Postirradiation Changes of White Blood Cells and Lymphocyte Subpopulations in Cancer Patients

Sung Ja Ahn, M.D., Woong Ki Chung, M.D., Taek Keun Nam, M.D.
Byung Sik Nah, M.D. and Young Hee Noh, M.S.

Department of Therapeutic Radiology, Chonnam University Medical School, Kwangju, Korea

= Abstract =

Purpose : Radiation-induced alteration in the immune function is well known phenomenon in cancer patients. Our purpose is to evaluate the extent of immune suppression immediately after mediastinal or pelvic irradiation, which include significant volume of active bone marrow in adults.

Materials and Methods : 48 cancer patients with mediastinal(N=29) and pelvic irradiation(N=19) were the basis of this analysis. Age ranged from 36 to 76 and mean and median value was 57 years, respectively. Sex ratio was 1.3(M:F=27/21).

The immunological parameters were the complete blood cell(CBC) with differential cell(D/C) count, T cell subset(CD3, CD4, CD8, CD19), NK cell test(CD16, CD56), and serum immunoglobulin(IgG, IgA, IgM) level.

Results : The mean value of white blood cell(WBC) was reduced from 7017 to 4470 after irradiation(p= 0.0000). In the differential count, the number of lymphocyte, neutrophil, and basophil was markedly reduced with statistical significance($p<0.01$) and the number of monocyte was not changed and, on the contrary, that of eosinophil was increased by irradiation.

In the lymphocyte subpopulation analysis, the number of all subpopulations, CD3(T cell), CD4(helper T cell), CD8(suppressor T cell), CD16(NK cell), CD19(B cell) was reduced with statistical significance. The mean ratio of CD4 to CD8 in all patients was 1.09 initially and reduced to 0.99 after radiotherapy(p=0.34), but the proportional percentage of all subpopulations was not changed except CD19(B cell) after irradiation.

In the immunoglobulin study, initial values of Ig G, Ig A, and Ig M were relatively above the normal range and the only Ig M was statistically significantly reduced after radiotherapy(p=0.02).

Conclusion : Mediastinal and pelvic irradiation resulted in remarkable suppression of lymphocyte count in contrast to the relatively good preservation of other components of white blood cells. But the further study on the functional changes of lymphocyte after radiotherapy may be necessary to conclude the effects of the radiation on the immunity of the cancer patients.

Key Words: Immunity, T-lymphocyte subpopulation, Radiotherapy

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INTRODUCTION

After Roentgen discovered the x-ray in 1895, radiation therapy was introduced and has been used as one of the important treatment modality of cancer until now. The lethal effect of irradiation is not selective to the tumor cell only, but produces normal tissue damage. Especially radiation-induced lymphocyte-immune deficiency was known in many reports[1-6] and clinical result secondary to immune deficiency was investigated[7-9] because immune system has been known to play a important role as cancer surveillance and cancer prevention[10]. Although the immune mechanism is inadequate to eradicate an established tumor, it is possible that the degree of competence of a patient's immune system may be an important factor in the clinical course of the disease. For this reason, it is meaningful to reveal the immunosuppressive effects of cancer therapy and the correlation, if any, of such immunosuppression with prognosis. But it is not yet clear whether the lymphocytopenic effect of radiotherapy influences adversely the prognosis of irradiated patients.

Human immune system has been known to have direct or indirect cancer cell killing effect. In recent studies, it is also known that the cell mediated immunity, which is usually mediated by T lymphocyte, has the central role in tumor immunology[11]. The immunologic status of a patient may be easily assessed by the analysis of peripheral blood lymphocytes T subsets[10] and this technique becomes very fine and very reliable in quantity as well as in quality now.

The aim of this study was to evaluate the radiation-induced changes of immunological function of cancer patients who were in the state of good general condition and were not treated by chemotherapy.

