Apoptotic Activity in Lymphocytes from Radiation-Treated Cervical Cancer Patients

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Abstract

Purpose : The per cent apoptotic activity in lymphocytes from pre-treated cervical cancer patients was cross-sectionally compared with post-treated patients at 1 month, 3 months, 1 year, and 5 years after completing the standard radiation therapeutic regimen. In addition, the differences in the per cent apoptotic activities among various stages of cervical cancer were simultaneously analyzed.

Material and Method : Blood samples were collected from five patients in each stage of cervical cancer before treatment, and at 1 month, 3 months, 1 year, and 5 years after completing the radiation therapy. The control samples were collected from healthy and aged-match female blood donors. The lymphocytes were separated and exposed to 0.5 Gy Co irradiation to induce apoptosis. The apoptotic cells were detected by *in situ* terminal deoxynucleotidyl transferase (TdT assay) and counted under a fluorescent microscope. Both the apoptotic index and per cent apoptotic activity were calculated.

Results : The per cent apoptotic activities in lymphocytes from all pre-treated patients with stage II and III cervical cancer were significantly lower than the controls ($p = 0.001$). The apoptotic activity in normal control, however, was not significantly different from the pre-treated stage I cervical cancer group. Following radiation therapy, the apoptotic activities at 1 month, 3 month and 1 year were increased in all stages. The per cent apoptotic activity, in all stages of cervical cancer at 5 years after treatment, was statistically higher than that of the pre-treated groups.

Conclusion : There was a decrease of per cent apoptotic activity in lymphocytes from all pre-treated cervical cancer patients in the present study, the change of which was reversed to normal after treatment in non-recurrent cases.

Key word : Apoptosis, Lymphocyte Apoptotic Activity, Cervical Cancer, Radiation Therapy for Cervical Cancer

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Apoptosis, or programmed cell death, is an active model of cell death that is essential to many biologic processes and functions. Apoptosis was shown to occur in various tumors and normal tissues under physiologic conditions as well as in response to ionizing radiation, ultraviolet irradiation, mild hyperthermia, hormone ablation, and certain chemotherapeutic agents[1,2]. In multicellular organisms, homeostasis is normally maintained through a balance between cell proliferation and cell death. Recent evidence, however, suggests that the failure of cells to undergo apoptosis might involve pathogenesis of a variety of human diseases, including cancer, autoimmune diseases, and viral infections. Moreover, a large number of diseases characterized by cell loss, including neurodegenerative disorders, AIDS (Acquired Immune Deficiency Syndrome), and osteoporosis may result from accelerated rates of physiological cell death[3,4]. Carcinoma of the cervix is so far the most common cancer found in women worldwide[5]; and radiation therapy (RT) is one of the cornerstone treatments of the disease. It is accepted that apoptosis is an important process, induced by radiation[2,6]. With low dose irradiation, a lymphocyte can be induced to undergo apoptosis, which can be detected by *in situ* terminal deoxynucleotidyl transferase assay (TdT assay), a rapid and sensitive test for the detection of apoptotic cells[7,8]. Furthermore, the process of apoptosis in many cell types is short-lived, however, the lymphocytes in the cell culture display arrested apoptosis after radiation exposure. The cells remain in the state for many days, and the accumulation of apoptotic cells leads to large signal-to-noise ratios[9]. In this cross-sectional study, the authors investigated radiation-induced apoptosis of lymphocytes from patients with stage I-III cervical cancer at various time intervals, while lymphocytes from age-matched female blood donors were used as controls.

MATERIAL AND METHOD

Patients and control group

Blood samples were collected from cervical cancer patients before the radiation therapy, timed at 1 month, 3 months, 1 year, and 5 years after completing the treatment. Before taking the first blood sample, each patient was fully informed both verbally and in a written document about the research project approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University. Written informed consent was signed by each patient before venepuncture. All the blood samples were collected under informed consent. The cervical cancer patients received radiation therapy at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, from 1999 to 2000. The radiation regimen was composed of external beam therapy, and high-dose rate intracavitary irradiation. External whole pelvis irradiation was performed with anteroposterior and posteroanterior parallel-opposed portals. The doses were 2 Gy per fraction, and the treatment was given five times per week; the total dose was 75 Gy per patient. The ages of the patients ranged from 29 to 72 years (mean = 53.3 ± 10.51 years). The control blood samples were collected from healthy female blood donors at the National Blood Bank, the Thai Red Cross Society, with their ages matched to each group of pre-treated patients. Their ages ranged from 21 to 59 years (mean = 39.8 ± 10.61 years).

