ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com
Journal DOI:10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of Gamma Ray on Number of T-cells In Blood Bags

AlauldeenMudhafarZubairAlqasim, M.D. (Hematopathology)
Associate Professor of Hematopathology, Department of Pathology, Al-MustansiriyaUniversity,College of Medicine, Baghdad, Iraq.

Manuscript Info

Manuscript History:

Received: 18 March 2016
Final Accepted: 26 April 2016
Published Online: May 2016

Key words:
TA-GvHD, gamma ray, T-lymphocyte

Corresponding Author

AlauldeenMudhafarZubairAlqasim.

Abstract

Introduction: Transfusion associated graft versus host disease is a serious complication of blood transfusion. The main effector cells which are responsible for this reaction are T-lymphocytes. To prevent this complication,the standard method is irradiation of blood bags using gamma ray.

Materials and methods:fifty blood bags were collected from donors. Hemoglobin concentration, total lymphocyte count, T-cell% and T-cell counts were measured for each blood unit before and after exposure to 25 Gray dose of gamma ray.

Results:there was a statistically significant reduction in means of total lymphocyte count, T-cell% and T-cell count ($4.267*10^9$/l ±1.438 vs $1.800*10^9$/l ±0.775, 57.467%±10.501 vs 44.533% ±9.265,$2.482*10^9$/l±1.081 vs $0.865*10^9$/l ±0.383) respectively.

On the other hand, mean hemoglobin concentration was reduced from14.9±1.6 g/dl to11.2±1.2 g/dl.

Conclusion:

1. There was a significant reduction in means of total lymphocyte count, T-Cell% and T-cell numbers after exposure of blood bags to 25 Gy gamma ray.
2. Hb concentration was mildly reduced during irradiation.
3. The reduction in T-cell numbers outweighs the reduction in Hb concentration if TA-GvHD is to be prevented.

Introduction:-

Graft-Versus-Host Disease(GvHD) occurs after allogeneic hematopoietic stem cell transplant and is a reaction of donor immune cells against host tissues.[1,2]It is normally connected with stem cell or bone marrow graft, but the term also applies to other sorts of tissue graft. Immune cells (white blood cells) in the tissue (the graft) recognize the receiver (the host) as "foreign." The transplanted immune cells then attack the host's body cells. GvHD can also happen after a blood transfusion of the blood products.[1,3] The induction of a GvHD is influenced by many factors such as type of graft used (bone marrow or peripheral blood stem cells), HLA typing, conditioning regimen, GvHD prophylaxis employed, etc. Acute GvHD develops in 30–60% of recipients of sibling matched allografts, and its mortality (direct or collateral) can hit 50%. [2]

Transfusion associated (TA-GvHD) was originally realized as a complication of intrauterine transfusion and transfusion to recipients of allogeneic bone marrow grafts. The most commonly reported setting for TA-GvHD is immunocompetent recipients of blood from biologically related (directed) or HLA identical donors.[2,4]

The main stay of preventing TA-GvHD is the ionization radiation therapy of blood products. Leukocyte depletion using current technology is inadequate for this purpose in order to kill all T- cells in the blood of the donor which will attack host tissue.[5] There is no effective treatment for TA-GvHD, and the irradiation of cellular blood

components prior to transfusion has been the only proven method of preventing this reaction.[6] Gamma and X-rays, both representing ionizing radiation, damage DNA of T lymphocytes and arrest responses to allogeneic cells.[7] Thus, these lymphocytes are unable toproliferate in the host and therefore cannot mediate TA-GvHD proliferate in the host and therefore cannot mediate TA-GvHD.[4] The mainstay of prevention is gamma irradiation, which inactivates T lymphocytes whilst preserving the function of other blood cells. Leucodepletion by current filtration technology is inadequate for this purpose.