MATERIALS AND METHODS

From January 1995 to April 1995 48 patients were registered in this study. We selected the patients completely treated to mediastinal or pelvic field and limited the registeration to the patients with good general condition(ECOG, 0 or 1) and without previous chemotherapy. Table 1 shows the patients' characteristics. Age ranged from 36 to 73 and mean and median age was 57 years, respectively. Sex ratio was 1.3(M:F=27/21).

The immunological status was checked using the test of complete blood cell(CBC) with differential cell(D/C) count, T cell subsets(CD3, CD4, CD8, CD19), NK cell test(CD16, CD56), and serum immunoglobulin(IgG, IgA, IgM). [2)]

Lymphocyte subset analysis was performed using two-color direct immunofluorescence flow cytometric analysis and Simultest™ kit. The heparinized venous blood 100 μL was mixed with the specific subtype monoclonal antibody kit* 20 μL for about 3 seconds and stained at a room temperature for 30 minutes and the red blood cells were lysed with FACS^R Lysing solution 2 mL and then centrifuged at 300 × g for 5 minutes. The harvested cells were mixed with buffered saline 2 mL for 3 seconds and centrifuged 200 x g for 5 minutes. The harvested cells were mixed

Table 1. Patients Characteristics(N=48)

Characteristics	No. of Pts(%)
Age(yrs)	
range	36 - 73
mean	57
median	57
Sex	
Male	27 (56%)
Female	21 (44%)
Operation	
Yes	19 (40%)
No	29 (60%)
Radiation Port	
Mediastinal Field	29 (60%)
Lung ca.	15
Breast ca.	3
Esophageal ca.	11
Pelvic Field	19 (40%)
Cervix	13
Rectal	6

* Simultest T and B cell test, Simultest T helper/ suppressor cell test, Simultest CD_3, Simultest CD_{16+56}, Simultest control

** Becton-Dickinson Co.(USA)

with 1% paraformaldehyde 0.5 mL and analyzed using flow cytometry**.

The venous blood was sampled for the tests within 1 week before radiotherapy and immediately after 4500-5000cGy of irradiation.

Radiotherapy was done with 6 or 10MV linear accelerator. The treatment port was individually designed conventional field using now generally for each disease. The effective fields was larger than $150cm^2$. All patients were treated on the schedule of daily 180 cGy with five fractions per week.

For statistical analysis, BMDP 3D(t-test) program was used to compare the mean values of each parameters between two groups[12].

RESULTS

Table 2 shows the arithmetric mean values of white blood cell(WBC) and differential cell(D/C) count. The mean value of WBC was reduced from 7017 to 4470 after irradiation(p=0.0000). In the differential count, the numbers of lymphocyte, neutrophil, and basophil were markedly reduced from 2046, 3922, 137 to 537, 3054, 27 with statistical significance($p<0.01$), respectively. The number of monocyte was not changed relatively and, on the contrary, that of eosinophil was increased from 276 to 320 after irradiation although there was no statistical significance.

Table 3 shows the cell number of lymphocyte subpopulations from the peripheral blood of patients before and immediately after radiotherapy. The number of all subpopulations, CD3, CD4, CD8, CD16, CD19 was reduced with statistical significance. The mean ratio of CD4 to CD8 in all patients was 1.09 initially and reduced to 0.99 after radiotherapy(p=0.34), but the proportional percentage of all subpopulations was not changed except CD19(B cell) after irradiation(Table 4).

We analyzed lymphocyte subset study according to the treatment field between mediastinal and pelvic irradiation. Mediastinal irradiation decreased the WBC and lymphocyte more remarkably than pelvic one and there was reverse finding in the eosinophil change(Fig. 1-3). The postirradiation change of lymphocyte subpopulation was not different between two areas(Table 5).