Cell culture and irradiation

Twenty milliliters of heparinized whole blood from each donor were layered onto Histopaque-1077, to separate the mononuclear lymphocytes[7]. The lymphocytes were washed twice in Hank's Balanced Salt Solution, and supplemented with 1 per cent fetal bovine serum, at room temperature. The lymphocytes were then cultured at $4x10^5$ cell/ml in RPMI 1640 medium containing 5.2 per cent fetal bovine serum, 0.5 per cent L-Glutamine, and 0.024 per cent Gentamycin. The cell cultures in T25 culture flask (10 ml) were then equilibrated to 37°C for approximately 30 minutes prior to irradiation. The culture flasks were then separated into two groups: the control group (non irradiation) and the irradiation group which was irradiated by 0.5 Gy of Cobalt-gamma ray[7]. Both groups were incubated in CO_2 incubator at 37°C, 97 per cent humidity, and 0.35 per cent CO_2. The lymphocytes were harvested at 24 and 48 hours after irradiation.

Detection of apoptotic cell using *in situ* terminal deoxynucleotidyl transferase labeling (TdT assay)

TdT assay was performed to access DNA fragmentation associated with apoptosis in lymphocytes using commercial detection kit (Apoptag Kit, Intergen. NY). The lymphocytes samples were dropped onto a clean slide, before the slides were allowed to air dry, and then fixed in absolute ethanol overnight

at -20°C. The slides were washed in PBS for 5 minutes at room temperature 3 times and then incubated with Apoptag equilibration buffer for 5 minutes prior to being incubated with terminal deoxynucleotidyl transferase (TdT) enzyme linkage of dUTP-digoxigenin to 3'-OH DNA ending at 37°C for 60 minutes. The reaction was then stopped in a stop/wash buffer at 37°C, for 30 minutes. After rinsing with PBS for 5 minutes, 3 times, the slides were then counter-stained with anti-digoxigenin-fluorescein for 30 minutes at room temperature in a dark room. Then, the slides were washed again with PBS for another 5 minutes, 3 times, and developed with DAPI-antifade solution.

A positive control slide was prepared by incubating the normal lymphocytes with DNase I, to induce DNA fragmentation. A negative control slide was obtained by omitting TdT enzyme from the labeling mix.

DAPI stained nuclei and fluorescein labeled apoptotic nuclei were scored using a fluorescent microscope equipped with a blue filter. Values for the TdT assay were expressed as "% Apoptosis", representing the number of apoptotic cells (%) in each sample, and designated as "Apoptotic index". The "Per cent Apoptotic Activity" was obtained by subtracting the spontaneous apoptotic cell (non irradiation) of the same sample from Apoptotic index(7).

Statistical analysis

Comparison between the per cent apoptotic activity of lymphocytes taken from pre-treated patients at various stages of cervical cancer and the controls, was analyzed by unpaired *t*-test. Comparison of the per cent apoptotic activity of the patients at various time intervals of the treatment was analyzed by one way Analysis of Variance (ANOVA), from the Statistical Packages for the Social (SPSS) program. A significant level of $p = 0.05$ was applied throughout.

RESULTS

1. The TdT assay of apoptotic cells

The DAPI stained lymphocytes were seen as a filled circle in blue under a fluorescent microscope. With a blue filter, only the fluorescein labeled apoptotic cells (light green color) appeared. The apoptotic bodies could be seen within the apoptotic cell.

To obtain reliable results, a quality control check was performed once a month by counting the number of apoptotic cells on the positive control slide. The mean of % OCV (% Optimal Condition Variance) was 5.21 ± 1.47 per cent (ranged 4.27-8.97%).

2. Per cent apoptotic activity at various time intervals within each stage of cervical cancer

The per cent apoptotic activity of stage I cervical cancer was detected in the lymphocytes at 24 hours (Fig. 1A) after the induction by low dose radiation. The results indicated that the per cent apoptotic activity of pre-treated patients was lower than the control group but had no statistical significance ($p = 0.116$). When the per cent apoptotic activity of pre-treated patients was compared with the post-treated patients at various time intervals, the data showed an increase at 1 month and 3 months, then slightly dropped at 1 year (Fig. 1A and 1B). The per cent apoptotic activity was increased after radiation treatment regimen. There was no significant difference between the 5 year post-treated group and the control group ($p = 0.713$). The results of the per cent apoptotic activity in lymphocytes at 48 hours (Fig. 1B), after the induction by low dose radiation, demonstrated a similar pattern as the above 24 hours data. The changes in per cent apoptotic activity of stage II (Fig. 1C) and stage III (Fig. 1D), had similar patterns, as found in stage I. Although the changed pattern of all the 3 stages were quite similar, the per cent apoptotic activity from the 5 year post-treated group of Stage II cervical cancer patients were statistically higher than the pre-treated group ($p = 0.007$, Table 1). Similarly, the 5 year post-treated Stage III group gave a significantly higher percentage of apoptotic activity than the pre-treated group ($p = 0.000$). The pre-treated group demonstrated a significantly lower per cent of apoptotic activity than the normal age-matched female blood donors ($p = 0.000$). Although the result of the 5 year post-treated group showed an increment, it was still significantly lower than the control group ($p = 0.015$, Table 1).