Gamma radiation (γ-ray) is a form of ionization electromagnetic radiation (nuclear radiation) produced by certain radioactive elements as they decay. Natural sources of gamma rays on Earth include gamma decay from naturally occurring radioisotopes such as potassium-40, and as a secondary radiation from various atmospheric interactions with cosmic ray particles. The most common Gamma source in the earth are ^{60}Co and^{137}Cs,gamma energy is 1.17Mev.[7] The biological effect of ionization radiation as gamma ray is divided into direct and indirect. In direct (γ-ray) hit the nuclei of cells causing damage of DNA. Radiation will cause fragmentation of DNA chains and lead to random cross linking in these chains, and also lead to inhabitation of cell efficiency, an interaction may affect the ability of the cell to reproduce and thus survive. If enough radiation damage reaches the cell, the chromosomes will not replicate.Then the cell may be destroyed by direct interference with its life-sustaining system or indirect effect which impact on cells and on water molecules in tissue. When radiation interacts with water, it may break the bonds that hold the water molecules together producing fragment such as Hydrogen (H) and Hydroxyls (OH), these fragments may recombine or may interact with other fragments or ions, which form toxic substance such as Hydrogen Peroxide (H_2O_2) leading to cell destruction.[8,9]

Aims of the study:-

To study the effect of blood bags exposure to 25 Gy gamma ray on hemoglobin concentration, total lymphocyte count, T-cell % and absolute T-cell count.

Materials and methods:

This study was approved by the ethical committee of department of pathology at Al-Mustansiriya University, College of Medicine in conformation to the Declaration of Helsinki. Informed consent was obtained from all participants.

Blood bags Irradiation:-

1- Fifty blood bags (490-500ml) of whole fresh blood which collected from donors, mostly men suffered from polycythemiaat the National Center of Hematology. Their age range was 24-55 years.

2- Three to fivemls of fresh blood were drawn from every blood bag and put in laboratory tubes to be considered as a control sample.

3-Irradiation of blood bags by 25 Gy was carried outusing gamma rays (ɣ-ray) (Coblat 60 ^{60}Co, model: virus serial No.R97009 /made in France. This source exists in a radiotherapy department / Al-Amal Hospital for Cancer, Baghdad, Iraq).

In gamma irradiation, the amount of dose depends on time of exposure. (γ) Source gives (19.516Gy/h) (dosimetryis repeated every week end in the hospital).

5- Hematology Analyzer used to measurer Lymphocytes number and Hemoglobin concentration (for all blood samples) [Hematology Analyzer Diagon D-cell5D, serial No.171021655D, media in Hungary: 2011]. This was carried out also at Al-Amal Hospital.

6- (T-cell %) in blood samples before and after irradiation was measured byflow cytometry using CD3 marker The machine is CyFlow, Cube 6, PARTEC. Serial No.111201134, made in Germany, 2011 (private laboratory).

7-The exact T-cell number has been calculated by using the following formula

$$No.ofT-cell = Lymphocyts\ number * T-cell\%$$

Statistical analysis:-

Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences- version 22). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means or Paired-t-test for difference of paired

observations (or two dependent means). Statistical significance was considered whenever the P value for the test of significance was equal or less than 0.05.

Results and discussion:-

The results are summarized in table 1 and figures 1-4.
It is clear from the results that there was a significant reduction in means of total lymphocyte number, T-cell percent and T-cell counts after exposure to 25 Gy of gamma ray. This will result in reduction of the incidence of TA-GvHD which is a serious complication of blood transfusion. The effect on hemoglobin was much less pronounced and this is essential to keep blood units useful in treatment of anemia.

Up to my knowledge, this is the first research in Iraq studying the effect of gamma irradiation on the number of T-cells in blood bags.

Similar results were reported by Pelszynski et al. [10] They stated that with 2,500 or 3,000 cGy, no T-cell growth (>5 log_{10} depletion) was detected. Rosen et al study concluded that a nominal dose of 3000 cGy is the appropriate amount of gamma radiation needed to eliminateT-lymphocyte-mediated graft-versus-host disease. [11] Also Góes et al [12] found in their study that the results showed that a dose of2500 cGy completely inactivates T cells in RBC unitsirradiated with cobalt-60 source.

