ORIGINAL ARTICLE

Assessment of in vitro chromosomal sensitivity to low doses of gamma irradiation in patients with acute lymphoblastic leukemia

Ramyar A[3] , Aghamohammadi A[4] , Mozdarani H[5] , Mahmoodi M[1*], Azimi C[2] , Safari Z[1]
Nedaei HA[6] ,Farzanfar F[2] , Rezaei N[4] , Asadi-Shekaari M[7] , Esfahani M[6] , Hosseini SK[1]
Yazdani M[6] , Abolhassani H[4] , Khalesi F[6] , Mohagheghi MA[1]

ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) constitutes a heterogeneous group of diseases characterized by abnormal proliferation and accumulation of immature lymphoblasts arrested at various differentiation stages. Increasing evidence suggests that chromosomal defects are present in these patients. The aim of this study is to investigate the chromosomal radiosensitivity in a group of ALL patients.

Materials and Methods: To analyze chromosomal radiosensitivity of ALL patients, lymphocytes of 20 patients were cultured followed by exposure to γ-ray irradiation to detect the chromosomal aberrations as an indicator of radiosensitivity. Cells were scored for the number of aberrations (chromatid breaks, chromatid gaps, chromosome breaks, chromosome gaps and chromatid exchanges). Results were compared with healthy individuals, and ataxia telangiectasia (AT) patients as positive control.

Results: On average number of aberrations in ALL patients was significantly higher than that in healthy controls. We found 65% of ALL patients appeared to be susceptible to *in vitro* irradiation. Chromosomal radiosensitivity of 35% patients was not different from healthy donors. Ataxia telangiectasia patients showed the highest degree of lymphocyte radiosensitivity. The results also indicate that there is a good correlation between the two assays, G2 and G0, using the same blood sample for both assays.

Conclusion: According to the result, we concluded that most of the ALL patients are sensitive to ionizing radiation and therefore should be protected from unnecessary diagnostic and therapeutic procedures using ionizing radiation.

Keywords: ***Chromosomal sensitivity, gamma irradiation, acute lympho, blastic leukemia***

زمینه و هدف: بیماری لوسمی لنفوبلاستی حاد (**Acute lymphoblastic leukemia**, **ALL**)، نوعی بیماری هتروژن است که با تکثیر غیر طبیعی لنفوبلاست ها و ازدیاد لنفوئیدهای نابالغ در خون و یا در مغز استخوان شناخته میگردد. شواهد متعدد نشان میدهد که ممکن است نقص کروموزومی در سلولهای این بیماران وجود داشته باشد. هدف از انجام این مطالعه بررسی اثر تابش اشعه گاما (اشعه پرتو درمانی) در پیدایش ناهنجاریهای کروموزومی در سلولهای بیماران مبتلا به **ALL** میباشد.

مواد و روشها: جهت انجام این بررسی، لنفوسیتهای خون محیطی از ۲۰ بیمار داوطلب مبتلا به **ALL** کشت داده شد و سپس مورد تابش اشعه گاما قرار گرفت و میزان ناهنجاریهایی از قبیل شکست های کروموزومی و کروماتیدی، شکاف های کروموزومی و کروماتیدی مورد بررسی و میانگین این ناهنجاریها با افراد گروه سالم و بیماران مبتلا به آتاکسی تلانژکتازی (**AT**) بعنوان گروه کنترل مثبت مورد مقایسه قرار گرفت.

یافته‌ها: نتایج این بررسی نشان داد که بیماران مبتلا به لوسمی لنفوبلاستی حاد در مقایسه با گروه افراد سالم از نظر میانگین ضریب ناهنجاریهای بررسی شده اختلاف معنی داری داشتند و از نظر در صد تعداد افراد حساس به اشعه، ٪۶۵ این بیماران حساس به اشعه گاما بودند و ٪۳۵ مشابه افراد گروه کنترل سالم بودند. بیماران مبتلا به **AT** بالاترین حساسیت به اشعه را داشتند. نتایج این بررسی همینطور نشان میدهد که بین دو روش بکار برده شده، **G2** و **G0**، جهت تعین نمودن حساسیت کروموزومی به اشعه، ارتباط مستقیمی وجود دارد.

نتیجه‌گیری: نتایج این بررسی نشان میدهد که در صد بالائی از بیماران مبتلا به لوسمی لنفوبلاستی حاد نسبت به اشعه رادیوتراپی حساس هستند و از اینرو بایستی مراقبت های لازم را از قبیل قرار دادن آنها در مقابل وسائل تشخیصی و درمانی غیر ضروری که اشعه گاما و یا اشعه **x** را مورد استفاده قرار میدهند اجتناب نمود.

