

Cytopathological and Immunohistochemical Study for Estimating Radiotherapeutic Effects in Uterine Cervical Cancer

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ABSTRACT

In order to estimate the effects of radiation on cancer tissue, cytopathological findings, BrdU labelling index (L.I.), tumor markers and C-myc oncogene products were examined in 30 patients of uterine cervical cancer, before radiation, at around 10Gy, 30Gy, 50Gy and at the end of the therapy. In order to assess the radiotherapeutic effects, the cytological, histological, immunohistological findings were evaluated by Papanicolaou's classification, Oboshi and Shimamoto's grading system and BrdU L.I.

In the cytology, the radiation effect, such as the enlargement and vacuolation of the nuclei and cytoplasm, was observed at around 10Gy of radiation, and significantly increased at around 30Gy showing lysis of the cells and pyknosis of the nuclei. The histological observation recorded remarkable change in the cancer tissue at around 30Gy of radiation. The BrdU L.I. showed a meaningful decrease around 30Gy in the radiosensitive cases compared with the radioresistant cases.

Moreover, immunohistochemical investigation indicated the location of SCC and SLX in the cytoplasm of the cancer cells and of C-myc product in the nuclei. A distinct radiation effect was found in the C-myc oncogene product at around 10Gy. Ras oncogene was not found in the adenocarcinoma cases investigated.

From these findings it is concluded that BrdU L.I., revealed by immunohistological study, is the most suitable indicator to estimate the response of radiation in cancer cells of the uterine cervix and that C-myc oncogene product has the potentiality to be used as a prognostic factor in uterine cervical cancer treated by radiation therapy.

Key words: *Radiotherapeutic effect, BrdU labelling index, Tumor antigen, Oncogene, Uterine cervical cancer*

Radiation therapy is one of the most effective treatments for uterine cervical cancer. The response of cancer cells to radiation is an essential factor in deciding the prognosis in each individual patient. For this reason it is necessary to use a parameter in evaluating the radiation effect. The morphologic change of cancer cells has been the only parameter for estimating radiotherapeutic effects for several decades. In this work, A study of the state of DNA synthesizing cells of cancer tissue was made by using Bromodeoxyuridine (BrdU) before and after radiation to produce a more useful parameter for radiotherapeutic effects on cancer cells.

Invasive squamous cell carcinoma of the uterine cervix is associated with the presence of tumor antigen SCC, and it is accepted that the presence of SCC antigen is related to malignancy^{8,9,11}. The C-myc oncogene product and ras oncogene accompany the squamous cell carcinoma of the uterine cervix, as reported by previous work^{13,15}.

With this background, the purpose of this research was to evaluate the radiation effects on cancer cells by a cytopathological and immunohistochemical study using BrdU L.I., tumor antigen SCC, SLX, C-myc and ras oncogene product during the period of radiotherapy in advanced cervical cancer.

MATERIALS AND METHODS

Thirty cases of advanced uterine cervical carcinoma were treated by radiation therapy in the Department of Obstetrics and Gynaecology, Hiroshima University School of Medicine, from January 1989 to December 1991. Of these 23 were squamous cell carcinoma cases (4 large cell keratinizing, 17 large cell non keratinizing and 2 small cell non keratinizing type) and 7 were endocervical adenocarcinoma cases.

All the patients were classified according to FIGO'S classification before radiation therapy with the following results: Six stage 11b, one

stage 111a, fourteen stage 111b, one stage 1Va, one stage 1Vb in squamous cell carcinoma, and one stage 1b, one stage 11a, one stage 11b and four stage 111b in adenocarcinoma. The ages of the patients ranged from 30 to 87 with an average at 65.2 years old.

The total dose of radiation was around 50Gy in most cases, but in some cases it was around 60Gy depending upon the size of the primary tumor and the extent of invasive lesion. The pelvis was irradiated externally with Mega voltage X-ray by Linear Accelerator (Linac). A center block to protect the bladder, rectum and small intestine was performed at around 30Gy of radiation in 26 patients but not in four patients (Table 4, squamous cell carcinoma cases no.3, 5, 16 and adenocarcinoma case no.3): After completion of external pelvic radiation, almost all patients were exposed to intracavitary radiation using Tandem and Ovoid. Low dose intracavitary radiation was given by Radium and the dose to A point ranged from 12.2Gy to 41.8Gy.

Cases with a biopsy free of cancer cells after therapy were considered as radiosensitive, and cases containing cancer cells in their biopsy after therapy were considered as radioresistant.

Table 1. Oboshi and Shimosato grading system (Grading of degenerative cancer cells after radiation)

Grade 0: No radiation effect found in cancer cell.
Grade 1: A little RT effect is present but the cancer cell nest remains completely undisturbed.
Grade 2: RT effect is found in individual cancer cells; Ca. cell nest pattern is destroyed.
(A) More than 1/4th of the cells of specimen have a strong probability of becoming active cancer cells if the radiation is stopped at this point.
(B) Less than 1/4th of the cells of the specimen have a strong possibility of becoming active cancer cells if the radiation is stopped at this point.
Grade 3: Non viable cancer cells are present only.
Grade 4: No cancer cells at all.
(A) Wide area of the specimen is necrotic.
(B) Granulation tissue.
(C) Fibrotic change.

Oboshi, S. and Shimosato, Y. Journal of Clinical and Experimental Medicine; Vol. 61, No. 10, 618-623 (1967)

RT: Radiation Therapy

Cytological study:

A cervical smear was taken with a cotton swab from each patient serially before radiation therapy, at around 10Gy, 30Gy, 50Gy, and at the end of radiation therapy. The smears were immediately fixed in 99% ethanol, stained by Papanicolaou's

Table 2. Protocol; Immunohistochemical study for BrdU*

Deparaffinization and dehydration
↓
Inactivation for endogenous peroxidase incubate in 0.3% H ₂ O ₂ added methanol solution, 30min
↓
Rinse in PBS, 5min × 2
↓
Rinse in PBS added 0.5% Tween-20, 5min × 2
↓
Denaturation of DNA in 2N HCl, 60min
↓
Neutralization with 0.1M borate buffer, 5min × 2
↓
Rinse in PBS added 0.5% Tween-20, 5min × 2
↓
Rinse in PBS added 5% normal horse serum incubation, 40min
↓
Rinse in PBS added 0.5% Tween-20, 50min × 2
↓
Anti-BrdU monoclonal antibody (1 : 20 dilution, Becton Dickinson), 60min
↓
Rinse in PBS added 0.5% Tween-20, 5min × 2
↓
Biotin labeled anti-mouse IgG (Vector Lab. Inc.)
↓
Rinse in PBS, 15min × 2
↓
ABC complex incubation, 30min
↓
Rinse in PBS, 15min × 2
↓
DAB-H ₂ O ₂ reaction
↓
Counter stain with methyl green

* All procedures were done at room temperature

method and evaluated according to Papanicolaou's classification.

Histological study:

Serial cervical punch biopsies were performed under the colposcopic direction after taking the cervical smear before, at around 10Gy, 30Gy, 50Gy during the course, and at the end of radiation therapy. Each punch biopsy was taken from the same quadrant of cervical lesion. Biopsy specimens were prepared for (1) Hematoxylin Eosin

(H.E.) staining and (2) Immunohistochemical staining. These biopsies, stained by H.E., were then graded for radiotherapeutic effect according to the Oboshi and Shimamoto grading system (Table 1).