In the immunoglobulin study, initial values of Ig

Table 2. Comparison of Results(mean±S.E.M. values) : White Blood Cell and Differential Counts(No)

Parameters	Pre-RT	Post-RT	Difference	P value
WBC	7017±372	4470±244	2546±319	0.0000
Lymphocyte	2046± 91	537± 35	1509± 96	0.0000
Neutrophill	3922±324	3054±200	868±319	0.0096
Monocyte	585± 37	536± 39	49± 40	0.23
Eosinophil	276± 42	320± 47	-44± 57	0.4420
Basophil	137± 35	27± 9	110± 37	0.0046

Table 3. Comparison of Results(mean±S.E.M. values) : Cell Number of Lymphocyte Subpopulation(per ml)

Parameters	Pre-RT	Post-RT	Difference	P value
CD3	1225±69	327±30	898±70	0.0000
CD4	413±33	98± 8	314±31	0.0000
CD8	408±28	133±23	275±31	0.0000
CD19	241±26	25± 3	216±26	0.0000
CD16	451±47	123±17	328±45	0.0000

Table 4. Comparison of Results(mean±S.E.M. values) : Percentage of Lymphocyte Subpopulations

Parameters	Pre-RT	Post-RT	Difference	P value
CD3	62±2	63±2	-0.29±2	0.86
CD4	33±1	32±2	1±2	0.50
CD8	33±1	36±2	-4±2	0.07
CD4/CD8	1.09±0.08	0.99±0.99	0.10±0.10	0.34
CD19	5±1	5±1	7±1	0.0000
CD16	24±2	24±2	-2±2	0.37

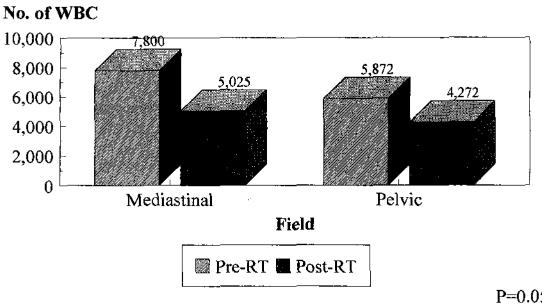
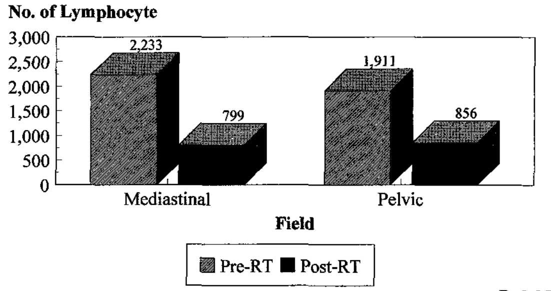
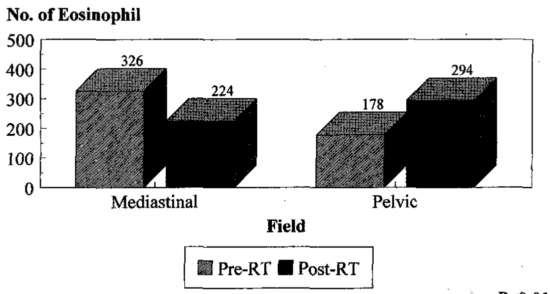


Fig. 1. Comparison of postirradiation change of White Blood Cell counts between mdiastinal and pelvic field.



P<0.05

Fig. 2. Comparison of postirradiation change of Lymphocyte counts between mediastinal and pelvic field.



P<0.01

Fig. 3. Comparison of postirradiation change of Eosinophil counts between mediastinal and pelvic field.

Table 5. Comparison of Postirradiation Changes (mean±S.E.M. values) according to the Radiation Fields : Cell Number of Lymphocyte Subpopulations(per ml)

Parameters	Mediastinum	Pelvis	P value
CD3	959±110	875±82	N.S.
CD4	337± 48	312±37	N.S.
CD8	278± 48	284±38	N.S.
CD19	249± 42	184±19	N.S.
CD16	371± 62	240±55	N.S.