3. Per cent Apoptotic activity at various time intervals, compared between stage I, II and III cervical cancer

The mean of per cent apoptotic activity in lymphocytes within the same time interval of Stage I, II and III cervical cancer patients was compared. The data were analyzed by unpaired *t*-test, the p-value of 0.05 or lower was considered statistically signifi-

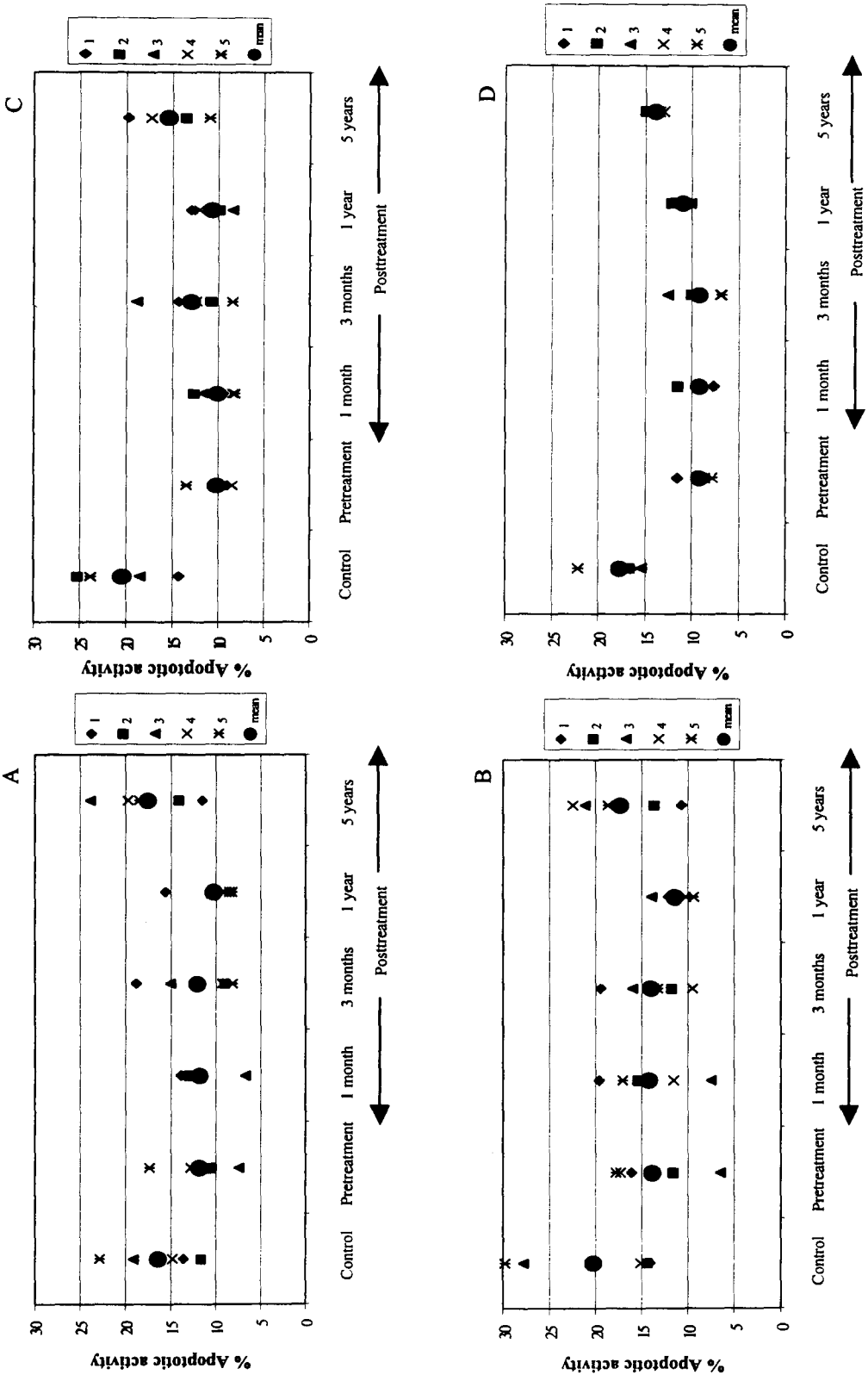

Fig. 1. Comparing percentage of apoptotic activities in lymphocytes taken from the controls, female blood donors, and patients with stage I cervical cancer at various time intervals. The apoptotic cells were detected by TdT assay at 24 hours (A) and 48 hours (B) after low dose irradiation. The percentage of apoptotic activities in lymphocytes taken from patients with Stage II (C), and Stage III (D) cervical cancer were analyzed at 48 hours.

cant. The comparison of the mean per cent apoptotic activity in lymphocytes between Stage I and Stage II of cervical cancer patients, demonstrated no statistical significance in the pre-treatment period, and at intervals of 1 month, 3 months, 1 year and 5 years post-treatment. Also no statistically significant difference was observed when comparing Stage II and Stage III, at similar time intervals (Table 2). There were statistically significant differences when comparing the mean per cent apoptotic activity in lymphocytes with Stage I to Stage III cervical cancer, at pre-treatment period and post-treatment intervals of 1 month and 3 months. The overall results in the present study indicated that the mean per cent apoptotic activity in lymphocytes taken from patients with early stage cervical cancer was higher than those of later stages. Pre-treatment of lymphocyte apoptotic activity was also lower than the activity found after completion of radiation therapy.

The per cent apoptotic activity of lymphocytes in the present study was standardized by exposure to 0.5 Gy Co-60 irradiation[7], and incubated for 48 hours before the TdT assay. The per cent apoptotic activity of each sample was the net result of the subtraction of the non Co irradiated per cent apoptotic index from the total per cent apoptotic index of its Co irradiated lymphocytes. The non Co irradiated per cent