TA-GvHD is a very rare but usually fatal complication following transfusion of lymphocyte-containing blood components. Although the first reports concerned cases where viable allogeneic lymphocytes had been transfused into immunosuppressed recipients, [13,14 15] it became apparent that non-immunosuppressed patients could also experience this problem, particularly if the blood components they received came from an HLA haploidentical unrelated donor or family member.[16,17,18,19,20]

TA-GvHD is a potential complication of transfusion of any blood component containing viable T lymphocytes when there is disparity in the histocompatibility antigens between donor and recipient. As well as the classical skin, gut and liver involvement seen in GvHD occurring after allogeneic stem cell transplantation, TA-GvHD is characterized by profound marrow hypoplasia and mortality in excess of 90%. [17,21]. There is a particular risk of TA-GvHD when the donor and patient share an HLA haplotype, as occurs within families,[22] or in populations with restricted haplotype diversity.[23] In the Japanese population, the incidence of TA-GvHD is 10–20 times higher than in the North American Caucasian population.[24]

Diagnosis is usually made by biopsy of skin, gut or liver supported by evidence of persistence of donor lymphocytes. The presence of cells of donor origin may be demonstrated by polymerase chain reaction in peripheral blood [25] or short tandem repeat analysis using peripheral blood and skin biopsies from affected and non-affected sites in the patient, and peripheral blood samples from the implicated donors. [26]

Gamma or X-irradiation of blood components, by validated systems, is the recommended procedure to prevent TA-GvHD. BCSH guidelines. Studies using sensitive-limiting dilution assays indicate that a dose of 25 Gy, measured at the mid-plane of a component, completely abolishes mixed lymphocyte response.[10] The American Association of Blood Banks (AABB) recommends a dose of 25 Gy to the central area of the component with no portion receiving <15 Gy (AABB 2006). The Japanese Society of Blood Transfusion's Guidelines recommend a similar dose. [27] In the UK, a minimum of 25 Gy is recommended, but with the dose to any bag in the container not exceeding 50 Gy. To ensure this dose distribution is achieved, consultation with supporting physicists is mandatory. [28,29]

Lymphocyte viability is retained in stored red cells for at least 3 weeks and TA-GvHD has been reported after transfusion of whole blood, red cells, platelets and granulocytes.[30] TA-GvHD has not been described following transfusion of frozen deglycerolized red cells, which are thoroughly washed free of leucocytes after thawing. TA-GvHD has not been described following transfusion of cryoprecipitate, fresh frozen plasma or fractionated plasma products, such as clotting factor concentrates, albumin and intravenous immunoglobulin.

Effects on Red Cells:-
Irradiation may affect the 24 hour recovery of transfused red cells, however, this effect is most pronounced only after prolonged storage. [31] Published data demonstrate that red cells irradiated within 24 hours of collection

maintain satisfactory viability up to 28 days, while units stored for 42 days had unsatisfactory viability. [32] (Other studies have suggested that gamma irradiation did not significantly affect the 24-hour post-transfusion recovery of red cells stored for 35 days; further, red cell viability was not affected by whether the blood was irradiated at one or 14 days after collection. [33]

Gamma irradiation increases the supernatant potassium level. Extracellular potassium levels increase more rapidly during storage in irradiated compared with non-irradiated red cells. [34] Further, the increase correlates with the initial radiation dose. Rapid infusion of potassium can have deleterious cardiac effects. Generally, with "top-up" transfusions infused at usual rates, the potassium load is of little clinical significance. However, it is of concern in infants and in large volume transfusions such as exchange transfusion, intrauterine transfusion and rapid massive transfusion in resuscitation settings. Therefore, in considering the clinical significance, both the speed and volume of the transfusion, as well as the age of the blood, must be taken into account.