Table 6. Comparison of Results(mean±S.E.M. values) : Serum Immunoglobulin(ng/ml)

Parameters	Pre-RT	Post-RT	Difference	P value
Ig G	2074±105	1986±103	88±95	0.36
Ig A	341± 25	309± 25	32±18	0.08
Ig M	228± 18	200± 15	29±12	0.02

G, Ig A, and Ig M were relatively above the normal range and the only Ig M was statistically significantly reduced(Table 6).

DISCUSSION

The radiation induced immune suppression is well-known phenomenon. The suppressive effect was reported to be dependent on the volume and therapeutic dose to active bone marrow, the number of lymph nodes in the radiation field, the volume of blood irradiated, and the irradiation of the thymic area[3, 4, 13].

The cells involved in immune defense are lymphocytes, plasma cells and macrophages and the most important cell is the lymphocyte[11]. Normally the lymphocytes comprise 20% of the blood leukocytes[11]. In this study, the proportion was ranged from 11.1% to 54.6% and the median value was 30.7%.

A survey of the present knowledge of bone marrow distribution was undertaken by Atkinson[14]. Using this data, we can estimate that the percentage of irradiated marrow to the total active marrow in the bones of adults aged above 40 is about 10% in head and neck, 20-25% in chest, and 30-35% in pelvic field, respectively. Comparing mediastinal irradiation with pelvic one, which is the object of this analysis, we consider that there is no significant difference in involved active bone marrow volume. But in the mediastinal field, the great vessels such as the aorta and the thymus were included. We think that this point is the only difference between two groups.

Localized large volume irradiation such as mediastinal and pelvic irradiation produced neutropenia, lymphopenia and eosinophilia and a marked consistent monocytosis and showed the pattern of remarkable suppression of lymphocyte in contrast to the relatively good preservation of other components of white blood cells[15]. In our study, we observed similar change that lymphocyte count was diminished to about 25% of the initial number, and monocyte count was not changed, and the eosinophil count was increased conversely. But because we did not perform the functional assay of the lymphocyte such as the proliferative responses to phytohemagglutinin or the allogenic mixed lymphocyte responses, it is hard to say the conclusion that the mediastinal and pelvic irradiation impairs the immunity of the patients.

The immunological status of a patient may easily be assessed by studying the results of the analyses of peripheral blood lymphocytes T subsets[10]. T cells have been physiologically characterized as "killer cells" when involved in cell mediated immunity, "helper cells" when involved in B cell instruction for the production of antibody, and "suppressor cells" when restraining reaction to a previous specific immunologic events. Previous studies have suggested that peripheral human T cells can be divided into two mutually exclusive functional subsets with the availability of specific monoclonal antibodies that define the inducer/helper(CD4) and cytotoxic/suppressor cell(CD8) in the peripheral blood of patients[10].

Irradiation of cancer patients has been shown to affect the proportion of lymphocyte subpopulations, and their in vitro reactivity[1-5]. Now it seems that the decrease of the helper/suppressor ratio is a general effect of radiation therapy and can be observed also under local radiotherapy[4]. In this analysis, all components of lymphocyte subsets were decreased significantly after irradiation, but the proportional percentage were not changed significantly except CD19(B cells). The CD4/CD8 ratio was decreased from 1.09 to 0.99(p=0.34).

If the influence of a particular treatment regimen on NK activity is to be studied, it is important to test age-matched, well-defined and otherwise healthy patient groups because variations in NK activity as a result of different factors such as age, pregnancy, viral and bacterial infections and day-to day variations in the test system have been shown[16]. The bias due to this restricted reproducibility of the test can be partly overcome by assaying the control and the experimental sample system simultaneously under the same conditions[16]. In the present study, these precautions are not taken, so we can not say that the postirradiation decrease of the CD16 cell number means the decreased activity of NK cell.

The reported result of the relative radiosensitivity of B cell and T cell is controversial. Some[2, 17] reported that B cell is more radiosensitive than T cell and some[1] reported the opposite result. The B lymphocyte(CD19) was diminished to a greater extent than the T lymphocyte(CD3) in this analysis. Since lymphopenia following radiation therapy is believed to result from a direct radiation induced killing of lymphocytes our results suggest that B lymphocytes are more radiosensitive than T cells.

