction as defined by Ki-67 immunostaining (Ki-67 index < 40%) were randomized into two groups. The first group of 30 patients were treated with irradiation alone and the second group of 31 patients were irradiated in combination with MMC. External irradiation was given with Linear accelerator or Co-60 to the whole pelvis, 180 cGy/fraction, 5 days a week, with a total dose of 5040 cGy in 5.5 weeks. This was followed by intracavitary afterloading irradiation within 2 weeks using High Dose Rate Co-60 sources, two applications, one week interval with a dose of 850 cGy at point A in each application. MMC was concurrently given as bolus injection on the first day of external irradiation and the first intracavitary application with a dose of body surface 15 mg/m^2 of each injection.

RESULTS Response of the tumors was determined within 3 months upon completion of irradiation. In the first group, complete response was achieved in 11 of 30 patients (36.6%), while in the second group, this was achieved in 26 of 31 patients (83.8%), showing significant difference between the two groups (p= 0.004). No marked difference of acute toxicity was observed.

CONCLUSIONS The results of the treatment suggested that low growth fraction tumors, which assumed as relatively hypoxic tumors, could be improved in their radiation response by Mitomycin-C.

Keywords Cervical cancer, Radiation response, Growth fraction, Ki-67 immunostaining, hypoxic tumor, Mitomycin-C.

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BACKGROUND

Uterine cervical cancer (UCC) represents the most frequently encountered malignant tumor in women in various developing countries. The majority of patients admitted for treatment were at locally advanced stage, and the curative treatment could only be performed with radiation[1] whose results were not satisfactory yet. It was reported that the survival rate of the patients receiving radiation therapy alone was 50% at Stage IIb, 28% at Stage IIIb, and 10% at Stage IVa.[2,3] In developing countries, the survival rate of Stage III UCC patients proved to be lower, i.e., 22% for 5 years, and 25.2% for 3 years.[4,5]

One of the causes assumed to be responsible for the failure in radiation therapy is hypoxic condition for which hyperbaric oxygen, hypoxic radiosensitizer, treatment with bioreductive materials, and hyperthermia are used to overcome it.

One of the methods for treatment using bioreductive materials is a combined therapy of radiation and Mitomycin-C (MMC), i.e., a long known type of antibiotics and was already in use since 1956.[6] In either in vitro or in vivo experiments, MMC has been shown to enhance the results of radiation since this cytostatics works more effectively in hypoxic environtment of the tumor.[7] However, no desirable results have been achieved in its clinical application, particularly in treating UCC. The combined therapy for locally advanced UUC resulting in complete response ranged between 74.5 and 84.61%, which did not show significant difference from radiation therapy alone, i.e., 48.9% compared to 70%. Such is the case with survival rate which reached 73.08% compared to 60% in three years, and 59.6% compared to 42.0% in 30 to 72 months.[8,9] The fact that these results are not satisfactory yet may be due to the less selective administration of therapy, and in order to perform better selection, some reliable indicators will be necessary.

Mendehlson, 1960[10] showed that in a tumor tissue there is a proportion of proliferating cells called tumor growth fraction. The proliferating cells exist in the phases of a cell cycle, i.e., phases G_1, S, G_2, and M, while the non -proliferating cells (the quiescent cells) are at phase G_0. The determination of the size of growth fraction can be made by the use of various methods, such as through immunohistochemical examinations.

Gerdes, 1984[11] performed immunohistochemical examination by means of Ki-67 monoclonal antibody, and through this method the proliferating cells could be identified. These cells express Ki-67 antigen in its nuclear surface and could bind the correspondent monoclonal antibody such that it can be stained specifically. By comparing the number of proliferating cells and the total tumor cells, including quiescent cells, the size of tumor growth fraction may be identified and defined by Ki-67 index.

Nakano, 1993[12] used this technique in UCC patients and observed that the patients with Ki-67 of 33 %, or more, showed better response to radiation and more significant survival rate than the patients with Ki-67 less than 33%.

Thus, tumor growth fraction defined by Ki-67 can serve as predictor for radiation response and as prognostic factor in radiation therapy for UCC patients.

