

Reduction of T-lymphocytes in Blood Samples Using X-ray of Two Different Energies

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Abstract

In this research the effect of X-ray with two different energies (6&10) MeV on the number of T-cells in whole blood samples collected from 50 donors (only men) was studied. Blood samples were exposed to (0,5,10,15,20,25,30,35,40) Gray doses of X-rays. The absolute lymphocytes number (Lym), T-cell % and T-cell number) were measured before and after irradiation, then the reduction of each value was calculated. By making comparison between the effects of two energies, it was observed that the use of X-rays 6MeV is more useful because T-cell number reduced with increasing the doses more than when using X-ray 10Mev.

Keywords: T-lymphocytes, X-ray, transfusion associated-GVHD.

1. Introduction

Lymphocytes are the most common white blood cells, which are 20-25% of the total number of white blood cells in the bloodstream. The lymphoid cells are spherical in shape, and have a nucleus spherical dense dyeing, occupy most of the space cell relatively small amount of cytoplasm base-line weak pigment [1]. Lymphocytes are two major kinds of cells, T-cells and B-cells

T-cells are a type of white blood cell that circulates around our bodies, scanning for cellular abnormalities and infections. They are called T cells because they mature in the thymus (although some also mature in the tonsils). The several subsets of T cells each have a distinct function. [2] There are several subsets of T cells, each with a distinct function.

- 1) **T helper cells (Th cells):** are a type of T cell that play an important role in the immune system are the major driving force and the main regulators of the immune defense. Their primary task is to activate B cells and killer T cells. [3,4] Every mature T cell expresses the CD3 molecule. [5]
- 2) **Cytotoxic T cells (T_C cells, or CTLs):** kill cancer cells, cells that are infected (particularly with viruses). [6] These cells destroy virus-infected cells and certain types of tumor cells in an antigen-specific manner.[3]
- 3) **Memory T cells:** are a subset of infection- as well as potentially cancer-fighting T cells.[4,5] Such T cells can

recognize foreign invaders, such as bacteria or viruses, as well as cancer cells.[7]

- 4) **Regulatory T cells (Treg cells):** Regulatory T cells engage in the maintenance of the thymus as a functionally mature subpopulation of T cells and can also be induced from naive T cells in the periphery. [8]
- 5) **Natural killer T cells (NKT cells):** Is a heterogeneous group of T cells that share properties characteristic of both T cells and NK cells, and possess a variety of unusual properties with regard to antigen recognition and function. [9] These cells provide innate protection by killing tumor cells and cells infected with intracellular pathogens. [7] Natural killer cells account for 10–15% of blood lymphocytes and are found in low numbers in the peripheral lymphoid system. [5] Killer T-cells find and destroy infected cells that turned into virus-making factories. To do this they need to tell the difference between the infected cells and healthy cells with the help of special molecules called antigens. Killer T-cells are able to find the cells with viruses and destroy them. [10]

Antigens work like identification tags that give your immune system information about your cells and any intruders. Healthy cells have 'self-antigens' on the surface of their membranes. They let T-cells know that they are not intruders. If a cell was infected with a virus, it has pieces of virus antigens on its surface. This is a signal for the Killer T-cell that lets it know this is a cell that must be destroyed.[2,4,9]

Graft-Versus-Host Disease (GVHD) occurs after allogeneic hematopoietic stem cell transplant and is a reaction of donor immune cells against host tissues.[11,12] 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. [11,13] 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%. [12]

TA-GVHD (transfusion associated 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. [12,14]

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 [15]. 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.[16] Gamma and X-rays, both representing ionizing radiation, damage DNA T lymphocytes and arrest responses to allogeneic cells.[17] Thus, these lymphocytes are unable to proliferate in the host and therefore cannot mediate TA-GvHD proliferate in the host and therefore cannot mediate TA-GvHD. [14]

2. Materials and Method

This study was approved by the ethical committees of both department of pathology at Al-Mustansiriya University, College of Medicine and department of physics at Faculty of Science, University of Baghdad. This is in conformation to the Declaration of Helsinki. Informed consent was obtained from all participants.

1)Twenty mls of whole blood have been collected from 50 donors, either from a vein in the arm using syringe size (20) ml, or by drawing (20) ml from blood bags collected from the National Center of Hematology. These donors, mostly suffered from high viscosity. In both cases the donors were men, their age range was 20-55 years.

2)Blood samples are divided into nine parts (2) ml for each part. Samples have been put in the laboratory tubes containing the anti-clotting EDTA.

3)(8) part of blood samples have been irradiated by (5,10,15,20,25,30,35,40) Gy of:

a- (6) MeV X-ray.

b-(10)MeV X-ray.