apoptotic index was the spontaneous or background lymphocyte apoptotic activity of an individual person. Comparison of the per cent apoptotic index in lymphocytes from various time intervals of all 3 stages was analyzed. The per cent apoptotic index of lymphocytes taken from Stage I, cervical cancer patients was lower than the age-matched controls, and it increased at 1 month, 3 months, 1 year and 5 years after completing the radiation therapy, but had no statistical significance (Fig. 2). The changes of the per cent apoptotic index of lymphocytes, from Stage II and Stage III cervical cancer patients exhibited the same pattern as in Stage I.

DISCUSSION

Lymphocyte is a nuclear hematocyte that can be induced by a low dose of radiation to undergo apoptosis. In the present study, lymphocytes were induced by 0.5 Gy of Co radiation that could cause

Table 1. The mean of per cent apoptotic activities of lymphocytes from cervical cancer patients of Stage I, Stage II and Stage III are presented. The apoptotic cells were detected by TdT assay at 48 hours after 0.5 Co irradiation and subtracted from individual non-irradiated control. The data is expressed as X ± SD.

Stage	Control	Pretreatment	Post-treatment			
			1 month	3 months	1 year	5 years
I	20.23 ± 7.86	13.86 ± 4.79	14.22 ± 4.76	14.02 ± 3.84	11.45 ± 1.74	17.31 ± 4.99
II	20.42 ± 4.37	10.17 ± 1.93[a]	10.09 ± 2.00	12.95 ± 3.96	10.66 ± 1.82	15.44 ± 3.40[b]
III	17.68 ± 2.63	9.20 ± 1.38[c]	9.16 ± 1.48	9.15 ± 2.42	10.89 ± 0.78	13.89 ± 0.78[d, e]

a The pretreatment group Stage II was significantly lower than the matched control (p = 0.001).
b The pretreatment group Stage II was significantly lower than the 5 year post-treatment group (p = 0.007).
c The pretreatment group Stage III was significantly lower than the matched control (p = 0.000).
d The 5 year post-treatment group Stage III was significantly lower than the matched control (p = 0.015).
e The 5 year post-treatment group Stage III was significantly lower than the pretreatment group (p = 0.000).

Table 2. Comparing the means of per cent apoptotic activities among Stage I, Stage II and Stage III cervical cancer patients analyzed by unpaired *t*-test. P-value with significance at 0.05 is marked with an asterisk (*).