Immunohistochemical study for BrdU:

Some of the tissues removed surgically from the cancer cases were prepared for immunohistochemical staining. The tissues were minced to 1 mm³ in size and cultured in a medium containing BrdU in a concentration of 5 microgram per milliliter at 37°C in 3 atmospheric pressure in the presence of carbogen gas for 2 hours, and fixed in 70% ethanol. The immunohistochemical procedure followed is shown in Table 2.

Around 2,500 cells were counted per slide and the percentage of BrdU stained cells was calculated before, during the treatment course at around 10Gy, 30Gy, 50Gy and at the end of radiation therapy. When the biopsies were free of cancer cells the BrdU L.I. could not be calculated and it was taken as zero. In six squamous cell carcinoma cases (Table 4-a, cases no.4, 5, 10, 18, 20 and 21) in which BrdU L.I. was detected at around both 10Gy and 30Gy, the decrease rate of BrdU L.I. from 10Gy to 30Gy was calculated to estimate radiosensitivity according to the following formula:

Decrease rate =

$$\frac{\text{L.I. at around 10Gy} - \text{L.I. at around 30Gy}}{\text{L.I. at around 10Gy}} \times 100$$

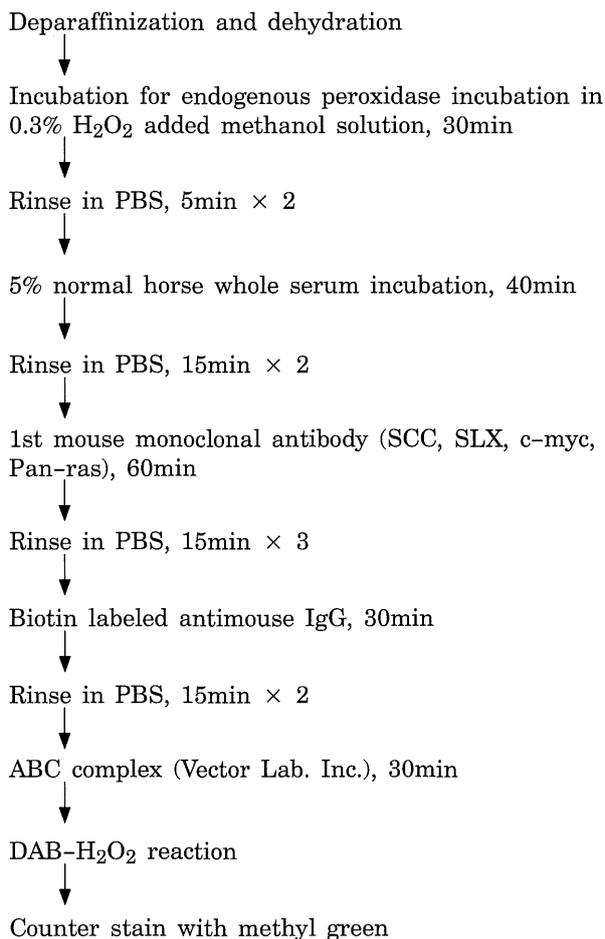
Study of tumor antigen and oncogene product:

The radiotherapeutic effect on tumor antigen (SCC and SLX) in the cancer tissue was examined by immunohistochemical staining by the ABC method in the biopsy before, at around 10Gy, 30Gy, 50Gy during the treatment course, and at the end of radiation therapy. Their serological level was also studied before and at the end of therapy. The presence or absence of tumor antigen in the cancer tissue with each dose of radiation therapy was observed. To study C-myc product and pan-ras oncogene the same process was followed.

Tissues for the immunohistochemical study of tumor antigen and oncogene product were fixed in a microwave for 30 sec in a fixation solution containing 2% formaldehyde, 0.05% glutaldehyde, 2.5 mM calcium chloride and 0.1 M phosphate buffer (pH 7.3).

Anti-SCC monoclonal antibody (Dinabott), anti-C-myc monoclonal antibody (Oncogene Science), anti-pan-ras monoclonal antibody (Oncogene Science), anti-SLX monoclonal antibody (Otsuka)

Table 3. ABC method of immunohistochemical staining*



* All procedures were done at room temperature

were prepared for immunohistochemical staining. SCC and C-myc were investigated for the squamous cell carcinoma cases and staining for SLX and ras was performed for the adenocarcinoma cases. The process of immunohistochemical staining is shown in Table 3.

RESULTS

Of the 23 squamous cell carcinoma cases, 21 were judged as sensitive to external radiation, 2 were sensitive only to intracavitary radiation (case no.5 and 21 in Table 4-a). The histological type of these 2 cases was large cell keratinizing, and a center block was held at 40.5Gy in case no. 21 but not performed in case no.5. Of the 7 cases of adenocarcinoma, 6 were very sensitive to radiotherapy but one was resistant (case no.2 in Table 4-b) and died from the cancer.

The Manifestations of radiotherapeutic effect were evaluated according to the findings in cytology, colposcopy, histology and the immunohistochemical study for BrdU L.I., tumor antigen and oncogene product.

Table 4. Radiation effect by Papanicolaou's classification, Oboshi and Shimosato grading system and corresponding BrdU L.I.

Case No.	Cytology / Histology / BrdU L. I.					
	Around 10Gy		Around 30Gy		Around 50Gy	
1	IIIb	2A / 15.1	IIIa	3 / N.D.	II	4A / N.D.
2	IIIa	I / 4.2	IIIa	3 / N.D.	II	4A / N.D.
3	IV	I / 3.0	IIIa	3 / N.D.	II	4A / N.D.
4	IIIb	I / 15.0	IIIa	I / 9.4	IIIa	4A / N.D.
5	III	I / 13.0	IIIa	I / 10.2	II	I / 11.6
6	IIIb	2B / 3.4	IIIa	3 / N.D.	IVa	4A / N.D.
7	V	2A / 10.5	IIIa	N.D. / N.D.	II	N.D. / N.D.
8	IIIa	I / 13.9	IIIa	4A / N.D.	IIIa	4A / N.D.
9	V	I / 11.6	III	4A / N.D.	II	4A / N.D.
10	IV	I / 12.3	III	2B / 4.5	III	4A / N.D.
11	IIIa	I / 7.0	III	4A / N.D.	IIIa	4A / N.D.
12	III	O / 16.0	III	4A / N.D.	II	4A / N.D.
13	III	N.D. / 14.3	III	4A / N.D.	N.D.	4A / N.D.
14	IV	I / 7.0	III	4A / N.D.	II	4A / N.D.
15	IIIa	I / 12.0	N.D.	4A / N.D.	N.D.	N.D. / N.D.
16	IIIb	I / 9.6	III	4A / N.D.	II	4A / N.D.
17	V	I / 9.2	III	3 / N.D.	N.D.	4A / N.D.
18	IV	2B / 6.5	IIIa	2B / 2.5	I	4A / N.D.
19	III	I / 3.2	IIIa	4A / N.D.	IIIa	N.D. / N.D.
20	III	O / 5.9	IIIa	2B / 1.6	II	4A / N.D.
21	V	I / 9.8	IIIa	I / 7.2	N.D.	N.D. / 6.0
22	IIIa	3 / 1.0	IIIa	4A / N.D.	IIIa	4A / N.D.
23	V	I / 10.9	N.D.	3 / N.D.	N.D.	4A / N.D.
a. Squamous cell carcinoma cases						
1	IIIa	I / 18.7	IIIa	4A / N.D.	II	4A / N.D.
2	IV	I / 17.0	IV	I / 10.0	IV	I / 11.5
3	IIIb	I / 6.4	IIIa	3 / N.D.	II	4A / N.D.
4	IV	3 / 9.7	IIIa	4A / N.D.	IIIa	4A / N.D.
5	IIIa	3 / 4.0	II	3 / N.D.	II	4A / N.D.
6	IIIb	I / 7.5	III	3 / N.D.	III	4A / N.D.
7	IIIa	2B / 3.6	II	2B / 3.3	II	4A / N.D.
b. Adenocarcinoma cases						