We analyzed whether there was any difference in the pattern of hematologic changes, when compared mediastinal and pelvic irradiation. White blood cell and lymphocyte was more decreased in the group of mediastinal field than pelvic one, but the eosinophil was increased after pelvic irradiation and decreased after mediastinal irradiation. There was no statistically significant difference between two group in the lymphocyte subpopulation changes. Through this results, we can assume that mediastinal irradiation more impairs the cell-mediated immunity than the pelvic field.

Stjernsward, reviewing the results of different clinical trials on breast cancer patients, found an increased incidence of distant metastasis and a higher mortality in patients treated by surgery plus radiotherapy compared to surgery alone[7]. This review has been disputed by others[8], both on a statistical basis and because the in vitro tests used were found to be unreliable as indicators for the general immunocompetence of the individuals. Recently the combination of immunomodulating agents with radiation therapy has been tried on the theoretical basis of buffering action to the radiation-induced immune deficiency[18].

Although the clinical significance mainly to the tumor control or survival has been controversial until now, it seems optimal to combine radiotherapy with immunotherapy concurrently to reduce the immunological compromise induced by irradiation.

REFERENCES

1. **Raben M, Walach N, Galili U, Schlesinger.** The effect of radiation therapy on lymphocyte

subpopulations in cancer patients. Cancer 1976; 37:1417-1421

2. **Blomgren H, Berg R, Wasserman J, Glas U.** Effect of radiotherapy on blood lymphocyte population in mammary carcinoma. Int J. Radiat Oncol Biol Phys 1976; 1:177-188
3. **Order SE.** The effects of therapeutic irradiation on lymphocytes and immunity. Cancer 1977; 39:737-743
4. **Job G, Pfreundschuh M, Bauer M, Winkel K, Hunstein W.** The influence of radiation therapy on T-lymphocyte subpopulations defined by monoclonal antibodies. Int J Radiat Oncol Biol Phys 1984; 10:2077-2081
5. **Choi GY, Choi WS.** Analysis of T lymphocyte subpopulation change defined by monoclonal antibodies immediately after radiotherapy. J Korean Soc Ther Radiol 1992; 10:129-135
6. **Schulof RS, Chorba TL, Cleary PA, Palazynski SR, Alabaster O, Goldstein AV.** T-cell abnormalities after mediasrinal irradiation for lung cancer: The in vitro influence of synthetic Thymosin alpha-1. Cancer 1985; 55:974-983
7. **Stjernsward J.** Decreased survival related to irradiation postoperatively in early operable breast cancer. Lancet 1974; 2:1285-1286
8. **Levitt SH, McHugh RB, Song CW.** Radiotherapy in the postoperative treatment of operable cancer of the breast. Cancer 1976; 39:933-940
9. **Meyer KK.** Radiation-induced lymphocyte-immune deficiency. A factor in the increased visceral metastases and decreased hormonal responsiveness of breast cancer. Arch Surg 1970; 101:114-120
10. **Burnet M.** Immunological surveillance. Pergamon Press, London, 1970;pp161-171
11. **Rosenberg SA.** Immunotherapy: Active nonspecific immunotherapy. In: De Vita VT, Hellman S, Rosenberg SA, eds. Principles and applications of biologic therapy. 2nd ed. Philadelphia: JB Lippincott Co. 1993:293-324
12. **B.M.D.P.** Statistical Software Inc: University of California, 1983
13. **Rubin P, Landman S, Mayer E, Keller B, Ciccio S.** Bone marrow regeneration and extension after extended field irradiation in Hodgkin's disease. Cancer 1973; 32:699-711
14. **Atkinson HR.** Bone marrow distribution as a factor in estimating radiation to the blood-forming organs: A survey of present knowledge. J. Coll Radiol Aust 1962; 6:149-154
15. **Rotman M, Ansley H, Rogow L, Stowe S.** Monocytosis: A new observation during radiotherapy. Int J Radiat Oncol Biol Phys 1977; 2:117- 121
16. **Onsrud M, Thorsby E.** Long-term changes in natural killer activity after external pelvic radiothrapy. Int J Radiat Oncol Biol Phys 1981; 7:609-614
17. **Idestroem K, Petrini B, Blobgren H, Wasserman J, Wallgren A, Baral E.** Changes of the peripheral lymphocyte population following radiation therapy to extended and limited fields. Int J Radiat Oncol Biol Phys 1979; 5:1761-1766
18. **Uchida A, Hoshino T.** Clinical studies on cell-mediated immunity in patients with malignant disease. Cancer 1980; 45:476-483