Comparison	Pre-treatment	Post-treatment			
		1 month	3 months	1 year	5 years
Stage I *versus* Stage II	0.083	0.057	0.635	0.428	0.417
Stage I *versus* Stage III	0.034*	0.024*	0.047*	0.572	0.150
Stage II *versus* Stage III	0.628	0.644	0.109	0.815	0.499

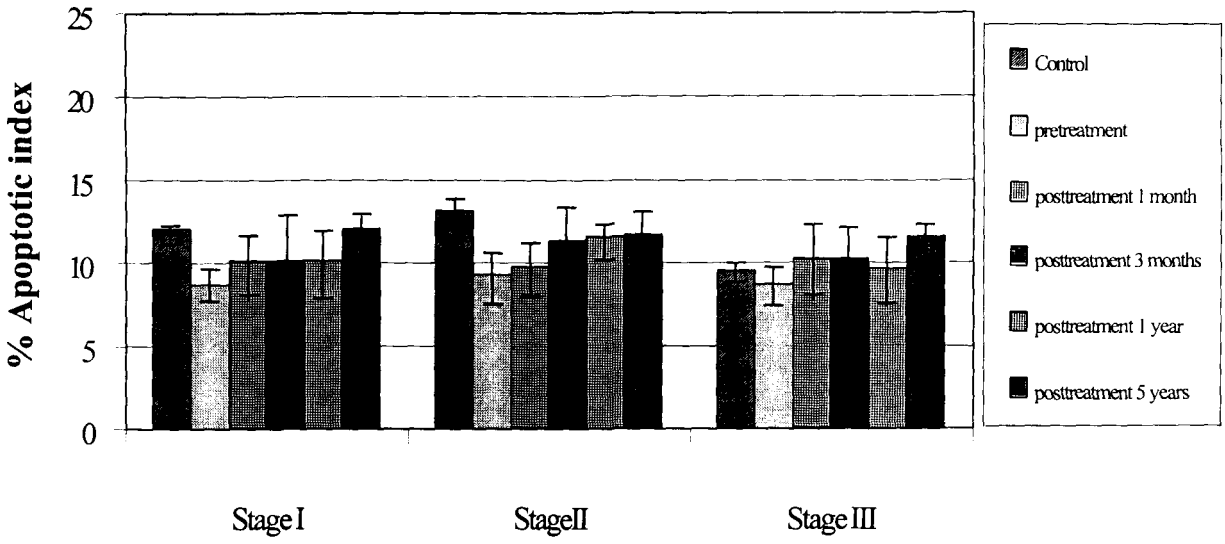

Fig. 2. Percentage of apoptosis indexes in lymphocytes among cervical cancer Stage I, II and III at various time intervals are presented as mean ± SD. TdT assay was analyzed at 48 hours incubation without 0.5 Co irradiation which represents the spontaneous apoptosis or background controls.

damage to their DNA, after the exposure to the ionizing radiation. The damage resulted in fragmentation of DNA within the cells undergoing apoptosis[7-11]. There are, however, many methods used for the detection of cell apoptosis. Some investigators prefer to use morphologic criteria[12,13], while others employ methods to detect changes in the integrity of DNA through pulsed-field gel electrophoresis[13-15] or TdT assay[7,13,14,16]. There is strong evidence that DNA fragmentation can be detected by the labeling of apoptotic nuclei using the TdT assay which is a rapid, sensitive, and accurate method[7,13,14,17]. Many studies have shown that apoptosis in lymphocytes requires many hours to develop. As for the incubation time needed for apoptosis to be best detected, studies suggest that 48 hours post-irradiation induction gives a high per cent of apoptotic cells, and a significance of apoptotic yield[7,9,18]. These results agree with the present study. The means of per cent apoptotic activity at 48 hours post low dose irradiation in all stages and at every time interval were greater than the means at 24 hours. Besides, the means of the 3 control groups at 48 hours were also greater than that at 24 hours.

Concerning the sensitivity of different cell types to low dose radiation induction which might affect the authors' model of study. There were studies which demonstrated that different cell types i.e., lymphocytes, skin cell[11], and fibroblast[19] taken from the same individual possess the same level of radiosensitivity. Boreham et al[7] postulated that the apoptotic response to low dose radiation of peripheral lymphocytes might be used as a biological dosimeter in each individual. Apoptosis in most cell types was short-lived, however, the lymphocytes in cell culture displayed an arrested apoptosis after radiation exposure[20]. The cells remained in this state for many days and therefore the apoptotic cells accumulated. However, it has been reported that approximately 0.3 ± 0.2 per cent of the lymphocytes exhibited apoptotic change immediately after the isolation process[7]. Another report[21] demonstrated that 8 per cent of lymphocytes in the cell culture would undergo spontaneous apoptosis. In order to obtain actual apoptotic changes in the present study, the authors double duplicated the lymphocyte cultures of each sample. One of the duplicates was irradiated with low dose irradiation, the other duplicate, without irradiation, was analyzed and counted for spontaneous apoptotic changes or the background. All the results presented in the present study are the actual apoptotic activities, since the background or spontaneous apoptotic changes were subtracted. Besides, the aging process might also decrease apoptotic activity in