N.D.: not detected

Cytological study: (by Papanicolaou's classification)

Figure one illustrates the cytology of squamous cell carcinoma before radiotherapy, showing cancer cells of various size and shape. The cytological appearance of the squamous cell carcinoma cases at 10Gy radiation showed mild morphological changes in which the nuclei of the cells were found to vary in size and contain granular chromatin materials, and have an irregular nuclear outline and slight enlargement of the cytoplasm (Fig. 2). The cervical smears in the squamous cell carcinoma cases before radiotherapy were diagnosed as class 1V to V. At around 10Gy of radiation they were class V in 5 cases, class 1V in 4 cases, class 111b in 4 cases, class 111 in 5 cases and class 111a in 5 cases (Table 4). Of the 23 cases 14 (60.9%) showed a mild effect of radiation, and active cancer cells were found in 9 (39.1%) cases at a dose around 10 Gy. The cytological findings of the squamous cell carcinoma at around 30Gy showed homogeneous nuclear chromatin materials and enlargement and vacuola-

tion in the cytoplasm of the cells. Cellular degeneration by radiation occurred to a greater extent (Fig. 3). At around 30Gy radiation all cases were class 111a, meaning a positive radiation effect with no cancer cells. At around 50Gy all cervical smears were class 1 to 111a.

The cytological findings in radiosensitive adenocarcinoma cases at around 10 Gy radiation revealed degenerative cancer cells having obscured nucleoli, enlarged cytoplasm, irregular cellular outline and homogeneous staining as a whole (Fig. 4). At around 10Gy of radiation, the smears showed class 1V in 2 cases, class 111b in 2 cases and class 111a in 3 cases. Thus 5 in 7 (71.4%) cases showed a radiation effect. Cancer cells were found in two cases at 10Gy, one of which was radioresistant (Table 4-b). At around 30Gy of radiation the cytological findings were class 11 in 2 cases, class 111a in 3 cases, class 111 in one case and class 1V in one case. A positive radiation effect was revealed in 6 of 7 (85.7%) cases. The Cytological findings had shown that all smears were free of cancer cells except in one



Fig. 1. Smear before radiation therapy, showing cancer cells of various shape and size in a squamous cell carcinoma case.

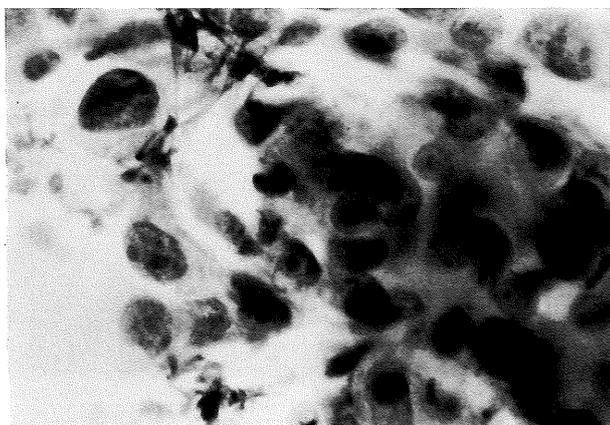


Fig. 2. Smear from a squamous cell carcinoma at around 10 Gy of radiation, showing the nuclei containing granular chromatin materials, an irregular nuclear outline, and the various size and slight enlargement of the cytoplasm.

case which was radioresistant (case no.2 in Table 4-b). At around 50Gy of radiation the smears showed class 11 in 4 cases, class 111a in one case, class 111 in one case and class 1V in the one radioresistant case.

Colposcopic study:

In most cases the colposcopic features before radiotherapy included cauliflower growth, excavated ulcer formation, and necrosis with massive bleeding. The colposcopic findings of the cancer lesion at 10Gy did not show any remarkable change in comparison with those before radiation (Fig. 5). At around 30Gy of radiation the cervix was found to have an almost normal appearance (Fig. 6), though occasionally abnormal vessels were found. At around 50Gy the cervix appeared to be totally normal with a white coat in the radiosensitive cases.

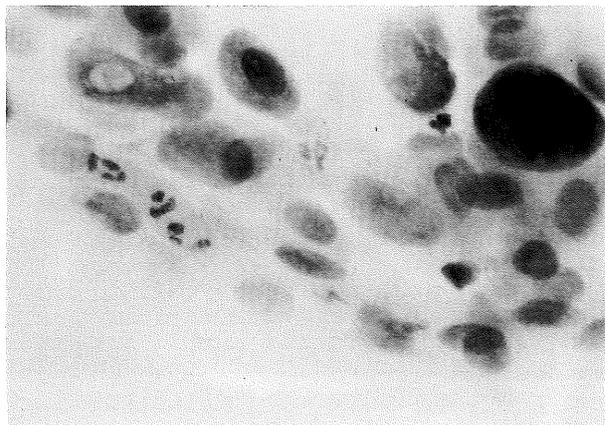


Fig. 3. Cytological findings from the same squamous cell carcinoma case (Fig.2.) at around 30Gy of radiation shows non granular nuclear chromatin materials and enlargement and vacuolation of the cytoplasm.

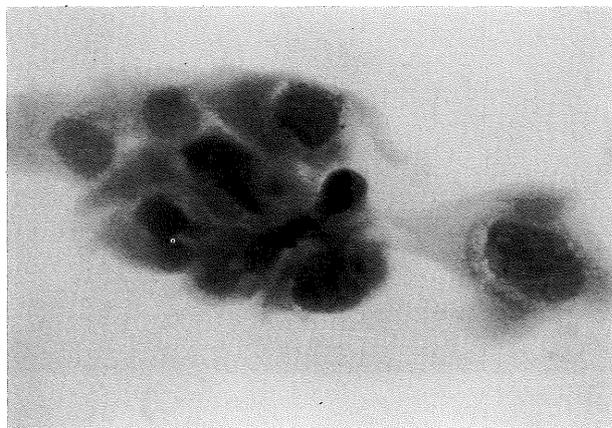


Fig. 4. The cytology of the smear in an adenocarcinoma case at around 10 Gy radiation shows obscured nucleoli, enlarged cytoplasm, irregular cellular outline, homogeneous staining and decreased stainability.

