

Lymphocyte count changes in cancer patients under radiation therapy - A prospective study

Type of manuscript: Research article

Running title: Lymphocyte count changes in cancer patients under radiation therapy

Nivashini G.S.V

Undergraduate student

Saveetha Dental College, Saveetha University, Chennai, India.

Brundha M.P

Department of Oral pathology

Saveetha Dental College, Saveetha University, Chennai, India.

Corresponding Author

Nivashini GSV

Saveetha Dental College,

Saveetha Institute of Medical and Technical Sciences

Chennai, India.

Total no of words: 2663

ABSTRACT:

AIM: The aim is to study the changes that occur in lymphocyte count in cancer patients undergoing radiation.

OBJECTIVE: The study is to know the numerical changes of lymphocytes analysed through automated analyser in cancer patients undergoing radiation.

BACKGROUND: In cancer patients undergoing radiation, the beneficial effects of radiation may extend beyond direct cytotoxicity to oncogenic cells. Delivery of these radiations in localised areas often leads to systemic responses in distinct areas; these mechanisms are diverse and include trafficking of lymphocytes into the tumour microenvironment, enhanced tumour recognition and killing via up-regulation of tumour antigens and antigen presenting machinery and, induction of positive immunomodulatory pathways.

REASON: The prevalence of cancer is increasing day by day and so to understand the effect and impact of radiation.

KEYWORDS: radiation, lymphocytes, cancer.

INTRODUCTION:

A carcinoma is a result of the mutations of the DNA that activate or deactivate the tumour suppressor genes. These may occur suddenly or as a result of being exposed to radiation or carcinogenic substances that are influenced by the genetic factor [1]. Any carcinoma or cancer is treated by the following radiotherapy, chemotherapy or surgical procedures. Radiation exposure can be acute or chronic, and the severity of symptoms depends on many factors, such as total dose, dose rate, distribution of dose, susceptibility of the person to radiation, and the type of organs (more radiosensitive tissues) [2,3]. The effect of radiation on healthy individuals depends on the total dose and dose rate of the exposure to radiation. High dose of ionising radiation is generally considered to be harmful, causing DNA damage, apoptosis and transformation of normal cells to tumour cells.[4] An increased susceptibility of infection is associated with the whole body exposure to moderate amount of ionising radiation[5]. Lymphocytes are the main type of WBCs which is a part of immune system. They are found from the lymph and hence prompted the name lymphocyte. They are of two types B cells and T cells. Radiation induced alterations in the function and number of peripheral blood lymphocytes have been described recently in patients treated with radiation therapy for carcinoma of the bladder, breast, lungs and uterine cervix [6].

Lymphocytes, which belong to a population of radiation sensitive cells, account for approximately 30% of the normal human white blood cell population and play an important role in antitumour immunity [7]. In a study by Nagai and colleagues, peritransplant lymphopenia was found to predict HCC recurrence after liver transplantation [8]. A low absolute lymphocyte count (ALC) was also used as an indicator of malnutrition and poor immune response in patients with chronic liver disease [9]. In addition, increasing evidence has shown that radiation-related lymphopenia is correlated with tumour progression and prognosis in several types of advanced cancers [10,11]. From these studies, we can hypothesize that peripheral ALCs will decline during RT, and the degree of decline may exhibit some clinical significance. However, the relationship between the minimum absolute lymphocyte count (Min ALC) during RT and clinical outcomes in patients with HCC has not been characterized.

Total body irradiation or TBI (where a person's entire body is treated with radiation) is the only type of radiation likely to cause very low white blood cell counts. This type of radiation may be used during the bone marrow or stem cell transplant process. The patients were grouped according to total dose received and clinical status after radiotherapy. Twenty-eight patients were treated at

the Department of Radiology, Group I was treated with 6500 to 6800 rads and remained tumor free for 5 years; Group II also received 6500 to 6800 rads but developed recurrent tumors after treatment; Group III (6 cases) was treated at Radiumhemmet Kamohinska Hospital, Stockholm, Sweden. A total dose of 8300 to 8500 rads was given as described elsewhere (3); all patients in this group had a residual tumor after therapy.