Irradiated red cells have been shown to contain more cell-free hemoglobin (approximately 50% increase) than control cells after equivalent periods of storage. There is no demonstrable, clinically significant effect of irradiation on red cell pH, glucose consumption, ATP or 2,3 DPG levels.[35]

Table 1.Comparison of Hb concentration, total lymphocyte count,T-cell % and T-cell number of blood bags before and after exposure to 25 Gy gamma ray

γ-ray doses in Gy	Mean±SD (Range)
Control Hb (g/dl)	14.9±1.6 (12.0-17.0)
Hb 25Gy(g/dl)	11.2±1.2 (9.0-13.0)
P value	0.0001*
Control Total Lymphocyte (*10^9/l)	4.267±1.438 (2.000-8.000)
Total Lymphocyte 25Gy(*10^9/l)	1.800±0.775 (1.000-4.000)
P value	0.0001*
Control T-cell%	57.467±10.501 (40.000-69.000)
T-cell% 25Gy	44.533±9.265 (30.000-58.000)
P value	0.0001*
Control T-cellnumber (*10^9/l)	2.482±1.081 (0.903-5.106)
T-cellnumber 25Gy (*10^9/l)	0.865±0.383 (0.335-1.814)
P value	0.0001*
*Significant difference between two dependent means using Paired-t-test at 0.05 level. *Significant difference between two independent means (type of exposure) using Student-t-test at 0.05 level.	