Histological study: (by the Oboshi and Shimamoto grading system)

The histological findings according to Oboshi and Shimamoto's grading system in squamous cell carcinoma cases at around 10Gy of radiation were grade 0 in 2 cases, grade 1 in 15 cases, grade 2A in 2 cases, grade 2B in 2 cases and grade 3 in one case. Thus 5 in 23 (21.7%) cases showed a radiation effect (Table 4), while 21 in 23 (91.3%) cases had shown active cancer cells in their biopsy. Figure 7 shows the histology of a squamous cell carcinoma case before radiation. The histological appearance at 10Gy is illustrated in Fig. 8, which shows that the individual cancer cells are present without degenerative features and that the epithelial pearl formation (the cell

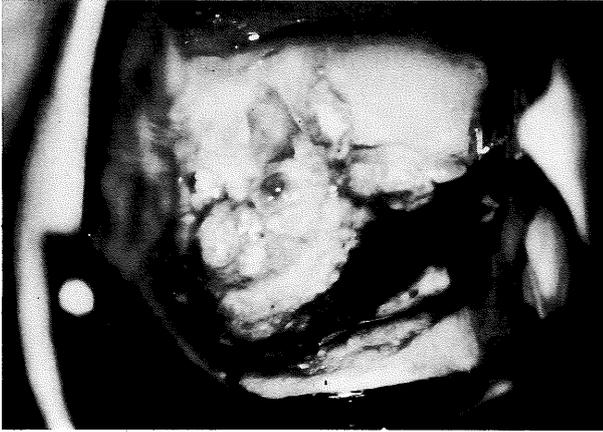


Fig. 5. Colposcopic features of a squamous cell carcinoma case at around 10Gy radiation, showing cauliflower growth from 6 to 9 o'clock position.

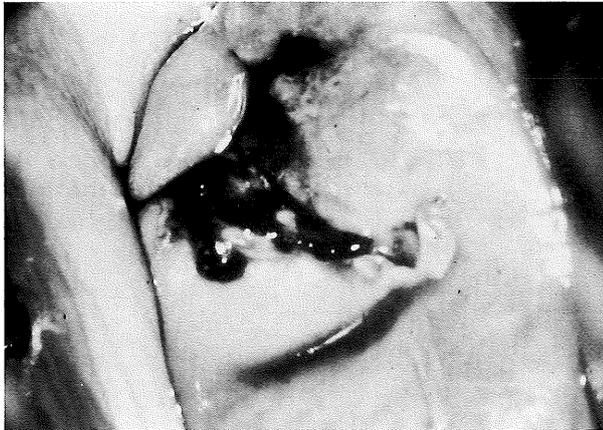


Fig. 6. The colposcopic features of a squamous cell carcinoma at around 30Gy radiation. Invasive cancer is not found. Mosaic and punctations are also not found.

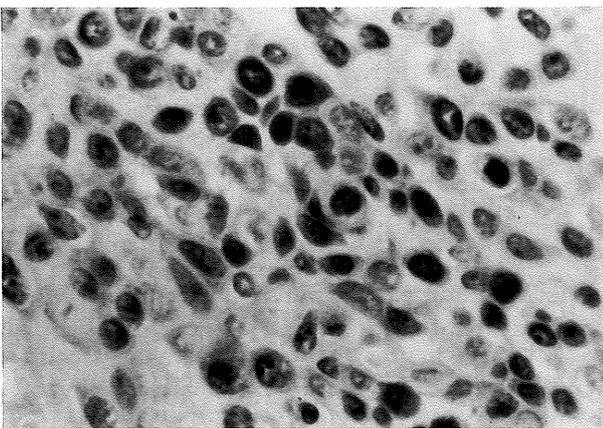


Fig. 7. Biopsy tissue of a squamous cell carcinoma case before radiation, showing non-irradiated intact cancer cells.

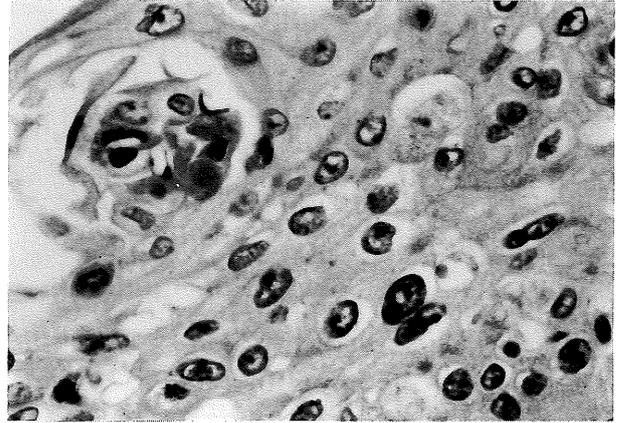


Fig. 8. Biopsy tissue at 10Gy of radiation in a squamous cell carcinoma shows partial destruction of the cell nest (pearl formation) but the individual cancer cells appear without degenerative features.

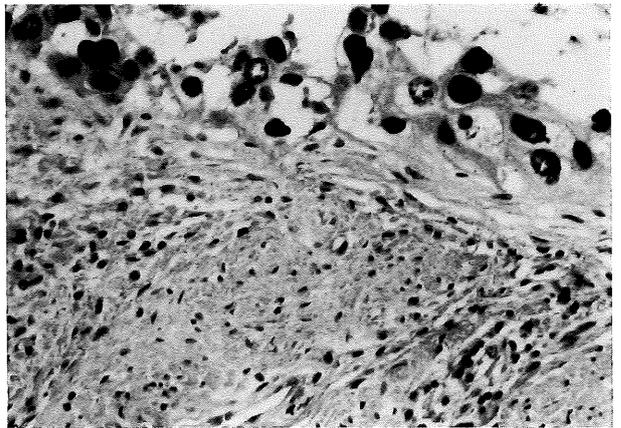


Fig. 9. Degenerative cancer cells with pyknotic nuclei and lysis of the cell sheet in a squamous cell carcinoma case at around 30Gy of radiation. Infiltration of inflammatory cells and fibrotic change are found in the surrounding tissue.

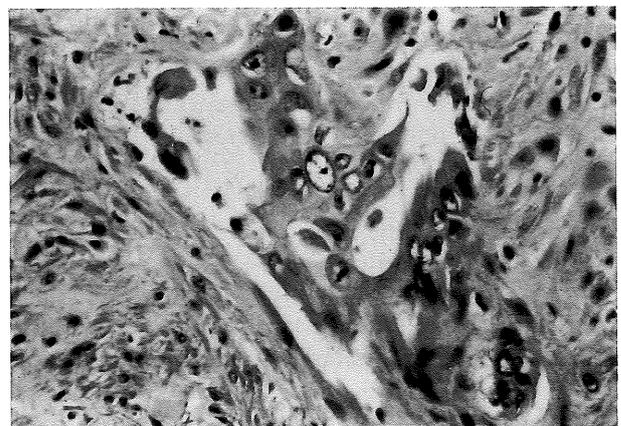


Fig. 10. Biopsy tissue from an adenocarcinoma case at around 30Gy of radiation shows non viable degenerative cancer cells with enlarged nuclei, and lysis of the cell sheet in the remnant of the damaged glands.

nest) has been affected in some radiosensitive cases. At around 30Gy radiation, 19 of 23 (82.6%) cases showed a radiation effect: grade 4 in 10 cases, grade 3 in 6 cases, grade 2 in 3 cases and grade 1 in 3 cases. Cancer tissue was found in 6 of 23 (26.0%) cases at this dose. The histological appearance of the cancer tissue that persisted around 30Gy radiation showed viable and degenerative cancer cells. Fibrous change with infiltrating inflammatory cells were found in the surroundings of the degenerative cancer tissue (Fig. 9). At around 50Gy all cases except case no.5 in Table 4-a showed grade 4, and were free of cancer tissue.