Sutherland, 1990[13] pointed out that hypoxic conditions could lead to changes at cellular level in tumor tissue. The condition of oxygen deficiency is generally accompanied by the nutritional deficiency and the occurrence of acid atmosphere in the environment of tumor cells. This

condition can result in inhibition to some of the proliferating cells that will terminate at phase G_1, and recede to phase G_0 such that tumor growth fraction is reduced, and thereby there is a likely correlation between hypoxia and low growth fraction.

Thus, it can be assumed that tumor growth fraction defined by Ki-67 index is an indicator for hypoxic tumor tissue.

Based on this assumption, values of Ki-67 index in locally advanced UCC may show the proportion of relatively hypoxic tumor group that is of some use for selecting therapy modality. The group with low Ki-67 index, which is considered to be relatively hypoxic, can be expected to yield favorable response to combined therapy of radiation and MMC, since the latter works more effectively in hypoxic conditions.

Hypotheses that can be formulated here is that the combined therapy of radiation and MMC in the patient group with low Ki-67 index provides better response to the tumor than the radiation therapy alone with insignificantly different toxicity.

The purpose of this study was to prove that the combined MMC and radiotherapy could enhance the results of radiation provided that this treatment is administered for the group of low growth fraction suspected to be hypoxic, and Ki-67 index can be used as the indicator for hypoxic conditions in locally advanced UCC.

MATERIALS AND METHODS

DESIGN

This study was a controlled clinical test, with basic design parallel for 2 groups of treatment in which each group served as the control group, respectively as:

a) Group of UCC patients with low growth fraction, receiving radiation therapy alone.
b) Group of UCC patients with low growth fraction, receiving combined therapy of radiation and MMC.

In this study, a number control variables were identified, i.e., age, clinical stage, histopathologic types, differentiation degree, type of growth fraction, tumor size, and Hb level. As control variables, which were also independent variables, were radiation treatment and combined treatment of radiation and MMC. Variables were dependent on response to tumor and therapy toxicity.

POPULATION AND SAMPLE

The population studied was all UCC patients at locally advanced stage according to the criteria established by FIGO (International Federation of Obstetrics and Gynecology) admitted for treatment at Division of Oncology, Department of Obstetrics and Gynecology, Faculty of Medicine University of Indonesia/Cipto Mangunkusumo General Hospital. In order to meet the criteria for the required sample size, all subjects fulfilling the criteria of the study were selected as samples through consecutive sampling.

INCLUSION CRITERIA

a) Performance status : 50-100 in Karnofsky scale [14]
b) Blood hemoglobin level : 10 g %, or higher
c) Number of lymphocyte : 4000/mm^3
d) Thrombocyte : > 100,000/mm^3
e) Renal and hepatic functions were normal
f) Lesion could be measured
g) Never receiving radiation and chemotherapy
h) No contraindications for therapy and chemotherapy.

EXCLUSION CRITERIA

a) Rejecting to participate in clinical tests
b) Cases with bleeding requiring immediate management
c) Age was > 70 years
d) Infection in pelvic cavity.

The calculation of sample size was done based on the assumption of the extent of radiation response in each group.
a) Group of low Ki-67 in radiation therapy alone : 35%
b) Group of low Ki-67 in combined therapy : 80%

The size of sample was counted using the calculation of response proportion in treatment group p (t) of 80%, and the control group p (c) of 35%, and by using the formula,[15] a sample of 22 subjects was obtained for each group.

Informed consent for medical intervention was obtained as necessary after the subjects of the study and their family were given explanation on the objective and steps of the study.

PATIENT SELECTION

Examinations were performed in patients suspected to develop locally advanced UCC admitted for treatment at the Oncology Polyclinic, Department of Obstetrics and Gynecology, Faculty of Medicine University of Indonesia/Cipto Mangunkusumo General Hospital, to determine the disease stage. Clinical and supporting examinations performed included thorax photos, intravenous pyelography, rectoscopy, cytoscopy, and laboratory examinations such as blood platelets, and renal and hepatic functions. Clinical stages were determined according to the system of FIGO of 1976.[16] Measurement of tumor size was performed with inspeculo, vaginal and rectal toucher. Patients with locally advanced Stage of IIb to IIIb meeting the inclusion criteria were included in the study. Patients at Stage IV were not included in the study since their management was subject to the prevailing protocols, and was not uniform.