واژه های کلیدی: حساسیت کروموزومی به اشعه گاما، لوسمی لنفوبلاستی حاد.

1. Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran.
2. Genetics Group, Cancer Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Hematology and Oncology of Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran.
4. Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran.
5. Department of Medical Genetics, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
6.Radiotherapy Oncology Department, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran.
7. Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran.
*** Corresponding author:** Majid Mahmoodi, Cancer Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran.
Tel:+98-21-61192501,
Fax:+98-21-66581638,
email:dmahmoodi@razi.tums.ac.ir

Introduction

Acute lymphoblastic leukemia (ALL) comprises a biologically and clinically heterogeneous group of diseases.[1] This disease show variations with respect to morphological, cytogenetic, and immunologic features of the transformed cells, that results in a diverse clinical behavior and different responses to therapy.[2-3] It is manifested by clonal proliferation, decreased apoptosis and the malignant proliferation and accumulation of immature lymphoid cells that are arrested at various differentiation stages within the bone marrow and lymphoid tissues.[4]

ALL patients usually have high white blood cell counts and may present with organomegaly, particularly mediastinal enlargement and CNS involvement.[5] ALL is the most common form of childhood malignancies but it can affect all age groups and constitutes 13% of acute leukemia in adults with slightly higher frequency in men than women.[5]

The etiology of ALL is unknown; however, Ionizing radiation especially in high doses and with acute exposure has been suggested as a possible risk factor in leukemogenesis.[6] In some studies, chromosomal defects and molecular abnormalities have been consistently observed in ALL patients.[7-8]

Furthermore, enhanced chromosomal radiosensitivity[9-13] and cancer-prone genetic disease such as ataxia-telangiectasia and Nijmegen breakage syndrome[14-16] has also been found in ALL patients. These patients do not tolerate conventional dose of radiation therapy.

Most studies performed on ALL, have focused on classifying patients into various risk groups based on known prognostic and predictive factors. The risk of severe reactions resulting from radiotherapy limit the total dose prescribed for patients, so it is important to identify these radiosensitive patients to avoid reactions[17] and to apply less invasive strategies in these patients.[18]

The current study was undertaken to investigate the chromosomal radiosensitivity by means of the G2 assay and the G0-micronucleus (MN) assay in a group of ALL patients. As a positive control we used ataxia telangiectasia (AT) patients and compared the results both with AT patients and healthy individuals as control.

Materials and Methods

A. Patients and controls:

Between March 2009 and June 2010, twenty consecutive patients with ALL, who had been referred to the Children's Medical Center Hospital, the main referral center for pediatric leukemia in Iran, took part in this study. Diagnosis of ALL was based on morphologic and immuno phenotypic criteria.[19-20] Clinical and laboratory data of the patients were documented. Thirty age-matched healthy individuals were randomly selected as control group. This study was reviewed and approved by the Ethics Committee of the Faculty of Medicine in Tehran University of Medical Sciences. After taking informed consent from the patients, venous peripheral blood samples were obtained stored in lithium heparin tubes. Blood cultures were started within 4 hours of collection.

B. Methods

1.The G2 assay: The assay was performed as described by Scott et al.[21] Briefly, heparinized blood was kept at room temperature prior to culturing, which was within 4 hours of the blood collection. For each sample, two tissue culture flasks (25 cm2) were set up: one for *in vitro* irradiation, the other served as control (un-irradiated). To each flask 0.5 ml of the blood was added to 4.5 complete RPMI-1640 culture medium supplemented with 10% fetal calf serum, 1% L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. The lymphocytes were stimulated to proliferate with 1% phytohemagglutinin (PHA, Invitrogen, final concentration 1 µg/ml). The flasks were incubated in a humidified air atmosphere at 37°C with 5% CO2 for 4 days. After incubation, the culture lymphocyte were exposed to gamma irradiation (Theratron 780e, MDS, Canada; 60Co, 70cGy/min) with a dose of 100 cGy at ambient temperature. After 2h post-irradiation, 0.2 ml Colcemid (Gibco, final concentration 0.1 µg/ml medium) was added to block cells at metaphase. Lymphocytes were harvested by centrifugation of the contents of each flask, supernatant was removed and for hypotonic shock, cell pellets were re-suspended in 5 ml of 0.075 M KCl for 15 min on ice. After further centrifugation, supernatant was again removed and cells were fixed three times in fresh fixative (methanol/glacial

acetic acid, 3/1). For making slides, cells were dropped on clean slides. Slides were stained with 2% Giemsa (in phosphate buffer) for 5 minutes. One hundred well-spread metaphases were analyzed from both irradiated and control samples and scored for aberrations such as chromatid breaks, chromatid gaps, chromosome breaks and chromatid exchange. Frequency of aberrations in control samples was subtracted from that in irradiated samples to give the induced yield.