In adenocarcinoma, at around 10 Gy the effects of radiation were found in 3 of 7 (42.9%) cases: grade 1 in 4 cases, grade 3 in 2 cases and grade 2B in one case (Table 4-b). However, morphological changes in the cancer cells were few. At around 30Gy, radiotherapeutic effects were found in all cases except one resistant case: grade 4 in 2 cases, grade 3 in 3 cases, grade 2B in one case and grade 1 in one case. Cancer tissue was still present in one case (excluding the resistant case). This persistent cancer tissue showed destruction of the glandular tissue, viable and degenerative cancer cells (Fig. 10). At around 50Gy all showed grade 4, but the one radioresistant case showed grade 1.

BrdU labelling index study: (by immunohistochemical method)

Positive cells for BrdU were stained in the nuclei of cancer cells at each dose of radiotherapy (Figs. 11, 12, 13 and 14).

The mean BrdU L.I. in radiosensitive squamous cell carcinomas was 12.7% before radiation, 8.6% at 10Gy, 4.5% at 30Gy and 0% at 50Gy. Similarly the mean BrdU L.I. in radiosensitive adenocarcinoma before radiation and at 10Gy, 30Gy, 50Gy

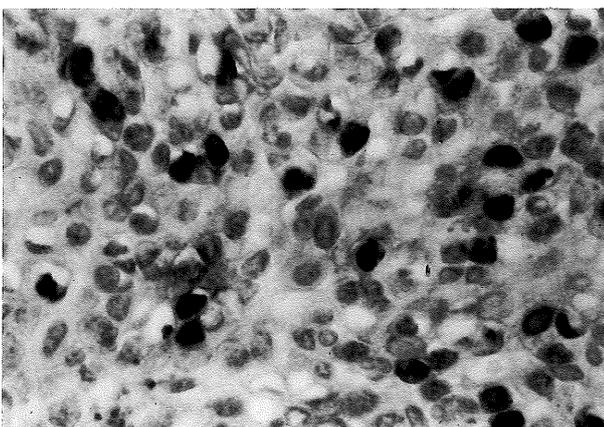


Fig. 11. A squamous cell carcinoma case before radiotherapy, showing the S-phase cells stained by anti-BrdU antibody

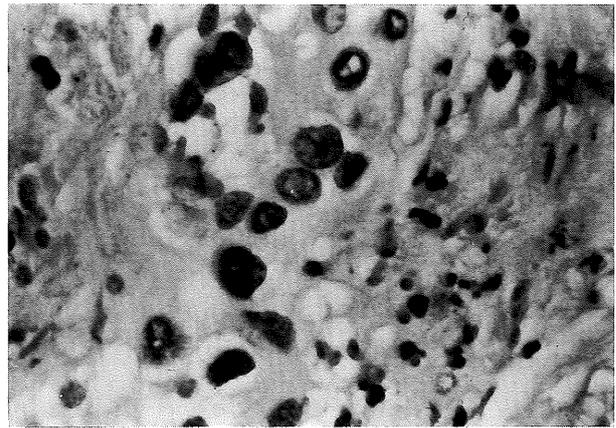


Fig. 12. Decreased S-phase cell population is seen at around 30Gy of radiation in a squamous cell carcinoma case.

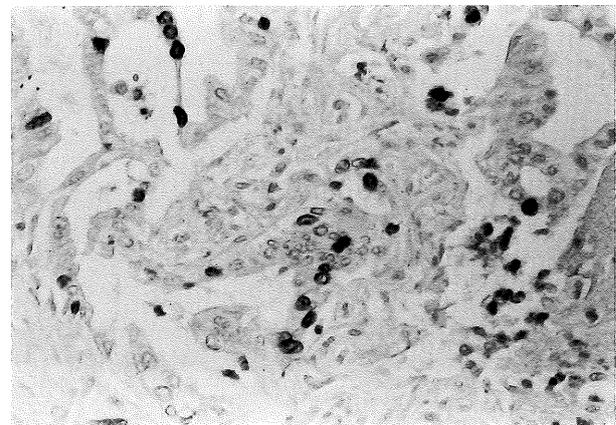


Fig. 13. An adenocarcinoma case showing the S-phase cells stained by BrdU before radiotherapy.

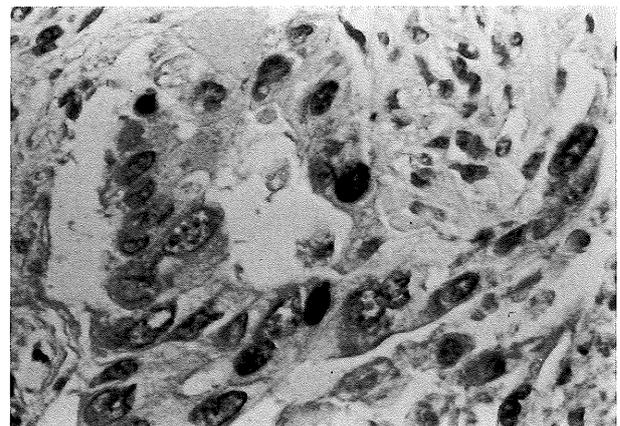


Fig. 14. A radioresistant adenocarcinoma case showing S-phase cells in high count at about 60Gy of radiation.