Biopsy was performed in patients who met the criteria of the study for routine histopathologic and immunohistochemical examinations. Biopsy was performed at two sites opposite to the uterine cervix in order to obtain the more representative tissues. Both biopsy tissues were divided into two parts, i.e., the first part for routine histopathologic examination (Hematoxyllin-Eosin specimen), and the second one for immunohistochemistry using Ki-67 monoclonal antibody. Both types of examinations were performed at Department of Anatomic Pathology, Faculty of Medicine University of Indonesia/Cipto Mangunkusumo Hospital. Routine histopathologic examinations were done to identify histopathologic types and descriptions on the differentiation degree as specified in the protocol of routine histopathologic examinations established by the Working Group of Gynecologic Tumors, Faculty of Medicine University of Indonesia.

IMMUNOSTAINING EXAMINATION

All the fresh biopsy specimens were divided into two. One was fixed with 10% formaldehyde solution for conventional hematoxyllin and eosin staining and the other specimen was quickly frozen for Ki-67 immunostaining. The specimens were cut with a cryostat in 6 μm thickness, air dried, and fixed with cold 4% formaldehyde solution for 30 minutes. Then the sections were reacted with anti-Ki-67 monoclonal antibody.[17,18] (DAKO-PC; Dako, Copenhagen, Denmark) for an hour at room temperature. The sections were followed by reaction with biotinylated anti-mouse immunoglobulin G and avidin-biotin complex[19] (Vector Laboratories, Burlingame, CA) for 30 minutes. The sections

were reacted with 3.3-diaminobenzidine tetrahydrochloride (DAB, Dojin Chemicals, Tokyo) solution with 0.01% (w/v) hydrogenperoxide 23 for 2-5 minutes at room temperature and counterstained with hematoxylin. Control staining was done by incubating with phosphate-buffered saline, instead of anti-Ki-67 antiserum.

ASSESSMENT OF KI-67 INDEX

More than 1000 tumor cells per specimen were counted on three x 200 color photograph for calculation of the Ki-67 index. The Ki-67 index was estimated by the percentage of Ki-67 positive cancer cells among all the counted tumor cells.

The selection of threshold of Ki-67 index was determined on the basis of the reference of Nakano and Oka[20] who set up the margin of Ki-67 for low growth fraction to be 33% and 42%. In the present study, this margin was set at 40% based on the previous studies in 11 patients. It was found that Ki-67 ranged from 28.4% to 80.0% with a mean of 50.3% and a median of 49.9%. By using confidence interval of 95%, the percentage obtained was 40-60%, such that the lowest threshold of 40% was used.[21] The difference in the thresholds may be due to the varying clinical stages, geographic distribution, and different laboratory techniques used.

UCC patients at Stage IIb to IIIb who had been examined and whose diagnosis had been established were sent to Radiotherapy Unit for radiation planning. Patients were then divided into two groups, i.e., the group with Ki-67 of 40% or higher, and the group with Ki-67 index lower than 40%. In the latter group, randomization was performed, i.e., half of the patients were administered radiation alone, and the other half was given combined therapy of radiation and Mitomycin-C (MMC).

The group with Ki-67 index (40% would be treated the same way and reported in the future.

RADIATION TREATMENT

Radiation treatment was administered in two stages. The first stage was external radiation given to the whole pelvis, and the second stage was intracavitary radiation given in 1 to 2 weeks upon completion of external radiation. External radiotherapy was administered with radiation field, with superior border in lumbal IV and V vertebral rifts, and the inferior border was at the inferior border of pubis symphysis, and lateral border was 1.5 cm from linea inominata. A dose of 180 cGy fraction was administered 5 times per week, totaling 5040 cGy in 6.5 weeks. Radiation was given through antero-posterior field with linear accelerator of 10 or 4 megavolt instrument, or telecobalt-60 instrument.