4)Linear Accelerator X-ray has been used for irradiation; primus Mid; Serial No. 3779; Siemens. /Germany) This device is present in radiotherapy department / Al-Amal hospital for cancer, designed for Therapeutic purposes). All tubes were put in plastic racks and placed under one parallel field two levels X-ray source .Time of exposure for every dose with (expect of control samples which were not irradiated) automatically depend on the dose.

5)By using Hematology Analyzer lymphocytes (Lym) were counted for all blood samples (controls and irradiated by X-ray (6and 10 MeV).

The Hematology Analyzer used in this project is a model: Diagon D-cell 5D, serial No.171021655D, made in Hungary: 2011.

6)The main aim of this research is measuring T-cell number in blood samples before and after irradiation, Flow Cytometry was used for accurate measurement of T-cell%.

The machine used is (CyFlow. Cube 6, PARTEC. Serial No.111201134, made in Germany, 2011 ((private laboratory)), and by using CD3) marker.

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

$$No. of T - cell = Lym account * T - cell\% \dots (1)$$

3. Results and discussion

As it's mentioned before the aim of the current study is to kill and reduce T-cells to the lowest value in the blood samples by using X-rays in order to avoid TA-GVHD. In order to determine the appropriate dose to irradiate blood bags it was necessary to test several doses of X-ray on blood samples of each donor. Lymphocyte counts have been measured in blood samples before and after irradiation with increasing X-ray doses. Also comparison was made between lymphocyte counts with different X-ray energies used. These results are statistically treated as in table 1.

3.1 Effect of X-ray on Lymphocytes Number

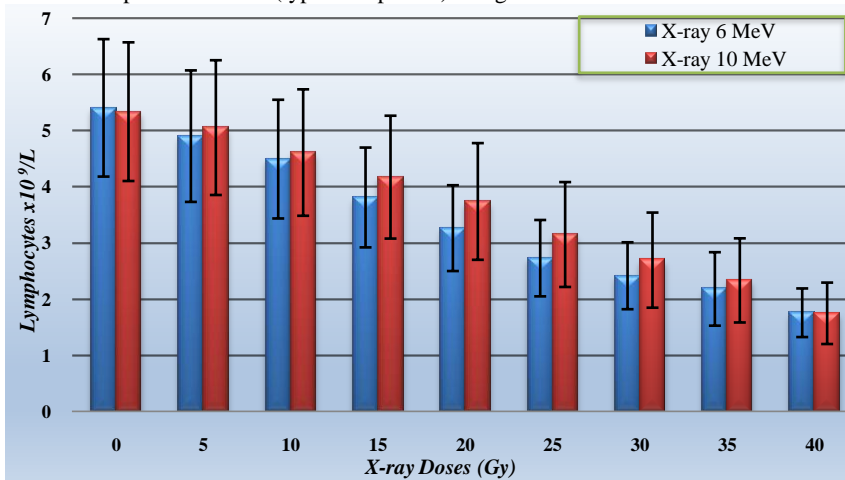
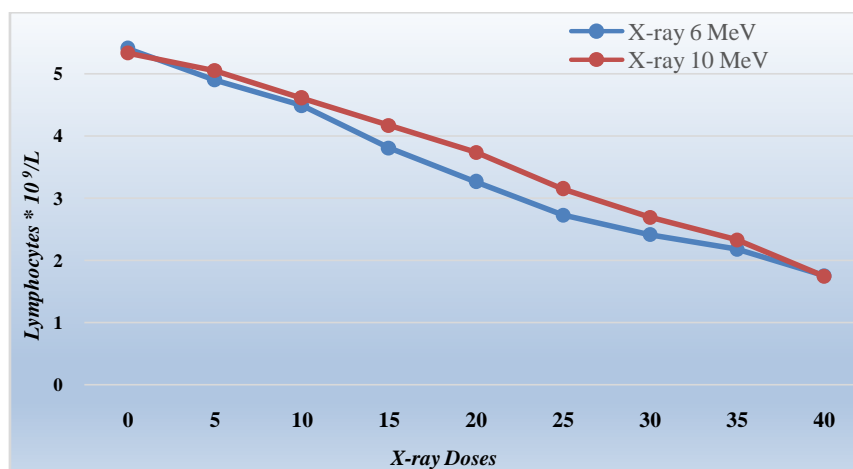
The change in number of Lymphocytes with increasing X-ray (6 &10) MeV, doses measured in blood samples before and after irradiation, also a comparison between the effect of the two energy was made .The results are statistically treated and arranged in a table 1, then plotted as in Figure 1, and linearly in Figure 2, these graphs clarify the inverse relation between Lymphocytes number and increasing doses, depending on X-ray energies (6&10) MeV.