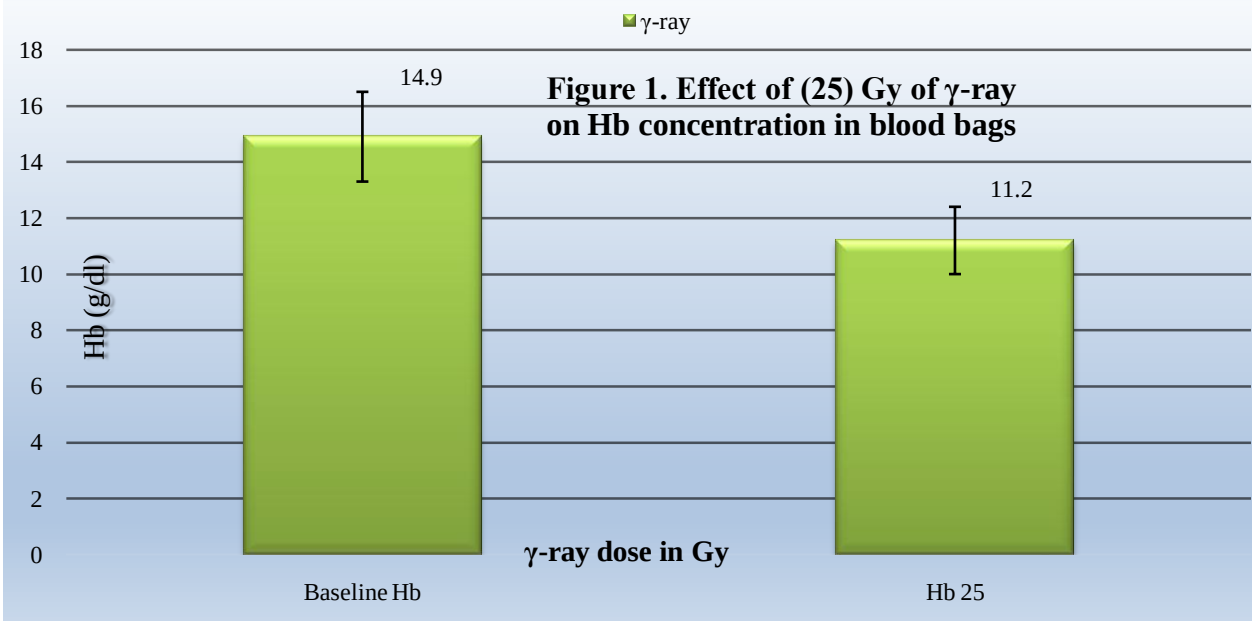


Figure 1. Effect of (25) Gy of γ-ray on Hb concentration in blood bags