Radiation is most often given to just one part of the body, so the whole immune system isn't damaged by radiation exposure. Still, depending on the dose and the part of the body being treated with radiation exposure, the skin or mucous membranes may be impaired, so you're less able to keep microorganisms out. Previous reports of the effects of intensive chemotherapeutic regimens on PB lymphocyte (PBL) populations are scarce. Strender et al⁷ reported decreased PBLs in women undergoing adjuvant therapy for breast cancer with chlorambucil, methotrexate, and 5-fluorouracil; however, T-cell subset analyses and T-cell phenotyping were not performed in this report. Although the prevalence of significant lymphopenia in similarly treated patients was not reported, Brunvand et al⁸ reported decreased CD4+ counts and concurrent pneumocystis pneumonia in two women treated with multiagent chemotherapy and radiation therapy for breast cancer. Because we have noted an increase in opportunistic infections in patients treated for cancer with dose-intensive regimens at our center in the past several years, we have systematically studied changes in PBL populations associated with intensive chemotherapy. Ten children and young adults treated with three separate dose-intensive protocols during the years 1990-1993 were evaluated by flow cytometric analyses of PBL populations obtained serially upon maximal hematologic recovery from successive cycles of chemotherapy. We report that although the time between cycles was adequate for granulocytic, monocytic, and platelet recovery, lymphocyte populations did not recover before the administration of successive cycles of therapy leading to severe B-cell and T-cell depletion in these patients.

Today, radiation treatments are most often given over many sessions rather than in one large dose. This helps minimise the amount of skin and tissue impairment, immune suppression, and the risk of infections. Radiation induced alterations in the number and function of peripheral blood lymphocytes have recently been described in patients treated with radiotherapy for carcinoma of the breast (1-6), lung (7-9), bladder (7- 10), uterine cervix (2, 6, 11), testicular seminoma and carcinoma (12, 13), Hodgkin's disease, (14-19) and in children receiving prophylactic craniospinal irradiation for acute lymphoblastic leukemia (13, 20). Most studies have demonstrated an acute lymphocytopenia and suppression of immune function, shortly after the initiation of treatment. Several investigators have shown that a partial recovery may occur within the first 18 months after the completion of treatment (6, 8, 10-14). There are, however, few data available on the long term effects of radiation therapy on the number and immune function of peripheral blood lymphocytes in patients in continuous remission many years after treatment.

In the present study, we assessed the variation of lymphocyte count during the full course of radiation in patients affected with different types of tumours.

MATERIALS AND METHODS:

The lymphocyte values of patients undergoing palliative radiotherapy with 5 fractions are given below. Complete Blood Count reports were collected from a local cancer clinic with the consent of patients and the counts were collected for 5 fractions. The technique of radiation used was 3DCRT technique for all the patients.

RESULT:

Throughout the radiotherapy the lymphocyte counts can vary from 16.33-21.53%. After the first, second, third, fourth and fifth fraction of radiotherapy the average range of lymphocytes are from 21.84-27.04, 16.16-21.36, 15.99-21.19, 16.08-21.28 and 11.59-16.79 respectively

Table 1: Results of platelet count changes in patients

Sl.no	age	sex	Fraction1	Fraction2	Fraction3	Fraction4	Fraction5
1	49	F	27.8	14	25	32	19
2	62	M	24	20	17	40	12
3	60	M	12	12	12	10	10
4	40	F	21	25	31	22	10
5	34	F	37	37	31	27	15
6	63	M	10	8	4	4	20
7	62	M	10	25	22.5	20	13
8	30	M	22.8	4.2	12.7	10	14
9	50	F	33.8	25	25	20	10
10	60	F	37	13	10	10	15
11	45	F	25.1	21.6	3.4	21.1	4
12	53	M	31	31	25	20	22.1
13	37	F	20	15	15	10	20
14	75	F	26	24	13	15	10
15	60	F	30	3	34	19	20
16	45	F	23.6	22.5	16.9	18.8	13

DISCUSSION:

A decline in the ALC after treatment has been identified as a prognostic biomarker for several malignancies. Saroha and colleagues suggested that a low ALC is associated with aggressive features and inferior survival in patients with clear cell renal cell carcinoma [Saroha et al. 2013]. Balmanoukian and colleagues reported that lymphopenia was an independent predictor for survival in patients

with resected pancreatic adenocarcinoma [Balmanoukian et al. 2012]. A decline in peripheral lymphocyte counts after RT was first described in the 1970s [Stratton et al. 1975], but the clinical significance has not been well studied. This retrospective analysis of HCC patients who received RT demonstrated that lymphocyte counts declined after RT, and this observation has also been found in other cancer types [Santin et al. 2000; Pinkawa et al. 2014].