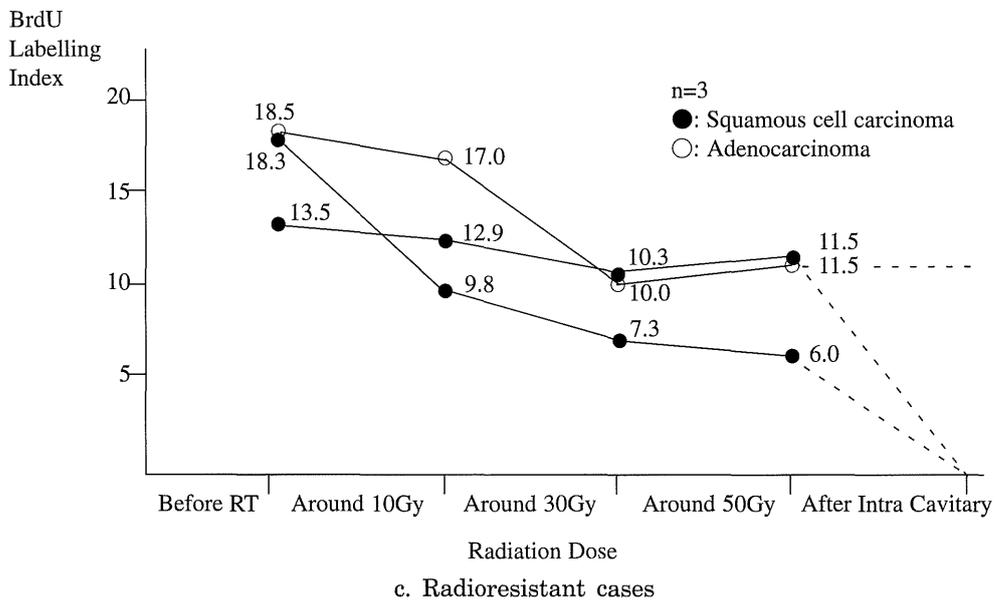
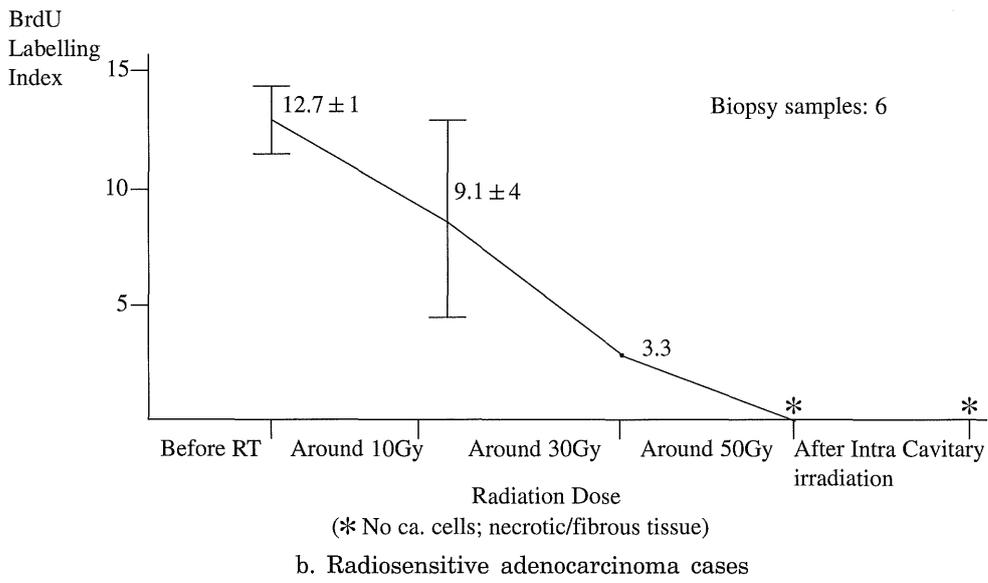
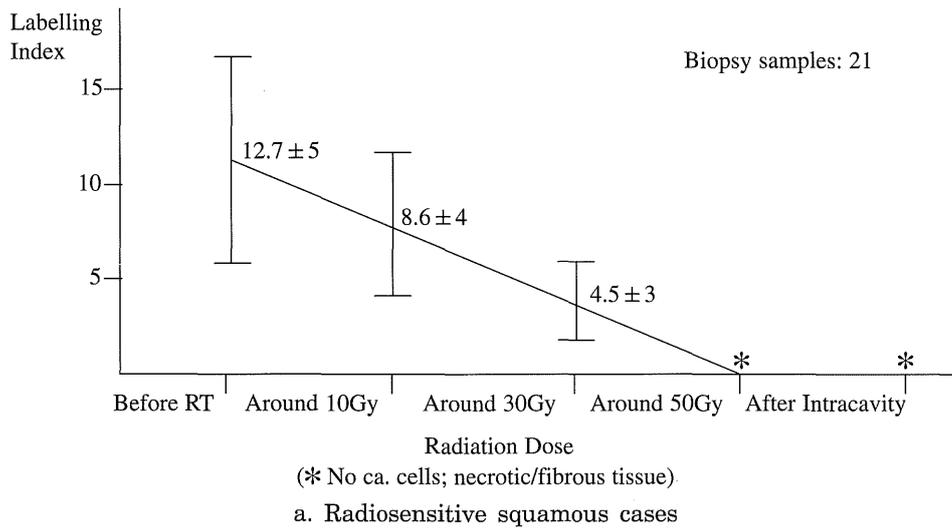


Fig. 15. BrdU labelling index corresponding to radiation dose

was 12.7%, 9.1%, 3.3% and 0% respectively (Table 4, Fig. 15a, b). On the other hand, in the radioresistant cases, the BrdU L.I. at 50Gy were 6.0% and 11.5% in squamous cell carcinoma, and 11.5% in adenocarcinoma (Fig. 15c).

By analysis of the results at around 30Gy it was observed that 17 of 23 (73.9%) cases of squamous cell carcinoma and 5 in 7 (71.4%) cases of adenocarcinoma cases showed BrdU L.I. 0%. The rate of decrease of BrdU L.I. from 10Gy to 30Gy was calculated as 37.3%, 21.5%, 63.4%, 61.5%, 72.9% and 26.5% in cases no. 4, 5, 10, 18, 20 and 21 respectively of the squamous cell carcinoma cases.

Analyzing the Oboshi and Shimosato grading system and BrdU L.I. (Table 5), it was observed that from grade 0 to grade 2A the BrdU L.I. was high ($13.3 \pm 6\%$, $12.8 \pm 2\%$). In grade 2B the BrdU L.I. was very low ($3.6 \pm 1\%$). In grade 3 the BrdU L.I. was $4.9 \pm 3\%$. (It should be noted that the BrdU L.I. could not be calculated in the cases having grade 3 cells around 30Gy as there was no cancer tissue in the biopsy and it was taken as zero).

Tumor antigen and oncogene product study:

Immunohistochemical positive cases of SCC were

Table 5. Correlation with Oboshi and Shimosato Grading System irrespective of radiation dose

	OSGS	BrdU L.I.
a. Squamous cell carcinoma cases	Grade 0	13.3 ± 6
	Grade 1	9.9 ± 3
	Grade 2A	12.8 ± 2
	Grade 2B	3.6 ± 1
	Grade 3	4.9 ± 3
	Grade 4	0
b. Adenocarcinoma cases	Grade 0	13.7 ± 2
	Grade 1	11.9 ± 4
	Grade 2A	N.F.
	Grade 2B	3.4
	Grade 3	4.9 ± 3
	Grade 4	0

OSGS : Oboshi and Shimosato Grading System

L.I. : Labelling Index

N.F. : Not found

found in 6 of 23 squamous cell carcinomas (Table 6). The SCC antigen was localized in the cytoplasm of the cancer cells. The tumor antigen SCC was positive in the specimen before and at around 10Gy and 30Gy of radiation. There was no remarkable difference in the immunohistological findings before and after radiation (Fig. 16), but the serum level of SCC decreased at the end of the therapy in the radiosensitive cases (Table 7).

Tumor antigen SLX was localized in the cytoplasm of the cancer cell and was positive in 6 of 7 adenocarcinoma cases before radiotherapy (Table 8). No remarkable change for SLX was found at 10Gy, 30Gy and at the end of radiation therapy (Fig. 17).

C-myc oncogene product was detected mainly in the nuclei of 7 squamous cell carcinomas before radiation (Table 9). With an increase in radiation dose nuclear staining for C-myc product was found to decrease, whereas the staining of the cytoplasm gradually increased (Figs. 18, 19).

Pan-ras product was not found in the cervical adenocarcinomas investigated in this study.

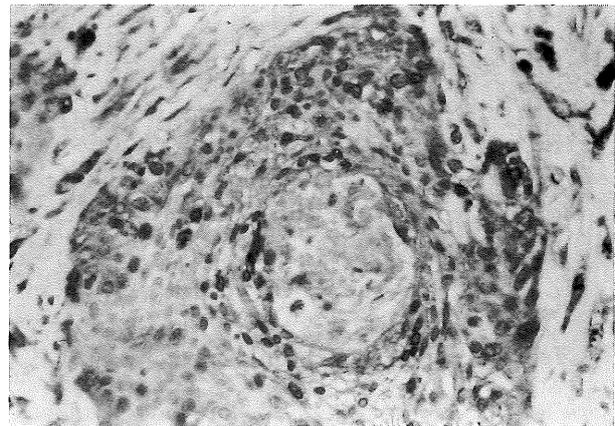


Fig. 16. A SCC antigen positive case showing staining in the cytoplasm of the cancer cells before radiation.