normal individuals. Since there was no normal data of apoptotic activity in Thai women to be used as a reference, the authors tried to match the age of the blood donor controls as close as possible to that of the cervical cancer patients of each stage. In the present study, the mean of per cent apoptotic activity in 15 healthy Thai women was 14.93 per cent (range 9.8% - 22.85%) and 19.44 per cent (range 14.4% - 29.80%) detected respectively at 24 hours and 48 hours after incubation.

The results from the present study, a comparison of the per cent apoptotic activity in lymphocytes taken from cervical cancer stage I, II, and III, at various time intervals, demonstrated that there was change in the apoptotic activity. The per cent apoptotic activity in the pre-treatment group of all 3 stages was lower than the controls with or without statistical significance. The phenomenon may be explained *via* a proposal that apoptosis may be considered as a carcinogenesis opposition, since, dead cells cannot turn into tumor cells. If by any reason, this defensive mechanism was disrupted, then, the body would be prone to carcinogenesis. However, there might be more than one defense mechanism and so the mechanism of carcinogenesis. This hypothesis may not be applicable to all. In the case of later stage carcinoma, where there is a spread of tumor cells or metastasis, the apoptotic change can be explained *via* other rationale. Zaghloul et al[22] proposed that, in the case of metastasis, tumor angiogenesis may contribute to a reduction of apoptosis activity. The ability of tumor cells to undergo apoptosis can either be enhanced or inhibited by neovascularization. The results from the present study, however, show that the per cent apoptotic activity in the pre-treatment groups of cervical cancer Stage II, III were significantly lower than the controls. As such, metastasis in cervical cancer possibly inhibits apoptosis[23]. The process might contribute to another proposal that, the apoptotic activity might be applied as a parameter to detect recurrent cervical cancer in long-term follow-up. Moreover, the present results showed that in spontaneous apoptosis in lymphocytes, spontaneous apoptosis in pre-treated groups was lower than the control groups without statistical significance in all stages. After radiation therapy, spontaneous apoptosis in the patients was increased. Wheeler et al[24] reported that cervical cancer patients with a high level of apoptosis had a better prognosis. Therefore, high spontaneous apoptosis might be a useful predictor of response to radiation therapy in cervical cancer.

The per cent of apoptotic activity in the post-treatment groups of all 3 stages showed an increase with longer intervals after the completion of radiation therapy. Nevertheless, at one year interval, the per cent of apoptotic activity in lymphocytes of all 3 stages of cervical cancer decreased again. Most of the patients did not exhibit any recurrent symptoms. Their common complaints included, constipation, and slight edema of the lower limbs. Literature reviews did not yield any satisfactory answers to this phenomenon. It might be possible that, after exposure to high dose radiation during the treatment period, most of the radiation sensitive tissues, including the tumor, were destroyed. The more peripheral cells and tissues that were sensitized by the lower dose radiation still survived. These cells, including the pro-lymphocytes, developed their radiation resistant property.

The results of per cent apoptotic activity in the 5 year post-treatment groups indicated an increase of the per cent apoptotic activity towards the level found in the control groups. Several studies have shown that high levels of spontaneous apoptosis were associated with improved survival of cervical cancer patients[6,24,25]. However, there was variability between persons for radiation induced apoptosis in lymphocytes. Individual apoptotic activity could change over a longer period of time, or be modified by certain factors, such as health status, environmental stress, and patients' life-styles[7]. In addition, hyperthermia treatments, a therapeutic raising of the body temperature equivalent to a fever, could also modify the response of lymphocytes to radiation-induced apoptosis[26]. It seemed that apoptosis played an important role in response to radiotherapy. It might also be a marker for the biological aggressiveness of tumors. Further research efforts are, therefore, needed to explain the loss of apoptotic response that invariably occurs during the evolution of a solid tumor, in the hope of providing more effective cancer therapy. In this regard, apoptosis in lymphocytes needs to be evaluated as a potential predictive assay for measuring tissue radiosensitivity, that could improve tumor cure rates by enabling radiation doses to be tailored to the individual.