At the end of external radiation, gynecologic examinations were performed to identify the response to radiation and to determine intracavitary radiation. Intracavitary radiation was performed when the at gynecologic examinations patients met the criteria, such as tumor had regressed in such a way that the applicator could be installed in the standard position. Intracavitary radiation was performed under spinal anesthesia if the first method failed. The applicator used was a rigid one; generally the applicator of RRTI (Rotterdam Radiotherapeutisch Instituut) model was applied with modified Manchester system.[22] The position of applicator was observed and improved with the aid of X-ray C-arm, and once it was well-positioned a calculation of isodose was performed to determine the required dose at point A, bladder, and rectum. The calculation of dose was done with Treatment Planning System and with Sidos-U computer from Siemens, or by using TSG-Radplan from IAEA that had both been calibrated with TLD.[23]

In general, intracavitary radiation was administered with High Dose Rate (HDR) afterloading techniques using Cobalt-60 source with HDR Selectron instrument (Nucletron). A dose of 850 cGy at point A was administered twice in one-week interval. In a number of patients, afterloading techniques were performed manually with Low Dose Rate (LDR), using Cs-137 source with a dose of 1300 cGy twice in one-week interval at point A. It was calculated that bladder and rectal doses did not exceed 80% of the dose at point A.

TUMOR RESPONSE

Tumor response was evaluated on the basis of the criteria of UICC[24] and determined at the end of radiation, in 1 month, and 3 months after completion of radiation. In addition to clinical examinations, biopsy interventions/histopathologic examinations were performed in 3 months after radiation.

Data were processed and analyzed with SPSS program with bivariate statistic test using Chi-square test for categorical variables and differentiation test of mean in continues variables. Furthermore, multivariate statistic test with logistic regression was used if bivariate statistic test was found to be significant.

RESULTS

From October 1, 1994 to November 30, 1996, 61 patients completed overall treatment and were evaluated up to 3 months after completion of radiation. The characteristics of 61 patients evaluated could be seen in (Table 1.)

TABLE 1. Patient's Characteistics

		No. of patients	%
Age	Mean	46.6	
	SD	9.3	
Stage	IIb.	38	62.2
	IIIa.	0	0
	IIIb.	23	37.7
Histologic type	Squamous cell Ca	48	78.6
	Adenocarcinoma	11	18.0
	Adenosquamous Ca	2	3.2
Histologic grade	Well differentiated	19	31.1
	Moderately differentiated	32	52.4
	Poorly differentiated	10	16.3
Tumor's size	< 4 cm	20	32.7
	4-6 cm	41	67.2
	> 6 cm	0	0
Hb level (pre-irradiation)	<12	37	60.6
	≥12	24	39.3
		61	100

Patients consisted of females aged between 26 and 69 years, with a mean of 46.6 years. The majority of them were at Stage IIb (62.2%). The most frequently found histopathologic type was squamous cell carcinoma (78.6%). However, the type of adenocarcinoma was found to be somewhat higher than the normal population, i.e., 18%, with the mostly frequently found differentiation of moderate degree (52.4%). Most of the patients had tumor measuring 4 - 6 cm (67.2%), and had Hb level < 12 g% (66.6%).

CHARACTERISTICS OF TREATMENT

Patients were divided into two types of treatment, i.e., 30 patients were treated with radiation alone, and another 31 patients with combined therapy of radiation and MMC.

TABLE 2. Characteristics of treatment

Type of treatment	No. of pts
Irradiation alone	30
Combined irradiation with MMC	31
Intracavitary irradation	
• High Dose Rate (HDR)	43
• Low dose rate (LDR)	18
• Intracavitary radiation dose	
Point A :	
HDR : mean 1700.0 cGy (TDF 50)	
LDR : mean 2600.0 cGy (TDF 50)	
Bladder :	
HDR : mean 1490,4 cGy (TDF 44)	
LDR : mean 1998,3 cGy (TDF 38)	
Rectum:	
HDR : mean 1351,3 cGy (TDF 39)	
LDR : mean 2003.5 cGy (TDF 38)	

TREATMENT OF INTRACAVITARY RADIATION

The majority of patients, i.e., 43 patients (70,5 %) received intracavitary radiation using HDR system, while another 18 patients (29,5 %) received manual afterloading application using LDR Cs-137 source. We resorted to this technique because HDR instrument was out of order for several months. The size and rate of the dose were calculated and tailored to HDR dose using Time Dose Fractionation (TDF) that was considered to have similar biological effects (Table 2). In the first group, the average dose administered was 2 x 850 cGy, or equivalent to TDF 50 at point A. The average bladder dose was 1490,4 cGy (TDF 44) and the rectal dose 1351,3 cGy (TDF 30). In the second group, dose at point A was 2 x 1300 cGy (TDF 50) with an average bladder dose of 1998,3cGy (TDF 38), and rectal dose of 2003.5 cGy (TDF 38).