Several large studies of patients with carcinoma of the cervix who were treated by radiotherapy have demonstrated that anemia at presentation is a poor prognostic factor predicting for decreased local control of disease and overall patient survival.¹⁻⁸ The association between anemia and poor patient outcome has been assumed to be causal: anemia leading to an increase in the tumor hypoxic fraction with subsequent relative radioresistance resulting from impaired tumor oxygenation.^{2, 9} The assumption of a causal relation had become the rationale for administering blood transfusions to anemic patients prior to and/or during radiotherapy; it also stimulated investigation into hypoxic cell sensitizers as well as methods that attempt to improve the oxygen carrying capacity of the blood through the use of hyperbaric oxygen, carbogen, perfluorochemicals, blood flow modifiers, and, more recently, recombinant human erythropoietin.^{3, 10-16}

Data from experimental animal studies, however, suggest that tumors can adapt to chronic anemia^{17, 18} and that an increase in the tumor hypoxic fraction resulting from anemia is transient. Moreover, many clinicians now believe that anemia at presentation may not be a treatment-related factor but may be solely or partially a tumor-related manifestation of more aggressive cervical carcinoma.¹⁹ Hence, the mechanisms underlying the clinical association between anemia and poor patient outcome may be more complex than originally believed or even may be unrelated to anemia-induced tumor hypoxia. Whether blood transfusion can improve the outcome for anemic patients who receive radical radiotherapy for carcinoma of the cervix remains unclear.

Although animal studies demonstrate that tumor radiosensitivity increases after blood transfusion,¹⁸ there are only two clinical studies of patients with carcinoma of the cervix that have suggested a benefit for blood transfusion.^{2, 4} Of these, the only randomized and widely quoted study concluded that maintaining patient hemoglobin levels > 125 g/L significantly improves pelvic control rates compared with the rates in patients with lower hemoglobin levels.² However, that study's conclusion of benefit is undermined by the small patient numbers and the lack of a multivariate analysis to evaluate the impact of other prognostic factors on patient outcome.

Concerns regarding the well-defined risks associated with blood transfusion have escalated over the past decade. Blood transfusion exposes recipients to infectious diseases, such as human immunodeficiency virus and hepatitis C, and may increase the risk of tumor recurrence by blood transfusion-induced immunosuppression.²⁰⁻²⁶

The primary objective of this multiinstitutional Canadian survey of anemia and blood transfusion practice in patients with carcinoma of the cervix was to examine the impact of anemia and blood transfusion on the outcome of patients who were treated with definitive radiotherapy. Patient hemoglobin levels at presentation and during radiotherapy were profiled to establish the prevalence and time course of anemia. The range of practice of blood transfusion in patients with carcinoma of the cervix as well as the change in practice after clinicians and patients became more concerned about the risks associated with blood transfusion also were examined. First, some studies have suggested that lymphopenia during RT might be caused by the apoptosis of lymphocytes that are caught in the radiation field [Yovino and Grossman, 2012]. Second, the correlation between the increasing infiltration of CD4+ T lymphocytes at tumour margins and a better prognosis of HCC patients has been demonstrated [Ma et al. 2016]. Third, *in vivo* studies have shown that tumour cells that are dead or dying due to RT with or without chemotherapy can present as tumour-associated antigens to host immune cells and thereby activate antitumour immune responses [Apetoh et al. 2007]. Some clinical data have revealed the presence of radiation-induced antitumour immunity in humans [Schaue et al. 2008]. Lymphocytes exert an antitumour effect in the tumour microenvironment and affect cancer development and progression [Fu et al. 2013]. HCC patients with severe lymphopenia have worse immune system function and antitumour immunity. This association has also been verified in other types of cancer [Cho et al. 2016a, 2016b]. Consequently, a lower serum Min ALC during RT, as a reflection of the host immunity network, may be used to predict the result of cancer treatment. However, we cannot ignore the effect of various factors such as host nutrition and chronic infection on the peripheral lymphocyte counts. HBV activation and other infections were not detected in any patients in our study during the course of RT; therefore, we could state that this value reflects the overall condition of the host to fight the tumour. The Min ALC may be a good marker of the host immune reaction to the tumour. Most of the reported studies on the effects of ionizing radiation on immune functions in mammals have dealt with single, whole body radiation exposures (38, 39). There have been only a few studies on the effects of localized or regional fractionated irradiation, such as employed in clinical radiotherapy, on immune functions in man.