Table 6. Immunological findings for SCC squamous cell carcinoma cases

Case No.	Before RT	Around 10Gy	Around 30Gy	Around 50Gy
4	+	+	N.C.T.	N.C.T.
14	+	+	N.C.T.	N.C.T.
18	+	+	+	N.C.T.
17	+	+	N.C.T.	N.C.T.
19	+	+	N.C.T.	N.C.T.
15	+	+	N.C.T.	N.C.T.
21	+	+	N.C.T.	N.C.T.

RT: Radiotherapy,

N.C.T.: No cancer tissue

Table 7. Serum level of SCC in advanced cervical cancer regarding radiation therapy

	Case No.	Before radiation	After radiation
Squamous cell carcinoma cases	1	25.4ng/ml	4.1ng/ml
	3	6.2ng/ml	1.3ng/ml
	4	16.2ng/ml	0.5ng/ml
	7	45.0ng/ml	1.1ng/ml
	8	0.5ng/ml	0.9ng/ml
	9	20.0ng/ml	2.4ng/ml
	10	1.5ng/ml	1.8ng/ml
	11	1.9ng/ml	1.3ng/ml
	12	2.8ng/ml	1.0ng/ml
	13	4.3ng/ml	1.3ng/ml
	14	68.0ng/ml	11.0ng/ml
	16	56.0ng/ml	17.7ng/ml
	18	7.6ng/ml	2.2ng/ml
	20	27.9ng/ml	2.9ng/ml

The value of cut off: 1.5ng/ml

Table 8. Immunological findings for SLX Adenocarcinoma case only

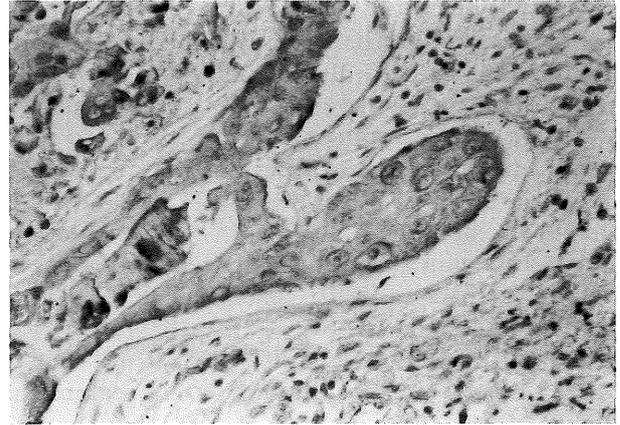
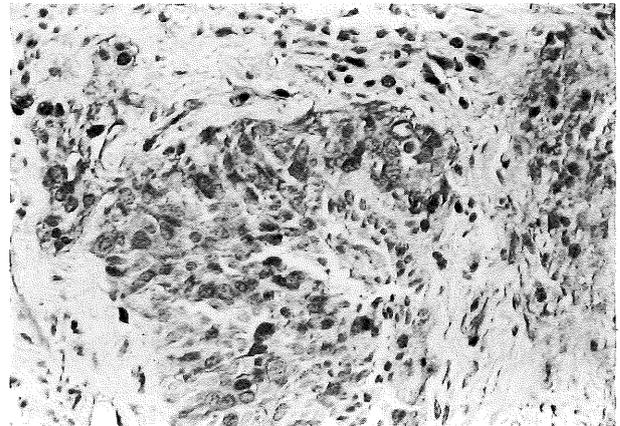
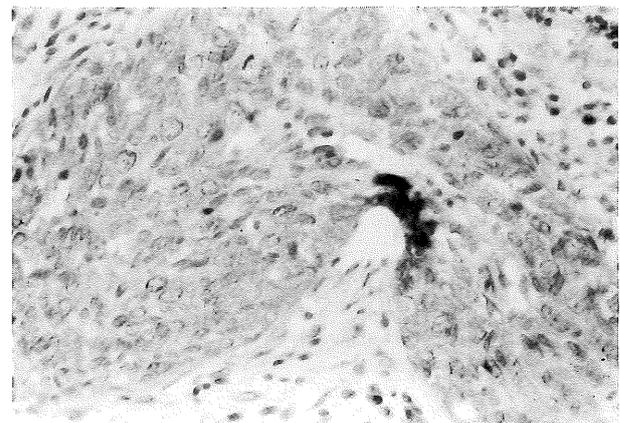
Case No.	Around 10Gy	Around 30Gy	Around 50Gy
1	+	N.C.T.	N.C.T.
2	+	+	+
3	+	- (degenerative cancer cell)	N.C.T.
5	+	+	N.C.T.
6	+	- (degenerative cancer cell)	N.C.T.
7	++	+	N.C.T.

N.C.T.: No cancer tissue

Table 9. Immunological findings for C-myc

Case No.	Before RT	Around 10Gy	Around 30Gy	Around 50Gy
15	N	+	-	-
	C	±	±	N.C.T.
17	N	+	-	-
	C	+	+	++
18	N	+	+	-
	C	+	±	++
1	N	+	±	±
	C	+	N.D.	+
19	N	+	+	-
	C	+	++	++
21	N	+	+	±
	C	+	++	++
23	N	++	+	±
	C	-	±	+

N.C.T. : No cancer tissue
 N : Nucleus
 C : Cytoplasm
 RT : Radiotherapy

**Fig. 17.** SLX antigen is positive in the cytoplasm of the cancer cells before radiation.**Fig. 18.** C-myc oncogene product is positive mainly in the nuclei before radiotherapy in a squamous cell carcinoma case.**Fig. 19.** Negative staining in the nuclei for C-myc product after 10Gy radiation in the same case as Fig. 18.

DISCUSSION

The success of radiotherapy depends mainly upon the radiosensitivity of the individual patient. There are a few useful parameters for evaluation of radiosensitivity. In head and neck squamous cell carcinoma there have been two research works on the predictive value of radiotherapy^{1,2}. One of them was an *in vitro* experiment² and in that study the author said that the S2 value of the cases that had recurrences was slightly higher (0.4) than that in those which appeared to have local tumor control. In the other study¹ the potential doubling time (T_{pot}) of the tumor was studied as the predictive value of radiotherapy using flow cytometry. In this work the author indicated that the cases having a T_{pot} below 4.6 days were fast growing tumors which were resistant to conventional radiotherapy. However, in gynaecological carcinoma there has been no report concerning the predictive value of radiotherapy. In earlier investigations^{12,16} the radiation response was evaluated cytologically and histologically. The cytological estimation was based on the effects of radiotherapy on the tumor cells such as enlargement and vacuolization of the cytoplasm and nuclei, multinucleation of cells, pyknosis, fragmentation and wrinkling of the nuclei, and lysis of the cell sheet. Histological estimation was made according to the Oboshi and Shimosato grading system¹², and revealed the invasion of inflammatory cells and connective tissue surrounding the cancer tissue. Similar effects of radiation in the cytology and in the histology were observed in this research.

The present study which aims at estimating the radiotherapeutic effects on uterine cervical cancer consists of two main works; one is a general pathological study and the second is an immunohistological study in terms of BrdU L.I., tumor antigen of SCC and SLX, and C-myc and ras oncogene product.

In the general study cytology at 10Gy showed the presence of cancer cells in only 39.1% cases whereas histology showed the presence of cancer cells in 91.3% cases. Again, where cytology at 30Gy showed all cases to be free of cancer cells, histology showed the persistence of cancer cells in 26.1% cases. From this evidence it is obvious that at around 10 to 30Gy of radiation the cervical smear does not reflect the exact condition of the cervical lesion. Cancer cells present at a deeper level can not be revealed by swabbing only the surface of the cervix. So histological investigation is more reliable than cytological investigation for evaluation of radiotherapeutic effects.