TABLE 3. Distribution of variables according to type of treatment

	Irradiation		Irradiation + MMC		n	p
Variables	Risk (+)	Risk (-)	Risk (+)	Risk (-)		
Stage	10	20	13	18	61	NS
Histol. Type	6	24	7	24	61	NS
Tumor's size	19	11	9	22	61	0,02 (S)
Hb level	17	13	20	11	61	NS
Histol. Grade	4	26	10	21	61	NS

COMPARABILITY OF GROUPS

Of the various factors studied, some of them had the risks that may affect the evaluation of treatment impact. Thus, in order to avoid bias in two groups of treatment, i.e., the group of radiation therapy and the group of combined therapy of radiation and MMC, the risk factors must be divided evenly.

Variables evaluated to have risk factors were:

1) Clinical stage: positive risk was stage III, negative risk was stage II
2) Histologic types : positive risk was adeno-carcinoma, negative risk was squamous cell carcinoma.
3) Histologic grade: positive risk was the patients with unfavorable histologic grade, negative risk was those with moderate and favorable histologic grade.
4) Tumor size: Positive risk was tumor with a diameter of ≥ 4 cm, and negative risk was that with a diameter of < 4 cm.
5) Hb level: positive risk was the patients with Hb level < 12 g%, and negative risk was those with Hb ≥ 12 g%.

EVALUATION OF TUMOR RESPONSE

Evaluation of tumor response based on clinical examinations was performed in all patients at the end of radiation, in 1 month, and 3 months after completion of radiation according to the criteria of UICC. In all patients, either complete or partial response was found, and no response categorized as no change or progressive disease was encountered. In 27 patients, biopsy was performed in 3 months following radiation. The results of clinical examinations in the form of complete response were found in 22 patients, compatible with 18 patients with negative anatomic pathology, while there existed a remaining tumor in 5 patients, and 4 patients showed positive anatomic pathology. In terms of sensitivity, clinical examinations reached 80 %, while specitivity reached 81,8 %.

Sixty-one patients who completed radiation with a 3-month follow-up consisted of 2 groups of treatment, i.e.,

1) Group I (radiation therapy alone) with complete response : 11 patients (36.6%).
2) Group II (combined radiation therapy) with complete response : 26 patients (83.8%).

Thus, different type of treatment yielded different tumor response. In this case, the combined therapy provided more significant response than the radiation therapy alone. (Table 4).

TABLE 4. Response of tumor according to type of treatment

Type of treatment	Complete Response		Partial Response		Total	
	n	%	n	%	n	%
Irradiation + MMC	26	83,8	5	16,2	31	100
Irradiation	11	36,6	19	63,4	30	100
Total	37	60,6	24	39,4	61	100

MULTIVARIATE ANALYSIS

Multivariate analysis was performed because there existed significant influence of tumor size on treatment response in different treatment groups. For that reason, logistic regression test was done with the following determination of variables:

Dependent variable : treatment response.
Independent variables : types of treatment and tumor size.

In statistical test, it was evident that type of therapy significantly determined therapy response with factor of 4 times, while tumor size determined the response by 2 in patients with low fraction growth.

TOXICITY OF TREATMENT

Toxicity evaluated was acute toxicity during radiation and up to 3 months afterwards, and divided into 5 degrees, i.e., from 0 to IV using the criteria of ECOG. In the present study, toxicity degree was rendered to be a simpler one:

1) Mild toxicity, degree 0 - I
2) Moderate toxicity, degree II - III
3) Severe toxicity, degree IV

Toxicity of digestive tract was the most frequently found toxicity observed in 45 patients (74.5%); however, this toxicity was generally mild and mostly found in degree I. This was followed by hematologic toxicity that was observed in 31 patients (50.8%), and mostly occurred in decreased Hb (26 patients), followed by reduced leukocyte (24 patients), and mostly found in degree I. The next toxicity was observed in urinary tract that was found in 15 patients (24.5%), and mostly occurred in degree I. By contrast, patients suffering toxicity of degree III were 3, and no one suffered toxicity of degree IV (Table 5).