SUMMARY

In summary, the present study showed that the per cent apoptotic activity in lymphocytes taken from pre-treated cervical cancer patients was lower than the control groups. After radiation therapy, the per cent apoptotic activity in all 3 stages of cervical cancer increased towards the control groups.

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REFERENCES

1. Kerr JFR, Wyllie AH, Currie AR. Apoptosis : A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-57.
2. Kerr JFR, Winterford CM, Harmon BV. Apoptosis its significance in cancer and cancer therapy. Cancer 1994; 73: 2013-26.
3. Duke RC, Ojcius DM, Young JDE. Cell suicide in health and disease. Sci Am 1996; 275: 80-7.
4. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995; 267: 1456-62.
5. Ponten J, Adami HO, Bergstrom R, et al. Strategies for global control of cervical cancer. Int J Cancer 1995; 60: 1-26.
6. Levine EL, Renehan A, Gossiel R, et al. Apoptosis, intrinsic radiosensitivity and prediction of radiotherapy response in cervical carcinoma. Radiother Oncol 1995; 37: 1-9.
7. Boreham DR, Gale KL, Maves SR, et al. Radiation-induced apoptosis in human lymphocytes : Potential as a biological dosimeter. Health Phys 1996; 71: 685-91.
8. Brinboim HC, Jevcak JJ. Fluorometric method for rapid detection of DNA strand breaks in human white blood cell produced by low doses of radiation. Cancer Res 1981; 41: 1889-92.
9. Crompton NEA, Miralbell R, Rutz HP, et al. Altered apoptotic profiles in irradiated patients with increased toxicity. Int J Radiat Oncol Biol Phys 1999; 45: 707-14.
10. Rupnow BA, Mortha AD, Alarcon RM, et al. Direct evidence that apoptosis enhances tumor response to fractioned radiotherapy. Cancer Res 1998; 58: 1779-84.
11. Nunez MI, Guerrero MR, Lopez E, et al. DNA damage and prediction of radiation response in lymphocytes and epidermal skin human cells. Int J Cancer 1998; 76: 354-61.
12. Milas L, Hunter NR, Mason KA, et al. Enhancement of tumor radioresponse of a murine mammary carcinoma by paclitaxel. Cancer Res 1994; 54: 3506-10.
13. Fuks Z, Persaud RS, Alfieri A, et al. Basic fibroblast growth factor protects endothelial cells against radiation-induced programmed cell death *in vitro* and *in vivo*. Cancer Res 1994; 54: 2582-90.
14. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death *in situ via* specific labeling of nuclear DNA fragmentation. J Cell Biol 1992; 119: 493-501.
15. Warters RL. Radiation-induced apoptosis in a murine T-cell hybridoma. Cancer Res 1992; 52: 883-90.
16. Eiseman JL, Rogers FA, Guo Y, et al. Tumor-targeted apoptosis by a novel spermine analogue, 1, 12-diaziridinyl-4, 9-diazadodecane, results in therapeutic efficacy and enhanced radiosensitivity of human prostate cancer. Cancer Res 1998; 58: 4864-70.
17. Kikuchai H, Ujiie S, Kanamaru R. The TdT-mediated dUTP nick end labeling assay precisely assesses the DNA damage in human tumor xenografts. Jpn J Cancer Res 1998; 89: 862-9.
18. Siles E, Villalobos M, Jones L, et al. Apoptosis after gamma irradiation. Is it an important cell death modality ?. Br J Cancer 1998; 78: 1594-9.
19. Burnet NG, Nyman J, Turesson I, et al. Prediction of normal-tissue tolerance to radiotherapy from *in vitro* cellular radiation sensitivity. *Lancet* 1992; 339: 1570-1.
20. Mitchel REJ, Birnhoim HC. Triggering of DNA strand break by 45°C hyperthermia and its influence on the repair of γ-radiation damage in human white blood cells. Cancer Res 1985; 45: 2040-5.
21. Dullea RG, Robinson JF, Bedford JS. Nonrandom degradation of DNA in human Leukemic cells during radiation induced apoptosis. Cancer Res