Table 1: shows the effect of X-ray (6&10)MeV for Lymphocytes number in blood samples

X-ray doses in (Gy)	X-ray 6 MeV		X-ray 10 MeV		X6xX10
	Lymphocytes*10 ⁹ /L				
	Mean±SD (Range)		Mean±SD (Range)		
Control 0	5.402±1.224 (3.000-7.430)		5.334±1.235 (2.970-7.640)		0.862
5	4.898±1.171 (2.860-6.870)		5.050±1.200 (2.760-7.260)		0.688
10	4.489±1.057 (2.420-6.230)		4.606±1.126 (2.260-6.720)		0.736
15	3.806±0.889 (2.070-5.320)		4.168±1.094 (2.010-6.280)		0.257
20	3.260±0.764 (1.850-4.350)		3.735±1.039 (1.840-5.630)		0.108
25	2.725±0.680 (1.470-3.630)		3.146±0.934 (1.560-4.920)		0.111
30	2.412±0.596 (1.350-3.220)		2.690±0.847 (1.220-4.140)		0.238
35	2.177±0.654 (1.120-3.980)		2.329±0.749 (0.940-3.650)		0.498
40	1.753±0.433 (0.920-2.460)		1.743±0.547 (0.690-2.860)		0.949

*All showed a highly significant difference between two dependent means using Paired-t-test at 0.05 level when compared to baseline or compared to previous measurement (P<0.0001).

*Significant difference between two independent means (type of exposure) using Student-t-test at 0.05 level.

**Figure 1: Graph showing statistic relation between Lymphocytes number and X-ray (6&10) MeV, doses****Figure 2: Graph showing linear relation between Lymphocytes number and X-ray (6&10)MeV, doses**

The decreasing percent in lymphocytes number (Lymphocytes %) can be extracted in two ways: First: depending on difference from control samples as shown in table 2 and plotted in Figure 3. The difference means that each value subtract from (0)Gy value as clear in table 1.

Table 2 explain the lowest value in reduction (Lymphocytes%) at dose 5Gy is (-9.49±3.16) at (6)MeV, while at (10)MeV (-5.45±2.32). The higher value of this

reduction at (40)Gy is (-67.69±2.03) at (6)MeV, (-67.92±4.38) at (10)MeV. It's well-known that ionization radiation of X-ray induces apoptosis in Lymphocytes, causing profound depletion of granulocytes and natural killer cells[1]. This degradation in Lymphocytes value attributed to direct and indirect effect of radiation means that either directly killing cells or generating free radicals that destroying these cells.

**Table 2: shows decreasing percent of Lymphocytes with effect of X-ray at 6&10 MeV (different from control value)
*minus sign refers to decreasing in Lymphocytes value**

X-ray Doses in Gy	Lymphocytes% number Difference from control		
	6 MeV Mean±SD	X-ray 10 MeV Mean±SD	X6 x X10
05	-9.49±3.16	-5.45±2.32	0.0001*
10	-17.04±2.51	-13.86±4.58	0.010*
15	-29.59±2.91	-22.33±5.25	0.0001*
20	-39.64±3.53	-30.58±5.72	0.0001*
25	-49.71±3.47	-41.62±6.38	0.0001*
30	-55.47±3.22	-50.22±6.78	0.003*
35	-60.13±4.92	-57.15±6.06	0.096
40	-67.69±2.03	-67.92±4.38	0.832

*All showed a highly significant difference between two dependent means using Paired-t-test at 0.05 level when compared to baseline or compared to previous measurement ($P < 0.0001$). *Significant difference between two independent means (type of exposure) using Student-t-test at 0.05 level.

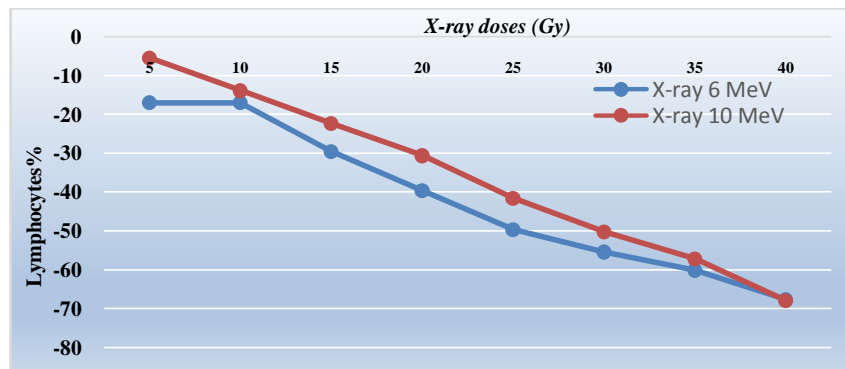


Figure 3: Graph showing the relation between the reduction of (Lymphocytes %) with doses of X-rays (6&10) MeV, (Depending on difference from control)

Second: depending on difference from previous as shown in table 3, which plotted in figure 4. The difference here means that each subtracted from the previous value.