국문초록 =

암환자의 방사선치료에서 흉부 및 전골반강 조사직후 백혈구 및 림프구아형 변화에 대한 연구

전남대학교 의과대학 치료방사선과학교실

안성자 · 정웅기 · 남택근 · 나병식 · 노영희

목 적 : 암환자에서 방사선치료에 의한 면역기능의 저하에 대해서는 많은 보고가 되어 있다. 저자들은 방사선치료부위중 비교적 활동성 골수를 많이 포함하고 있는 흉부 및 골반강조사 직후 어느정도 면역력의 저하가 오는지 알아보고자 하였다.

대상 및 방법 : 1995년 1월부터 1995년 4월까지 등록된 61 명의 환자중 48 명을 대상으로 분석 하였다. 이중 흉부(조사문, > 150cm^2)에 방사선치료를 시행한 환자는 29명이었고 전골반강부에 방사선치료를 시행한 환자는 19명 이었다. 연령분포는 36세에서 73세 였으며 평균 및 중간값 모두 57세 였으며 남녀비는 1.3(27/21)이었다.

환자의 면역기능의 지표는 말초혈액검사에서 전혈구 및 감별혈구계산(CBC with D/C), 간기능검사, 신장기능검사 및 림프구아형검사(CD3, CD4, CD8, CD16, CD56, CD19)를 시행 하였으며, 검사시기는 방사선치료 직전과 4500 cGy - 5000 cGy 선량에서 동일 검사를 반복시행하였으며, 1980cGy에서는 전혈구 및 감별혈구계산 만 시행하였다.

결 과 : 전체환자의 치료전 백혈구 총수는 7017이었으며 방사선치료직후 평균 4470으로 감소하였다(p=0.0000). 감별혈구계산에서는, 림프구수는 평균 2047 에서 537 로(p=0.0000) 로 감소하였고, 호중구, 호염구세포의 절대수도 통계학적으로 유의한 감소를 보였으나, 단핵세포는 변화가 없었으며, 호산구세포는 오히려 방사선치료후 증가하였으나 통계적인 의의는 없었다.

림프구아형에 대한 검사결과는, 모든아형의 절대수가 통계학적으로 유의한 감소를 보였으며, CD4/CD8비는 치료전 평균 1.09에서 0.99로 감소 하였으나 통계적인 유의성은 없었다. 전체 림프구에 대한 비율의 변화를 보면, B림프구(CD 19)는 감소하였으나, 그외 아형의 비율은 방사선치료후 변화를 보이지 않았다.

혈청면역글로불린은 초기 Ig G, Ig A, Ig M 모두 정상값보다 높은 수치였으며, 방사선치료에 따른 변화는 Ig M에서만이 통계적으로 유의한 감소를 보였으며, Ig G, A는유의한 변화가 없었다.

결 론 : 흉부나 골반강부위의 방사선치료는 림프구의 급격한 저하를 초래하는 반면 단핵구등은 비교적 잘 유지 되었으며, 호산구는 오히려 증가 됨을 알수 있었으나, 인체의 면역과의 관계를 설명하기 위해서는 림프구의 기능변화가 함께 연구 되어져야 하겠다.