The finding that uncorrected anemia before or during radiotherapy in patients with carcinoma of the cervix compromises pelvic control and survival rates has led to several hypotheses regarding the causal mechanisms underlying the association. Many believe that anemia impairs the tumor's response to radiotherapy by increasing the tumor hypoxic fraction and resulting in relative radioresistance. However, others have questioned this point of view, suggesting that anemia is primarily a tumor-related poor prognostic factor that cannot be overcome by administering blood transfusion. This latter hypothesis is supported by data from several studies suggesting that anemic patients with carcinoma of the cervix who are treated with surgery also appear to have a poor prognosis.⁸ These studies have shown that anemia at presentation is associated with other tumor-related poor prognostic factors, such as advancing stage of disease^{2, 8, 9} and large tumor size.⁸ Also, animal studies have demonstrated that tumors exposed to low levels of oxygen for prolonged periods of time, as seen in the chronic anemia of malignancy, do not necessarily remain hypoxic but are able to adapt to their microenvironment. Although the exact mechanisms by which tumor adaptation occurs are unknown, evidence suggests that tumors are capable of reducing the greatest dimension of tumor cords and changing the level of red blood cell 2,3-diphosphoglycerate concentration in response to anemia, thereby restoring tumor oxygenation.¹⁸ However, although the

latter observations have been seen in the animal model, it is unknown whether these mechanisms operate in human cancer. Furthermore, the anemia of carcinoma of the cervix often is acute superimposed on chronic, and animal experiments suggest that acute anemia consistently causes radioresistance,^{17, 18, 32} which may be overcome by blood transfusion.^{32, 33}

Interpreting the results from reports of other clinical data gathered from examining the correlation between anemia and poor outcome is difficult not only because of the uncertainties discussed above but also because varying definitions of anemia are used, e.g., levels as low as 100 g/L and as high as 125 g/L. Also, single measurements of hemoglobin or hematocrit levels taken before treatment often are used to reflect the hemoglobin level throughout the course of treatment. As observed in this study, levels may change due to hemorrhage, chronic decline/depletion of iron stores, and/or blood transfusion. Blood transfusions are given at varying levels of hemoglobin that may or may not be influenced by local policy and by patient and/or doctor concern regarding the transmission of infectious diseases or the declining availability of blood. Hence, from the currently available information it is not possible to distinguish the degree to which anemia is a treatment or a tumor-related poor prognostic factor; in fact, it may be both. Most of the reported studies on the effects of ionising radiation on immune functions in mammals have dealt with single, whole body radiation exposures^{17, 81}. There have been only a few studies on the effects of localised or regional fractionated irradiation, such as employed in clinical radiotherapy, on immune functions in man. In this study we have described the changes in the lymphocyte number in cancer patients undergoing radiation.

CONCLUSION:

Radiation exposure in cancer patients and its impact on lymphocyte count have been investigated for 16 patients. There is a drastic decrease in the lymphocyte count, therefore it is most affected. The pathological complete response is yet to be analysed by obtaining more than 50 patients.