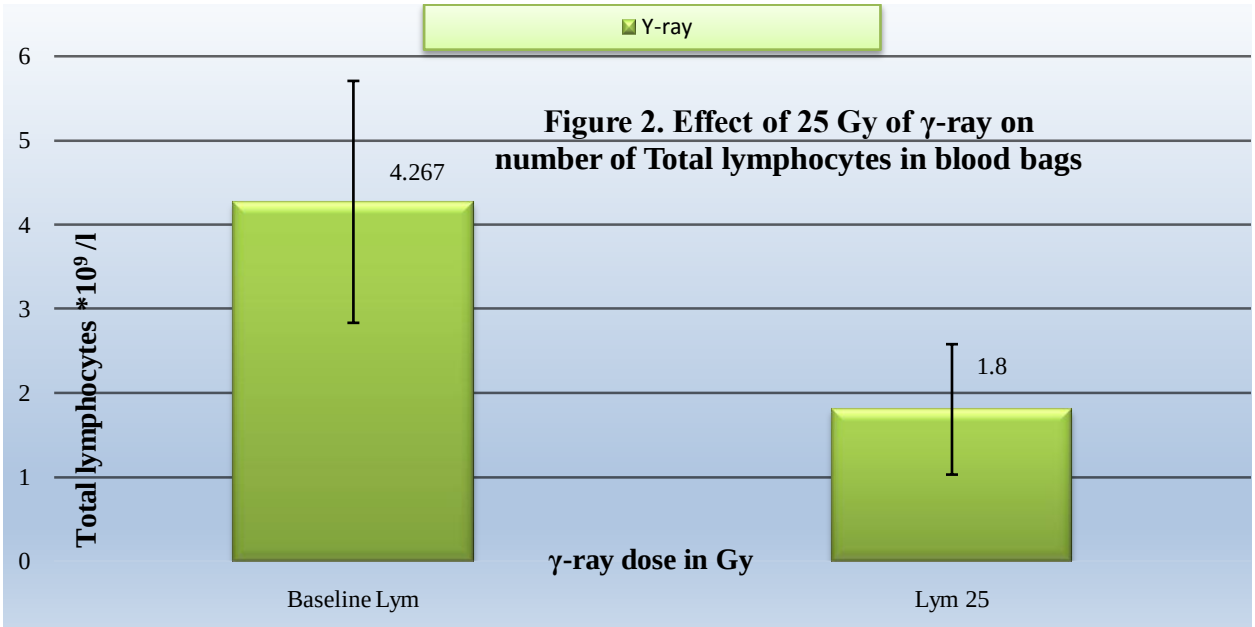
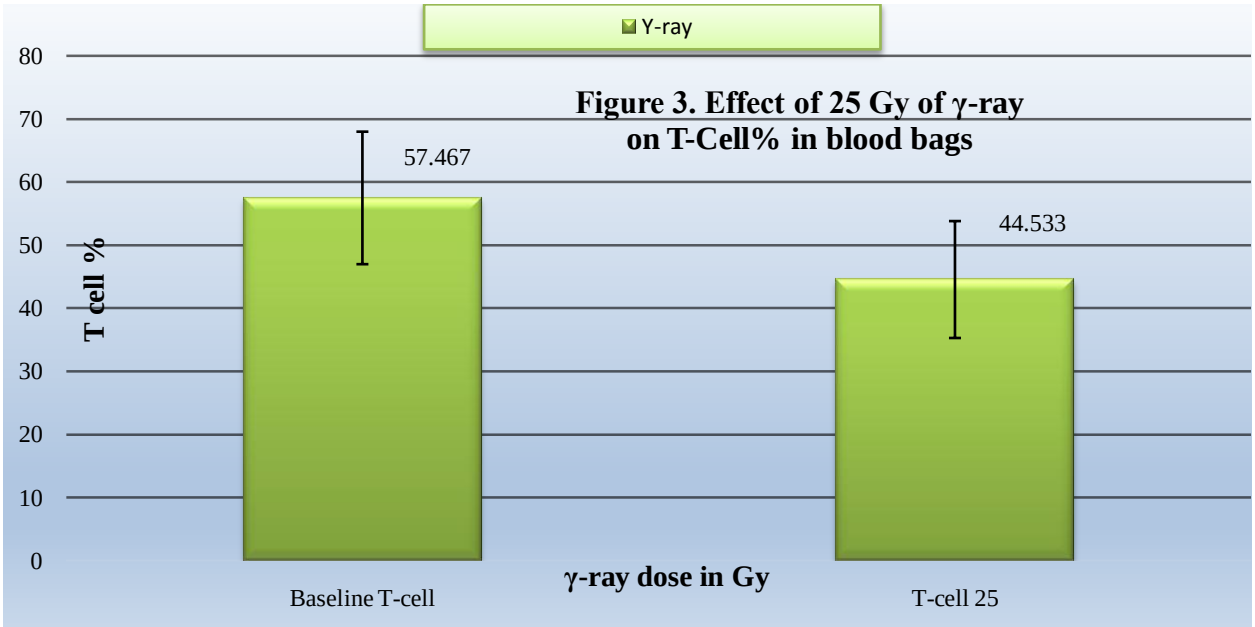
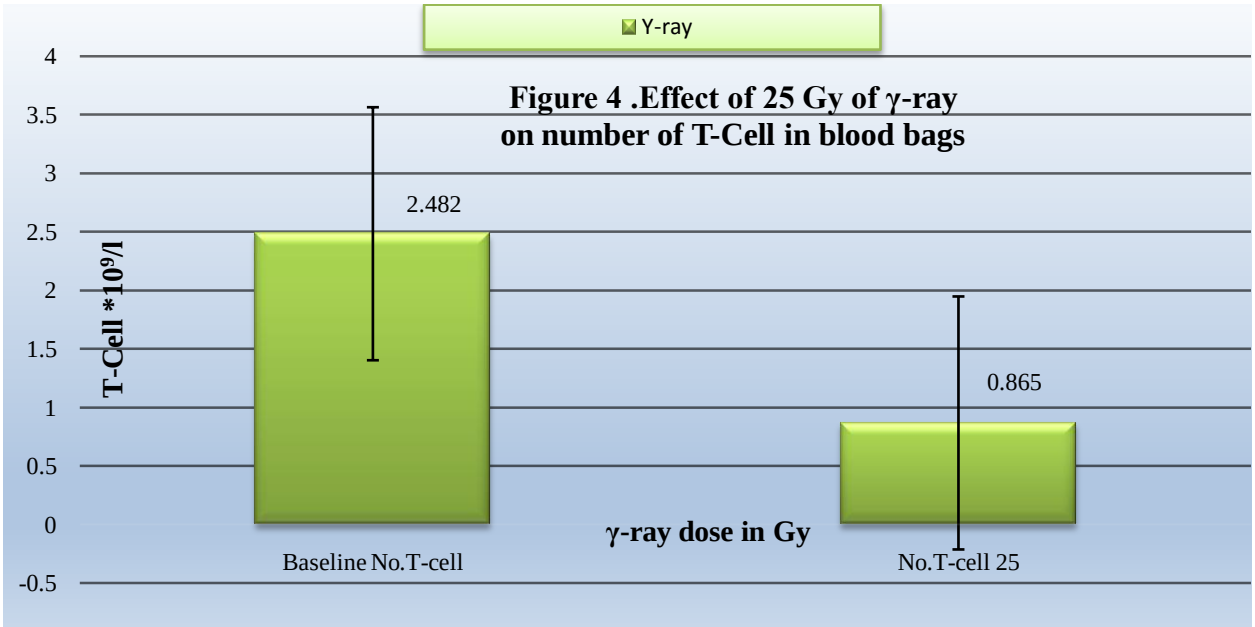