Table 3: shows the results of decreasing of Lymphocytes% with increasing of X-ray different from previous

X-ray doses in Gy	Lymphocytes% different from previous		X6 x X10
	X-ray 6 MeV Mean±SD	X-ray 10 MeV Mean±SD	
5	-9.49±3.16	-5.45±2.32	0.0001*
10	-8.28±3.03	-8.91±4.11	0.589
15	-15.10±3.33	-9.86±2.79	0.0001*
20	-14.24±4.48	-10.66±2.86	0.005*
25	-16.61±4.96	-16.04±3.69	0.685
30	-11.28±5.94	-14.86±5.12	0.048*
35	-10.25±11.59	-13.56±9.27	0.324
40	-18.27±7.12	-24.91±5.28	0.002*

*All showed a highly significant difference between two dependent means using Paired-t-test at 0.05 level when compared to baseline or compared to previous measurement ($P < 0.0001$). *Significant difference between two independent means (type of exposure) using Student-t-test at 0.05 level.

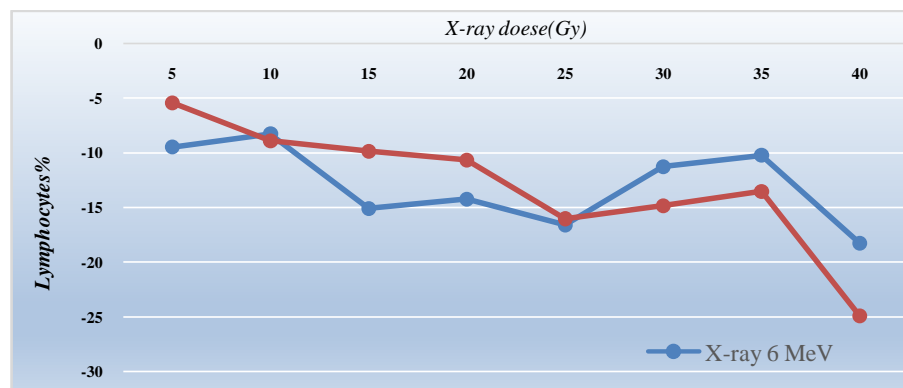


Figure 4: Graph showing the relation between the reductions of (Lymphocytes %) with every dose of X-rays (depending on difference from previous).

It is observed from Figure 4 the dose 25Gy at (6) MeV is the most effected dose, where the dose 35Gy at 10) MeV is the most effective dose. The inverse relation between Lymphocytes number and X-ray doses is the same at (6 &10) MeV, but by comparing the effect of two energies it observed that the reduction in number of lymphocytes in X-ray (6MeV) greater that in (10MeV).

4.3 Effect of X-ray on T-cells% and T-cells Number

The percentage value of T-cells in blood samples were measured by flow cytometry, before and after irradiation with X-ray (6&10) doses, the results are arranged in a table 4, which statistically plotted in figure 5, and linearly in figure 6.

Table 4: shows the change T-cells% with the effect X-ray (6 &10) MeV doses.

X-ray doses In Gy	T-Cell%		X6xX10
	X-ray 6 MeV	X-ray 10 MeV	
	Mean±SD (Range)	Mean±SD (Range)	
0 control T-Cell%	60.813±12.276 (40.920-77.560)	59.259±12.148 (40.450-77.560)	0.690
05	56.116±10.566 (37.620-74.400)	55.854±12.282 (38.320-76.470)	0.943
10	50.360±8.482 (37.280-68.240)	52.537±12.174 (35.230-73.560)	0.516
15	44.666±8.753 (32.560-63.550)	48.046±11.546 (30.250-68.740)	0.303
20	40.886±7.201 (32.140-57.320)	43.033±10.312 (27.650-62.590)	0.450
25	34.660±6.234 (23.620-48.410)	38.174±9.004 (25.680-54.370)	0.159
30	30.789±5.793 (22.120-43.540)	33.300±8.351 (21.420-47.660)	0.276
35	27.371±5.364 (17.390-37.830)	28.400±7.781 (17.470-42.450)	0.629
40	23.953±5.823 (14.540-35.310)	23.494±7.379 (12.560-40.300)	0.828

*All showed a highly significant difference between two dependent means using Paired-t-test at 0.05 level when compared to baseline or compared to previous measurement (P<0.0001).*Significant difference between two independent means (type of exposure) using Student-t-test at 0.05 level.