TABLE 5. Toxicity of treatment

Organ	n	%
Digestive tract	45	73,7
Hematological	31	50,8
Urinary tract	15	24,5
Others	3	4,9
Total	61	100

There was no significant difference in toxicity between the group of patients treated with radiation only or combined irradiation with MMC (Table 6).

TABLE 6. Comparison of toxicity according to type treatment

Toxicity	Irradiation	Irradiation + MMC	Total
Mild	24	21	45
Moderate	6	10	16
Total	30	31	61

DISCUSSION

It was evident that the radiation therapy alone in the group of patients with low Ki-67 index, i.e., less than 40%, resulted in unfavorable tumor response, i.e., complete response was found only in 11 of 30 patients (36.6%). As known, radiation response in UCC patients correlated with the tumor local control in the pelvis,[25] and the patient's survival rate.[26] With this less favorable prognosis, it would be appropriate to administer more intensive therapy. Combined therapy of radiation and MMC proved to provide more significant response in the group of patients with low Ki-67 index., i.e., 26 of 31 patients (83.8%) experienced complete response.

In addition, tumor growth fraction defined by Ki-67 index represented an independent predictor factor.[27] This was evident from biva- riate analysis, suggesting that tumor size played a part in radiation response. However, in multi- variate analysis, it showed that such influence was not significant. Other study [28] focusing on the group of patients with large tumor suggested a less satisfactory radiation response. Thus, tumor size constituted a prognostic factor with an adverse impact on UCC.

Previously, we assumed that there existed a close correlation between low growth fraction and hypoxic condition of the tumor tissue. This finding was based on an in vitro study[29] that was subsequently supported by the recent clinical studies.[30] With such assumption in mind, the group of patients with low growth fraction, suspected to be hypoxic, could be treated more definitely. Treatment modality adopted in this study was combined therapy of radiation and MMC that was considered to be more effective for hypoxic tumors. It proved that the combined therapy of radiation and MMC for the low growth fraction tumors yielded significantly better results than the radiation therapy alone, i.e., each with complete response of 83.8% and 36.6% respectively ($p = 0.004$).

With better results obtained from the combined therapy for UCC patients with low Ki-67 index, other treatments to overcome the problem of hypoxia was made possible, such as the use of hypoxic radiosensitizer, hyperthermia, and so forth, which in the past had not yielded satisfactory results. Such unsatisfactory results may be attributed to the fact that the treatment was performed in hypoxic tumors without cautious selection. Thus, the opportunity to perform a study with this mode of study may be more feasible. It is hoped that such a study could be performed in other tumors receiving radiation therapy as a curative treatment with hypoxic problems, such as nasopharynx cancer. There exists a number of

theories that correlate hypoxic condition with low growth fraction, such as: passive mechanism and active mechanism.

In passive mechanism, tumor cells situated more distant from blood vessels would suffer more oxygen and nutrition deficiency, and with adverse environmental impacts the metabolism of those cells would come to an end. Even if they still survive they would remain quiescent until the atmosphere improves. The weak cells would not survive, be necrotic and die.[31] Other theory held that in hypoxic condition due to disrupted vascularization or nutrition deficiency, a mechanism of cell preservation serving as genetic regulation would evolve to protect the cell from any possible threat. Here, the role of p^{53} as "watchman" was evident in regulating the cell's defense system. In severe hypoxic condition, p^{53} would direct the cells to perform apoptosis (programmed death). However, in milder hypoxic condition, through cyclin-dependent kinase inhibitor, i.e., p^{21}, p^{53} would inhibit various dependent kinases, i.e., CDK2, CDK4/6 and cdc2 that correlate with various cyclins, such as cyclins A, B, D, and E, which in turn change the activities of key proteins that control the cell's entry into cell cycles.[32] One of the inhibitions is to prevent cells that have completed mitosis from entering into phase G_1, such that those cells will recede into phase G_0, preventing them from proliferating and thereby reducing tumor growth fraction. Apoptosis itself could serve as predictor for the response to radiation, including locally advanced UCC.[33] This may be attributed to the extent of severity of hypoxic condition in the tumor tissue. Thus, the relationship between apoptosis and growth factor could provide important information on the severity of hypoxic condition.