Figure 2. Effect of 25 Gy of γ-ray on number of Total lymphocytes in blood bags


Y-ray
Figure 3. Effect of 25 Gy of γ-ray on T-Cell% in blood bags
T cell %
80
70
60
50
40
30
20
10
0
57.467
44.533
Baseline T-cell
γ-ray dose in Gy
T-cell 25


Y-ray
Figure 4 .Effect of 25 Gy of γ-ray on number of T-Cell in blood bags
T-Cell *10⁹/l
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
2.482
0.865
Baseline No.T-cell
γ-ray dose in Gy
No.T-cell 25

Conclusion:-

1. There was a significant reduction of total lymphocyte count, T-Cell% and T-cell numbers after exposure of blood bags to 25 Gy gamma ray.
2. Hb concentration was mildly reduced during irradiation.
3. The reduction in T-cell numbers outweighs the reduction in Hb concentration ifTA-GvHD is to be prevented.

Recommendation:-

Conducting similar study using x-ray.

References:-

1. Jacobsohn DA, Vogelsang GB. Acute graft versus host disease. Orphanet J Rare Dis. 2007 Sep 4;2:35.
2. Bolaños-Meade J, Vogelsang GB. Acute graft-versus-host disease. ClinAdvHematolOncol. 2004 Oct;2(10):672-82.
3. RE Dinsmore, DJ Straus, MS Pollack, JM Woodruff, TJ Garrett, CW Young, BD Clarkson and B Dupont. Fatal graft-versus-host disease following blood transfusion in Hodgkin's disease documented by HLA typing. Blood. 1980 May; 55(5): 831-834.
4. E. G. Góes, D. T. Covas, R. Haddad, C. A. Pelá, C. E. Formigoni& J. C. Borges. Quality control system for blood irradiation using a teletherapy unit.VoxSanguinis. 2004 ;86: 105–110.
5. Dennis O'Neil (1999). Blood Components. Palomar College. Available from: http://anthro.palomar.edu/blood/blood_components.htm
6. Chapman J, Finney RD, Formam K. Guide on gamma irradiation of blood components for prevention of transfusion-associated graft-versus-host disease. Transfusion 1996; 6:261–271.
7. James E. Turner. Atoms, Radiation, and Radiation Protection. Third Edition, 2007 WILEY-VCH Verlag GmbH & Co.,KGaA, Weinheim,ISBN 978-3-527-40606-7, pg 68.
8. Park B, Yee C, Lee KM. The Effect Radiation on the Immune Response to Cancers.Int J Mol Sci. 2014 Jan 10;15(1):927-43. doi: 10.3390/ijms15010927.
9. Seah BT .Effect of Gamma Ray on Hemoglobin for Some Patients with Thalassemia in vitro.M.Sc.Thesis submitted to the department of Physics /Faculty of Science / Mustansiriya University (2000).
10. Pelszynski MM, Moroff G, Luban NL, Taylor BJ, Quinones RR. Effect of gamma irradiation of red blood cell units on T-cell inactivation as assessed by limiting dilution analysis: implications for preventing transfusion-associated graft-versus-host disease. Blood. 1994 Mar 15;83(6):1683-9.
11. Rosen NR,Weidnerh JG, Boldt D, and Rosen DS. Prevention of transfusion-associated graft-versus-host disease:selection of an adequate dose of gamma radiation. Transfusion 1993;33(2):125-127.
12. Góes EG, Borges JC,Covas DT, Orellana MD, Palma PVB,Morais FR, andPelá CA. Quality control of blood irradiation: determination T cells radiosensitivity to cobalt-60 gamma rays. Transfusion. 2006 January; 46(1): 34-40.
13. von Fliedner V, Higby DJ, Kim U. Graft-versus-host reaction following blood product transfusion. Am J Med. 1982 Jun;72(6):951-61.
14. Burns LJ, Westberg MW, Burns CP, Klassen LW, Goeken NE, Ray TL & Macfarlane DE. Acute graft-versus-host disease resulting from normal donor blood transfusions. ActaHaematol. 1984;71(4):270-6.
15. Anderson KC & Weinstein HJ. Transfusion-associated graft versus-host disease. N Engl J Med. 1990 Aug 2;323(5):315-21.
16. Ohto H, Yasuda H, Noguchi M & Abe R. Risk of transfusion-associated graft-versus-host disease as a result of directed donations from relatives. Transfusion. 1992 Sep;32(7):691-3.
17. Aoun E, Shamseddine A, Chehal A, Obeid M, Taher A.Transfusion-associated GVHD: 10 years' experience at the American University of Beirut-Medical Center. Transfusion. 2003 Dec;43(12):1672-6.
18. Serefhanoglu K, Turan H, Saba T, Ozer I, Tosun E &Arslan H. Transfusion-associated graft-versus-host disease in an immunocompetent individual following cardiac surgery. J Natl Med Assoc. 2005 Mar;97(3):418-20.
19. Triulzi D, Duquesnoy R, Nichols L, Clark K, Jukic D, Zeevi A &Meisne D. Fatal transfusion-associated graft-versus-host disease in an immunocompetent recipient of a volunteer unit of red cells. Transfusion. 2006 Jun;46(6):885-8.
20. Agbaht K, Altintas ND, Topeli A, Gokoz O &Ozcebe O. Transfusion-associated graft-versus-host disease in immunocompetent patients: case series and review of the literature. Transfusion. 2007 Aug;47(8):1405-11.