2.The G0-micronucleus (MN) assay: Full details of this assay are given elsewhere.[11] Briefly, heparinized blood samples were stored at room temperature for 4 hours. Two tissue culture flasks (25 cm2) were set up: one for *in vitro* irradiation, the other served as control (unirradiated). An aliquot of 0.5 ml of the blood was diluted with complete RPMI-1640 culture medium in the ratio of 1:9. Complete culture medium was supplemented with 10% fetal calf serum, 1% L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. One of the flasks of each donor was irradiated, total dose 3 Gy (source: Theratron 780e, MDS, Canada; 60Co, 70cGy/min) at ambient temperature. After irradiation lymphocytes were stimulated with 1% PHA (final concentration 1 µg/ml). Flasks were incubated at 37°C (with 5% CO2). Forty-four hours later, cytochalasin B (Sigma) was added to a final concentration of 6 µg/ml. After further incubation, cells were harvested at 92 h post-stimulation by hypotonic shock with 0.075 M KCl, followed by fixation, three times, in methanol/acetic acid (3:1) solution. For analysis, slides were coded and randomized. Per slide 500 binucleated cells (BNCs) were scored for micronucleus (MN) frequencies.

Statistical analysis

Statistical analysis of data was performed using SPSS statistical software package (version 16.0). Results were presented as the mean ± standard deviation. Independent-samples t-test was performed to compare means between the groups.

P-values of less than 0.05 were considered statistically significant.

Results

Mean number of chromosomal aberrations such as chromatid breaks, chromatid gaps, chromosome breaks, chromosome gaps and chromatid exchange for the ALL patients, the control healthy group and the positive AT patients are presented in **Table 1**. As shown, the mean number of any kind of aberrations, except Chromosome breaks, in ALL patients was significantly higher than that in healthy controls. Besides, 65 percent of these patients appeared to be susceptible to *in vitro* irradiation. Chromosomal radiosensitivity of 35% patients was not different from healthy donors. Ataxia telangiectasia patients showed the highest degree of lymphocyte radiosensitivity than ALL patients and healthy individuals.

Discussion

Acute lymphoblastic leukemia is the most frequent cancer in childhood and is associated with good outcomes. [22] Currently, 80% of children with ALL treated with anticancer therapies are alive and disease-free after 5 years of therapy completion.[23]

Recently, number of patients with second neoplasia has increased among long-term survivors of childhood ALL. [24-26] There is also compelling evidence that specific exposure to radiation and chemotherapy are the risk of developing secondary malignancy.[27]

In the current study, the chromosomal radiosensitivity by means of the G2 assay and the G0-micronucleus assay was measured in a group of ALL patients and the results were compared with those of healthy individuals and AT patients as a positive control group. We found that ALL patients have a significantly higher mean number of *in vitro* radiation-induced chromosomal aberrations than those in healthy individuals. Besides, with respect to the proportion of sensitive patients,65 percent of these patients were sensitive to ionizing radiation.

The relevance of *in vitro* increased frequency of chromosomal aberrations in peripheral lymphocytes of patients exposed to gamma radiation as indicator of cancer risk is supported by several studies.[28-29] Moreover, enhanced chromosomal radiosensitivity has been demonstrated in certain cancer-prone diseases such as ataxia telangiectasia, ligase IV deficiency, Nijmegen breakage syndrome, hereditary retinoblastoma and primary immunodeficiency disorders.[15-16, 30]

The elevated chromosomal radiosensitivity has also been reported in a significant proportion of cancer pa-

Table 1- Mean values of chromosomal aberrations per 100 metaphases scored in peripheral blood lymphocytes exposed to .gamma rays for the ALL patients, healthy control group and the positive ataxia telangiectasia (AT) patients

Aberration	ALL patients Group I (N = 20)	Healthy controls Group II (N = 30)	AT patients Group III (N = 7)	P(I vs. II)	P(I vs. III)
Chromatid breaks	33.5±16.1	23.5±9.6	50.1±12.2	0.003	0.02
Chromatid gap	46.8±16.8	37.2±11.5	70.2±14.1	0.01	0.007
Chromosome breaks	17.1±12	18.3±10.7	33.1±7.4	0.19	0.1
Chromosome gap	23.7±15.1	16.6±7.3	19.3±7.5	0.01	0.35
Fragmentation	12.5±7.1	9.5±4.1	15.5±3.1	0.001	0.15
Rearrangement	4.8±4.5	2.5±3.6	6.8±5.5	0.03	0.44
Micronucleus	73.9±21.1	17.3±5.9	96.5±13.7	0.001	0.009