To prove further the clinical relationship between growth fraction and hypoxic condition, a study on the pO_2 measurement using direct microelectrode[34] and immunohistochemistry using Ki-67 in the treatment of UCC is necessary. This will be more relevant if an examination on vascular density and intercapillary distance is also performed,[35] such as the relationship among the microenvironment factors resulting from tumor development and vascular changes may be proved. The recent report of the study performed by Nakano et al, 1997, suggested that there existed a correlation between pO_2 measured by microelectrode and tumor growth fraction defined by Ki-67 index.[36]

Clinical toxicity due to the combined application of radiation and MMC can be classified into acute and chronic toxicities. In the present study, acute toxicity did not reveal any significant increase between the group receiving combined radiation and the group with radiation alone.

In the previous studies,[37] effects of bone marrow depression in the administration of MMC were noticeable. However, these effects were not evident in the current study. This may be due to the fact that the dose administered was sufficient and the interval of administration was sufficiently long (approximately 8 weeks), such that hematologic toxicity of the first MMC administration had been lessened before the second administration. The dose given referred to the MMC administration in a prospective study on cervical and head and neck cancers, i.e., 15 mg/m^2 of body surface[38] in a 6-week interval for subsequent MMC administration. With such dose and method, the therapy response was sufficiently good with minimum toxicity. In addition, in this study radiation was administered with relatively lower dose per fraction, i.e., 180 cGy, administered 5 times a week during approximately 5.5. weeks with a total dose of 5040 cGy. Routine radiation was administered with a dose 200 cGy per fraction in the period of 5 weeks, with a total of 5000 cGy. The administration of different dose per fraction would result in different biological effects

if it was not compensated with identical amount of time of radiation and identical total dose. To achieve the similar biological effects, a TDF (Time Dose Fractionation) factor from Orton, 1973,[39] was used. In the current study, a TDF smaller than the conventional radiation was used. However, it was still within the limits of the radiation dose frequently administered conventionally to the pelvis area or parametria, i.e., between 4500 and 5000 cGy. It is hoped that with such dose, the radiation administered was effective, and thereby the cumulative toxicity from both treatment modalities could be reduced.

Clinical toxicity of chronic nature could not be assessed yet since the evaluation was made in 3 months after completion of therapy. In general, chronic toxicity in UCC was assessed after 6 months following the therapy. Chronic toxicity due to MMC was frequently observed in the lungs, heart and kidney. Nevertheless, those areas were not affected by radiation during the radiation of UCC, such that no cumulative toxicity occurred in those organs.

Based on the above findings, it was evident that the combined therapy of radiation and MMC could be practically applied. The possible barrier to be faced is that the examination with monclonal antibody Ki-67 requires frozen section that calls for a special management. Such special management requires a close coordination among the relevant departments, i.e., Departments of Obstetrics and Gynecology, Anatomic Pathology, and Radiology. The crucial problem is that how the biopsy tissue could be frozen in low temperature immediately after the tissue removal, and be kept in a low temperature before immunohistochemistry is performed. However, most of those barriers may be overcome, because currently the specimens using paraffin block can be made, as they have been performed for routine HE examination. The antibody used in this examination was MIB-1 monoclonal antibody or Ki-67 polyclonal antibody. Both antibodies have the same specificity in determining the size of growth fraction in the tumor with Ki-67 monoclonal antibody.[40]

CONCLUSIONS

In conclusion, the above findings suggested that the low growth fraction, which resulted in poor response to the radiation, yielded better results through the administration combined radiation and MMC. We assumed that the low tumor growth fraction provides important description on the hypoxic tumor tissue, such that MMC working more effectively in hypoxic condition will enhance the results of radiation. However, in order to prove conclusively that the growth fraction correlates with hypoxic condition, a further study employing, among others, O2 measurement with microelectrode will be necessary.

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