21. Williamson LM, Stainsby D, Jones H, Love E, Chapman CE, Navarrete C, Lucas G, Beatty C, Casbard A & Cohen H.The impact of universal leukodepletion of the blood supplyon hemovigilance reports of posttransfusionpurpura and transfusion-associated graft-versus-host disease. Transfusion. 2007 Aug;47(8):1455-67.
22. Petz LD, Calhoun L, Yam P, Cecka M, Schiller G, Faitlowicz AR, Herron R, Sayah D, Wallace RB&Belldegrun A. Transfusion-associated graft-versus-host disease in immunocompetentpatients: report of a fatal case associated with transfusion ofblood from a second-degree relative, and a survey of predisposingfactors. Transfusion. 1993 Sep;33(9):742-50.
23. Yasuura K, Okamoto H& Matsuura A. Transfusion-associatedgraft-versus-host disease with transfusion practice in cardiacsurgery.J CardiovascSurg (Torino). 2000 Jun;41(3):377-80.
24. Shivdasani RA, Haluska FG, Dock NL, Dover JS, Kineke EJ&Anderson KC. Brief report: graft-versus-host disease associatedwith transfusion of blood from unrelated HLA-homozygousdonors. N Engl J Med. 1993 Mar 18;328(11):766-70.
25. Utter GH, Reed WF, Lee TH & Busch MP. Transfusionassociatedmicrochimerism. Vox Sang. 2007 Oct;93(3):188-95.
26. Sage D, Stanworth S, Turner D & Navarrete C. Diagnosis oftransfusion-associated graft-vs.-host disease: the importance of shorttandem repeat analysis. Transfus Med. 2005 Dec;15(6):481-5.
27. Asai T, Inaba S, Ohto H, Osada K, Suzuki G, Takahashi K,Tadokoro K & Minami M. (2000) Guidelines for irradiation ofblood and blood components to prevent post-transfusion graft vshost disease in Japan.Transfus Med. 2000 Dec;10(4):315-20.
28. Moroff G&Luban NL. The irradiation of blood and bloodcomponents to prevent graft-versus-host disease: technical issuesand guidelines. Transfus Med Rev. 1997 Jan;11(1):15-26.
29. Moroff G, Leitman SF &Luban NL. Principles of bloodirradiation, dose validation, and quality control.Transfusion. 1997 Oct;37(10):1084-92.
30. Weiden PL, Zuckermn N, Hansen JA, Sale GE, Remlinger K,Beck TM & Buckner CD. Fatal graft-versus-host disease ina patient with lymphoblastic leukemia following normal granulocytetransfusion. Blood. 1981 Feb;57(2):328-32.
31. FDA Recommendations Regarding License Amendments and Procedures for Gamma Irradiation of Blood Products. Food and Drug Administration. 1993
32. Davey RJ, McCoy NC, Yu M, Sullivan JA, SpiegelDM &Leitman SF. The effect of pre-storage irradiation on post-transfusion red cell survival. Transfusion.1992 Jul-Aug; 32(6), 525-8.
33. Mintz PD & Anderson G. Effect of gamma irradiation on the in vivo recovery of stored red blood cells. Ann Clin Lab Sci. 1993 May-Jun;23(3):216-20.
34. Dinning G, Doughty RW, Reid MM & Lloyd HL. Potassium concentrations in irradiated blood. BMJ. 1991 Nov 2; 303(6810): 1110.
35. Australian and New Zealand Society of Blood Transfusion Ltd. Guidelines for Prevention of Transfusion-Associated Graft versus Host Disease (TA-GVHD). 1st Edition, January 2011.