Chromosomal aberrations consisted of chromatid breaks, chromatid gaps, chromosome breaks, chromosome gaps, fragmentation, chromatid exchange and micronucleus.

tients such as cases with breast cancer, colorectal cancer and various head and neck cancer including patients with oral cavity, pharynx and larynx cancers that all show the *in vitro* increased mean number of radiation-induced chromosomal aberrations compared with the healthy subject group.[31-33] There is no similar report on performing the *in vitro* cytogenetic analysis, by G2 and G0-MN chromosomal sensitivity assay, in patients with ALL to evaluate the long-term effects of radiation therapy, however, our data are in agreement with the enhanced chromosomal radiosensitivity observed in a group of the young breast cancer patients who showed the highest percentage of radiosensitive cases in the G2 assay as well as in the G0-MN assay.[34]

In conclusion, our data show that ALL patients are more sensitive to ionizing radiation than normal individuals as indicated by the G2 and by G0-MN assay, therefore they should be protected from unnecessary diagnostic and therapeutic procedures using ionizing radiation. In addition, the results of this study indicate that a good correlation exists between G2 and G0 assay using the same blood sample for both assays.

References

1. Czuczman MS, Dodge RK, Stewart CC, Frankel SR, Davey FR, Powell BL, et al. Value of immunophenotype in intensively treated adult acute lymphoblastic leukemia: cancer and leukemia Group B study 8364. Blood. 1999 Jun 1;93(11):3931-9.

2. Bruchova H, Kalinova M, Brdicka R. Array-based analysis of gene expression in childhood acute lymphoblastic leukemia. Leuk Res. 2004 Jan;28(1):1-7.

3. Uckun FM, Sensel MG, Sun L, Steinherz PG, Trigg ME, Heerema NA, et al. Biology and treatment of childhood T-lineage acute lymphoblastic leukemia. Blood. 1998 Feb 1;91(3):735-46.

4. Ahmad F, Dalvi R, Chavan D, Das BR, Mandava S. Cytogenetic profile of acute lymphocytic leukemia patients: report of a novel translocation t(4;13) (q21 x 3; q35) from an Indian population. Hematology. 2008 Feb;13(1):28-33.

5. Chiaretti S, Foa R. T-cell acute lymphoblastic leukemia. Haematologica. 2009 Feb;94(2):160-2.

6. Greaves MF. Aetiology of acute leukaemia. Lancet. 1997 Feb 1;349(9048):344-9.

7. Cytogenetic abnormalities in adult acute lymphoblastic leukemia: correlations with hematologic findings outcome. A Collaborative Study of the Group Francais de Cytogenetique Hematologique. Blood. 1996 Apr 15;87(8):3135-42.

8. Ferrando AA, Look AT. Clinical implications of recurring chromosomal and associated molecular abnormalities in acute lymphoblastic leukemia. Semin Hematol. 2000 Oct;37(4):381-95.

9. Riches AC, Bryant PE, Steel CM, Gleig A, Robertson AJ, Preece PE, et al. Chromosomal radiosensitivity in G2-phase lymphocytes identifies breast cancer patients with distinctive tumour characteristics. Br J Can-

cer. 2001 Oct 19;85(8):1157-61.

10. Distel LV, Neubauer S, Keller U, Sprung CN, Sauer R, Grabenbauer GG. Individual differences in chromosomal aberrations after *in vitro* irradiation of cells from healthy individuals, cancer and cancer susceptibility syndrome patients. Radiother Oncol. 2006 Dec;81(3):257-63.

11. Scott D, Barber JB, Levine EL, Burrill W, Roberts SA. Radiation-induced micronucleus induction in lymphocytes identifies a high frequency of radiosensitive cases among breast cancer patients: a test for predisposition? Br J Cancer. 1998 Feb;77(4):614-20.

12. Papworth R, Slevin N, Roberts SA, Scott D. Sensitivity to radiation-induced chromosome damage may be a marker of genetic predisposition in young head and neck cancer patients. Br J Cancer. 2001 Mar 23;84(6):776-82.

13. Mozdarani H, Mansouri Z, Haeri SA. Cytogenetic radiosensitivity of g0-lymphocytes of breast and esophageal cancer patients as determined by micronucleus assay. J Radiat Res (Tokyo). 2005 Mar;46(1):111-6.