The exact dose of radiation necessary for creation of an adequate radiation response in the cancer tissue is still unknown. In this study, radiosensitivity started at around 10Gy and reached a maximum around 30Gy of radiation.

In a previous study⁶ it was shown that discrimination is possible between histologically benign and malignant meningiomas by using the value of BrdU L.I. The author said that meningiomas having a L.I. greater than 1% indicate biological malignancy as they grow fast and recur more frequently. In another study¹⁴ it was reported that poorly differentiated adenocarcinoma of the lung showed a higher value of BrdU L.I. In the field of Gynaecology past studies^{3,7} have reported that in cases of cervical cancer that showed a good response to irradiation, the BrdU L.I. decreased during the course of therapy, and that cases that responded poorly to irradiation showed a high value of BrdU L.I. during the course of therapy. In view of previous research work it is obvious that the BrdU L.I. definitely indicates the proliferating activity that is the biological status of the cancer tissue, and that the degree of malignancy is directly proportional to the value of BrdU L.I. Highly malignant tissue shows a high value of BrdU L.I. The decrease in the value of BrdU L.I. during radiotherapy suggests the evidence of radiosensitivity. In this research work, cases evaluated by the Oboshi and Shimosato grading system as radiosensitive showed a gradual decrease of BrdU L.I. toward the end of therapy and those evaluated as radioresistant showed a persistent high value of BrdU L.I. Two squamous cell carcinoma cases (case no.5 and 21 in Table 4-a) were found to have grade 1 till completion of the external irradiation and were diagnosed as radioresistant according to the grading system, and these cases finally became radiosensitive after application of intracavitary radiation therapy. The BrdU L.I. was found to be high during the treatment course of external irradiation in these two cases and finally became zero after application of intracavitary irradiation. One adenocarcinoma case (case no. 2 in Table 4-b) was classified as grade 1 till the end of therapy and diagnosed by the grading system as totally radioresistant. This case showed a high BrdU L.I. all along the treatment course. The cases resistant to external irradiation and the totally resistant case showed a high value of BrdU L.I. during the course of radiotherapy in contrast to the radiosensitive cases. The decrease in the value of BrdU L.I. in the radiosensitive cases during the course of therapy proves that radiotherapeutic effects on cancer cells can be evaluated by the BrdU L.I. By calculating the decreasing rate of BrdU L.I. at 30Gy it has been ascertained that the rate of decrease of BrdU L.I. from 10Gy to 30Gy was more than 60% in the radiosensitive cases and less than 30% in the radioresistant cases. From this discussion it is clear that the radiosensitive and the radioresistant cases can be predicted by observing the value of BrdU L.I. at a 30Gy radiation dose, and thus this

study would define the 30Gy radiation dose as the dose of radiosensitivity.

A reasonable correlation (Table 5) has been discovered between the Oboshi and Shimosato grading system and the BrdU L.I. by analysis. There was no conflict between the two systems from grade 0 to grade 2B but contradiction was found at grade 3. Logically, the grade 3 cells should not show any BrdU L.I. as they are defined as non viable by the grading system. But, in fact, the grade 3 cells (case no. 22 in Table 4-a and case no.4 and 5 in Table 4-b) showed a L.I. of $4.9 \pm 3\%$ in this study, and this finding indicates that grade 3 cells are still in cell cycle activity. This information about grade 3 cells could not be obtained by the Oboshi and Shimosato grading system.

The BrdU L.I. can identify the cancer cells histologically and at the same time can describe the biological behavior of the cancer cells, so it is possible to see the histology as well as the biological status of cancer cells simultaneously in a single investigation. The grading system is unable to disclose the real condition of grade 3 cells. The advantages of BrdU L.I. merit over the Oboshi and Shimosato grading system, so this work suggests that as a parameter of radiotherapeutic effects on cancer cells the BrdU L.I. is more useful than the Oboshi and Shimosato grading system.

The tumor antigen SCC is a specific marker for squamous cell carcinoma of the uterine cervix, and the serum level of SCC presents a correlation with prognosis of the disease reported by several authors^{8,9,11}). Reports of research on SCC and its tissue level are very few. In this work, by immunohistological study the tumor antigen SCC was found positive in 6 of the 23 squamous cell carcinoma cases and the radiation effect was observed. In the tissue, SCC was localized in the cytoplasm of the cancer cells. It was difficult to recognize the radiation effect on SCC by observing the post radiation specimen. In the serum level of SCC a decline to the end of the therapy was found to reflect the gradual destruction of SCC due to radiation therapy. Here it is obvious that in positive case of SCC the serum level is more useful for assessing the effects of radiation.

The tumor antigen SLX is a type 2 chain carbohydrate antigen and is associated with various human adenocarcinomas^{4,5}). A high serum level of SLX was found in ovarian cancer and other human adenocarcinomas^{4,5}). The serum level of SLX was found useful in monitoring the condition of ovarian cancer reported by one author¹⁰). However, no report is available concerning the study of human uterine cervical adenocarcinoma with SLX. To observe the radiation effect in the cervical adenocarcinoma cases SLX antigen was chosen. The antigen was found positive in 6 of 7

adenocarcinoma cases and was localized in the cytoplasm of the cancer cells. The radiation effect in the specimen was found to be indistinguishable at each dose of radiation by immunohistochemical study. A high serum level of SLX was detected in a few cases and SLX was found to be non specific for cervical adenocarcinoma.

The C-myc oncogene is detected on the long arm of chromosome number 8. It is believed to be associated with the events of normal and neoplastic cellular proliferation. Expression of the oncogene product is also found in malignancy of the uterine cervix. Previous research work¹⁵) has suggested that the myc oncogene product and EGF-R have a close relation with the malignant transformation of the squamous epithelium of the uterine cervix. No previous report is available concerning the prognostic value of C-myc product. The C-myc oncogene product was chosen to recognize the effect of radiation, and results suggested that nuclear activity was depressed as a result of radiation. This was reflected by decreased nuclear staining for the C-myc product. On the other hand, increasing of cytoplasmic staining for C-myc product with the increase of radiation dose seemed to be non specific reaction due to the degeneration of cancer cells by radiotherapy. From the above results it is clearly apparent that the immunohistochemical study of C-myc has the potentiality to be used as one of the tools for the estimation of radiotherapeutic effects.

CONCLUSION

Analysis of the results indicates that cervical cytology is not sufficient to detect the radiation effect on the cervix as a whole, and histology (by the Oboshi and Shimosato grading system) gives reliable but comparatively incomplete information about the radiation effects on cervical cancer. The BrdU L.I. is able to confirm the presence of cancer cells histologically and also their proliferating activity after radiation therapy. This study also indicates that a radiation dose of 30Gy is the dose of radiosensitivity because at this dose it is possible to differentiate the radiosensitive and the radioresistant cases by observing the value of BrdU L.I. The study showed that the radiosensitive cases had a decrease rate of BrdU L.I. of more than 60% and that the radioresistant cases had one of less than 30%. Therefore, this study strongly proposes that the BrdU L.I. at around 30Gy radiation is able to be used as a differential diagnostic tool for detection of radiosensitive and radioresistant cases of uterine cervical cancer.

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