References:

1. R. J. Dufraim, L. G. Littlefield and E. E. Joiner No. 3, 1979, pp. 279-289.
2. NRCNA (National Research Council of the National Academies), "Health Risks from Exposure to Low Levels of Ionizing Radiation," Beir VII Phase 2, 2006.
3. R.W. Field, "Radon: An Overview of Health Effects," Encyclopaedia of Environmental Health, Vol. 23, 2011, pp. 745-753.
4. Bonggoo Park, Cassian yee, and Kyung-Mi Lee. The effect of radiation on the immune response to cancers. International journal of molecular sciences, ISSN 1422-0067
5. Thomas R. Holley, BUS, Dennis E. Van Epps, PhD, Ronald L. Harvey, MD, Robert E. Anderson, MD and Ralph C. Williams Jr, MD. Effect of high doses of radiation on human neutrophil chemotaxis, phagocytosis and morphology. American journal of pathology, Vol. 75 No. 1, April 1974.
6. Zvi Fuks, Samuel Strober, Arthur M. Bobrove, Takehiko sasazuki, Andrew McMichel and Henry S. Kaplan. Long term effects of radiation on T and B lymphocytes in peripheral blood of patients with hodgkin's disease. The journal of clinical investigation, Vol.58, October 1976. 803-814.
7. Topalian S., Weiner G., Pardoll D. (2011) Cancer immunotherapy comes of age. *J Clin Oncol* 29: 4828–4836.
8. Nagai S., Abouljoud M., Kazimi M., Brown K., Moonka D., Yoshida A. (2014) Peritransplant lymphopenia is a novel prognostic factor in recurrence of hepatocellular carcinoma after liver transplantation. *Transplantation* 97: 694–701.
9. Fernandez-Ruiz M., Lopez-Medrano F., Romo E., Allende L., Meneu J., Fundora-Suarez Y., et al. (2009) Pretransplant lymphocyte count predicts the incidence of infection during the first two years after liver transplantation. *Liver Transpl* 15: 1209–1216.
10. Kitayama J., Yasuda K., Kawai K., Sunami E., Nagawa H. (2011) Circulating lymphocyte is an important determinant of the effectiveness of preoperative radiotherapy in advanced rectal cancer. *BMC Cancer* 11: 64.
11. Cho O., Oh Y., Chun M., Noh O., Hoe J., Kim H. (2016a) Minimum absolute lymphocyte count during radiotherapy as a new prognostic factor for nasopharyngeal cancer. *Head Neck* 38: E1061–E1067.
12. Talianferro, W.H., L.C. Talliferro, and B.N. Jaroslow. 1964. Radiation and immune mechanisms. Academic Press, Inc., N.Y. 17-50.
13. Effects of radiation on the immune response in ionizing radiation: Levels and effects. A report of the United Nations Scientific Committee on the effects of atomic radiation to the general assembly. 1972. United Nations publication, N. Y. 2: 303-378.
14. Sri Vasavi Kadiyala, Dr. M.P Brundha. Platelet Count Changes in Cancer Patients Under Radiation Therapy - A Prospective Study. *Int. J. Pharm. Sci. Rev. Res.*, 48(1), January - February 2018.
15. Dr. C. Harshitha, Dr. Laliytha K.B. Effect of Radiotherapy on the Oral Cavity. *J. Pharm. Sci. & Res.* Vol. 9(12), 2017, 2332-2334.
16. Fleming, P. Dental Management of the Pediatric Oncology Patient., *Pediatric Dentistry*, 1991
17. Osteoradionecrosis of mandible and mastoiditis after radiotherapy for parotid mucoepidermoid carcinoma.
18. Osteoradionecrosis of the mandible : Etiology, Prevention, Diagnosis and treatment. Sukh S Rayatt et al.
19. The role of cytokines in development of normal tissue reaction after radiotherapy. *Strahlenther Onkol* 1998, 174:12-5
20. Management of trismus following radiation therapy by cost-effective approach. Kamleshwar Singh,¹ Upadhyay Snehal Rashmikant,¹ Habib Ahmed Alvi,¹ Rajeev Kumar Singh²
21. PREVENTION AND TREATMENT OF THE CONSEQUENCES OF HEAD AND NECK RADIOTHERAPY. A. Vissink^{1*}, F.R. Burlage² F.K.L. Spijkervet¹ J. Jansma¹, R.P. Coppes^{2,3}
22. Heasman PA, Thomason JM, Robinson JG. The provision of prostheses for patients with severe limitation in opening of the mouth. *Br Dent J* 1994;176:171–4.