1999; 59: 3712-8.
22. Zaghloul MS, Naggar ME, Deeb AE, et al. Prognostic implication of apoptosis and angiogenesis in cervical uteri cancer. Int J Radiat Oncol Biol Phys 2000; 48: 1409-15.
23. Lyng H, Sundfor K, Trope C, et al. Disease control of uterine cervical cancer : Relationships to tumor oxygen tension, vascular density, cell density, and frequency of mitosis and apoptosis measured before treatment and during radiotherapy. Clin Cancer Res 2000; 6: 1104-12.
24. Wheeler JA, Stephens LC, Tornos C, et al. Astro research fellowship : Apoptosis as a predictor of tumor response to radiation in stage IB cervical carcinoma. Int J Radiat Oncol Biol Phys 1995; 32: 1487-93.
25. Levine El, Davidson SE, Robert SA, et al. Apoptosis as predictor of response to radiotherapy in cervical cacinoma. The Lancet 1994; 344: 472.
26. Boreham DR, Maves SR, Miller S, et al. Heat induces thermal tolerance and radiation resistance to apoptosis in human lymphocytes. 44th Annual Meeting of Radiation Research Society. Chicago 1996.

อะพอพโทสิส แอกติวิตีในเซลล์เม็ดเลือดขาวจากผู้ป่วยมะเร็งปากมดลูกที่ได้รับรังสีรักษา

ธาดา สืบหลินวงศ์, พบ, พิสุทธิ์ ปามุทา, วทม**

วัตถุประสงค์ : ศึกษาแบบตัดขวางของเปอร์เซนต์อะพอพโทสิสในเม็ดเลือดขาวจากผู้ป่วยมะเร็งปากมดลูกก่อนได้รับรังสีรักษา เปรียบเทียบกับเม็ดเลือดขาวจากผู้ป่วยมะเร็งปากมดลูกหลังได้รับการฉายรังสีครบแล้วที่ระยะเวลา 1 เดือน 3 เดือน 1 ปี และ 5 ปี พร้อมทั้งเปรียบเทียบเปอร์เซนต์อะพอพโทสิสในเม็ดเลือดขาวจากผู้ป่วยมะเร็งปากมดลูกที่ระยะต่าง ๆ

วัสดุและวิธีการ : เก็บตัวอย่างเลือดครบจากผู้ป่วยมะเร็งปากมดลูกระยะละ 5 ราย โดยเจาะก่อนได้รับการฉายรังสีและภายหลังฉายรังสีครบแล้ว 1 เดือน 3 เดือน 1 ปี และ 5 ปี ตัวอย่างเลือดกลุ่มควบคุมกลุ่มละ 5 รายเช่นกัน ได้จากผู้บริจาคโลหิตสตรี ซึ่งมีอายุใกล้เคียงกับกลุ่มผู้ป่วยมะเร็งปากมดลูกแต่ละระยะ แยกเม็ดเลือดขาวลิมโฟซัยท์ออก แล้วนำไปฉายรังสีโคบอลท์ (รังสีแกมมา) ขนาด 0.5 Gy เพื่อชักนำให้เกิดการตายแบบอะพอพโทสิส ตรวจสอบเซลล์ที่เกิดอะพอพโทสิสโดยใช้เทคนิค in situ terminal deoxynucleotidyl transferase (TdT assay) และนับจำนวนเซลล์ที่เกิดอะพอพโทสิสภายใต้กล้องฟลูออเรสเซนท์ คำนวนค่า apoptotic index และ per cent apoptotic acticity

ผลการวิจัย : เปอร์เซนต์อะพอพโทสิสของลิมโฟซัยท์จากผู้ป่วยมะเร็งปากมดลูกระยะที่ 2 และระยะที่ 3 ก่อนได้รับการฉายรังสี มีค่าต่ำกว่ากลุ่มควบคุมที่มีอายุใกล้เคียงกันอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) แต่อะพอพโทสิสแอกติวิตีของลิมโฟซัยท์ ในกลุ่มควบคุมไม่ต่างจากในผู้ป่วยมะเร็งปากมดลูกระยะที่ 1 ที่ยังไม่ได้รับการรักษา อะพอพโทสิสแอกติวิตีเพิ่มขึ้นในมะเร็งปากมดลูกทุกระยะภายหลังเสร็จสิ้นการรักษาแล้ว 1 เดือน 3 เดือน และ 1 ปี เปอร์เซนต์อะพอพโทสิสในผู้ป่วยมะเร็งปากมดลูกทุกระยะภายหลังเสร็จสิ้นการรักษา 5 ปี จะเพิ่มขึ้นสูงกว่าก่อนการรักษา

สรุป : พบเปอร์เซ็นต์อะพอพโทสิสของลิมโฟซัยท์ลดลง ในผู้ป่วยมะเร็งปากมดลูกทุกระยะก่อนการรักษา การเปลี่ยนแปลงนี้กลับสู่ปกติได้ในผู้ป่วยที่ไม่กลับเป็นซ้ำหลังจากการรักษา

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