14. Palanduz S, Palanduz A, Yalcin I, Somer A, Ones U, Ustek D, et al. *In vitro* chromosomal radiosensitivity in common variable immune deficiency. Clin Immunol Immunopathol. 1998 Feb;86(2):180-2.

15. Gatti RA, Boder E, Good RA. Immunodeficiency, radiosensitivity, and the XCIND syndrome. Immunol Res. 2007;38(1-3):87-101.

16. Aghamohammadi A, Moin M, Kouhi A, Mohagheghi MA, Shirazi A, Rezaei N, et al. Chromosomal radiosensitivity in patients with common variable immunodeficiency. Immunobiology. 2008;213(5):447-54.

17. Tucker SL, Geara FB, Peters LJ, Brock WA. How much could the radiotherapy dose be altered for individual patients based on a predictive assay of normal-tissue radiosensitivity? Radiother Oncol. 1996 Feb;38(2):103-13.

18. Uckun FM, Sather H, Gaynon P, Arthur D, Nachman J, Sensel M, et al. Prognostic significance of the CD10+CD19+CD34+ B-progenitor immunophenotype in children with acute lymphoblastic leukemia: a report from the Children's Cancer Group. Leuk Lymphoma. 1997 Nov;27(5-6):445-57.

19. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol. 1976 Aug;33(4):451-8.

20. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995 Oct;9(10):1783-6.

21 Scott D, Barber JB, Spreadborough AR, Burrill W, Roberts SA. Increased chromosomal radiosensitivity in breast cancer patients: a comparison of two assays. Int J Radiat Biol. 1999 Jan;75(1):1-10.

22. Baccichet A, Qualman SK, Sinnett D. Allelic loss in childhood acute lymphoblastic leukemia. Leuk Res. 1997 Sep;21(9):817-23.

23. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008 Mar 22;371(9617):1030-43.

24. Felix CA. Secondary leukemias induced by topoisomerase-targeted drugs. Biochim Biophys Acta. 1998 Oct 1;1400(1-3):233-55.

25. Liang DC, Yang CP, Lin DT, Hung IJ, Lin KH, Chen JS, et al. Long-term results of Taiwan Pediatric Oncology Group studies 1997 and 2002 for childhood acute lymphoblastic leukemia. Leukemia. 2010 Feb;24(2):397-405.

26. Vrooman LM, Neuberg DS, Stevenson KE, Asselin BL, Athale UH, Clavell L, et al. The low incidence of secondary acute myelogenous leukaemia in children and adolescents treated with dexrazoxane for acute lymphoblastic leukaemia: a report from the Dana-Farber Cancer Institute ALL Consortium. Eur J Cancer. 2011 Jun;47(9):1373-9.

27. Dann EJ, Rowe JM. Biology and therapy of secondary leukaemias. Best Pract Res Clin Haematol. 2001 Mar;14(1):119-37.

28. Bonassi S, Hagmar L, Stromberg U, Montagud AH, Tinnerberg H, Forni A, et al. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. European Study Group on Cytogenetic Biomarkers and Health. Cancer Res. 2000 Mar 15;60(6):1619-25.

29. Hagmar L, Stromberg U, Bonassi S, Hansteen IL, Knudsen LE, Lindholm C, et al. Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer Res. 2004 Mar 15;64(6):2258-63.

30. Sun X, Becker-Catania SG, Chun HH, Hwang MJ, Huo Y, Wang Z, et al. Early diagnosis of ataxia-telangiectasia using radiosensitivity testing. J Pediatr. 2002 Jun;140(6):724-31.

31. Baria K, Warren C, Roberts SA, West CM, Scott D. Chromosomal radiosensitivity as a marker of predisposition to common cancers? Br J Cancer. 2001 Apr 6;84(7):892-6.

32. Howe OL, Daly PA, Seymour C, Ormiston W, Nolan C, Mothersill C. Elevated G2 chromosomal radiosensitivity in Irish breast cancer patients: a comparison with other studies. Int J Radiat Biol. 2005 May;81(5):373-8.

33. De Ruyck K, de Gelder V, Van Eijkeren M, Boterberg T, De Neve W, Vral A, et al. Chromosomal radiosensitivity in head and neck cancer patients: evidence for genetic predisposition? Br J Cancer. 2008 May 20;98(10):1723-38.

34. Baeyens A, Thierens H, Claes K, Poppe B, Messiaen L, De Ridder L, et al. Chromosomal radiosensitivity in breast cancer patients with a known or putative genetic predisposition. Br J Cancer. 2002 Dec 2;87(12):1379-85.