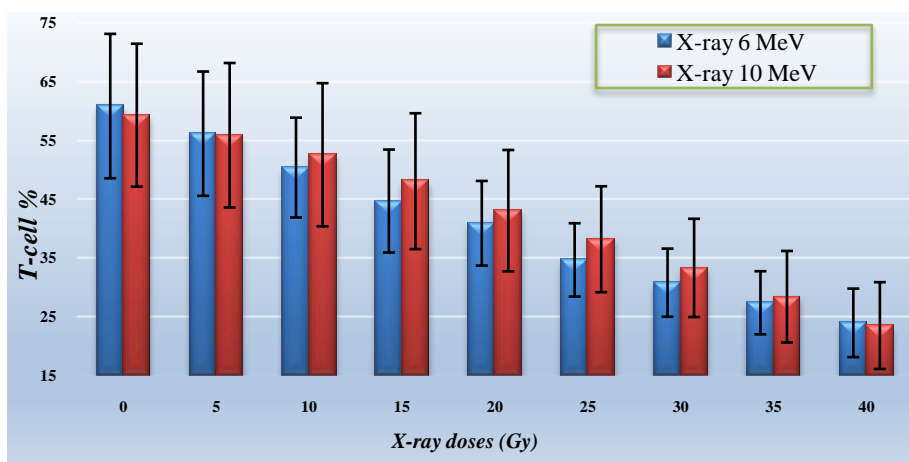


Figure 5: Graph showing statistical relation between the effect of X-ray (6&10) MeV, doses on T-cell%

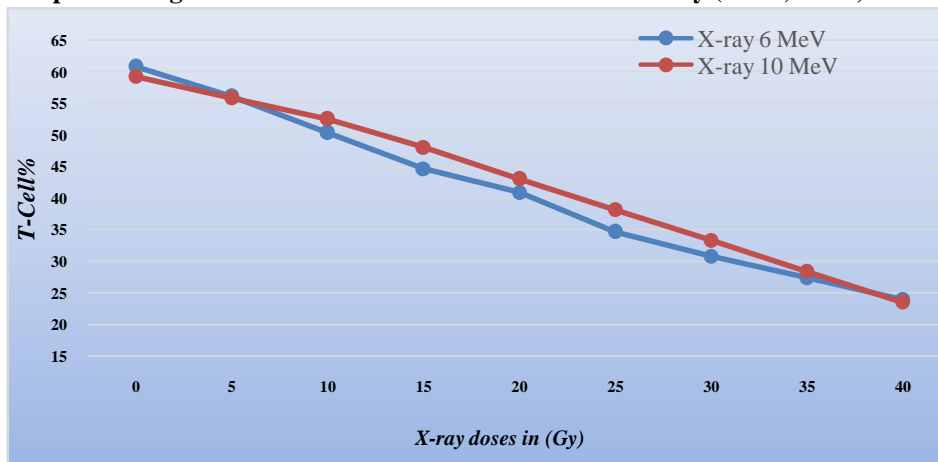


Figure 6: Graph showing linear relation between the effect of X-ray (6&10)MeV doses on %T-cell.

Equation (3-1) used to find the number of T-cells/L, depending on results in table 1 and 4, which are statistically treated in table 5 and plotted, in Figure 7, and linearly in Figure 8.

Table 5: shows the change of T-cell number with increasing X-ray doses (6&10) MeV

X-ray Doses (Gy)	Number of T-cells*10 ⁹ /L		X6xX10
	X-ray 6 MeV	X-ray 10 MeV	
	Mean±SD (Range)	Mean±SD (Range)	
0	3.295±1.004 (1.228-5.235)	3.238±1.194 (1.201-5.235)	0.870
5	2.758±0.869 (1.137-4.613)	2.895±1.127 (1.058-4.825)	0.670
10	2.275±0.714 (0.952-3.897)	2.490±1.012 (0.819-4.303)	0.443
15	1.700±0.514 (0.703-3.019)	2.064±0.888 (0.677-3.721)	0.121
20	1.339±0.416 (0.600-2.385)	1.663±0.744 (0.542-3.035)	0.098
25	0.947±0.312 (0.439-1.757)	1.247±0.577 (0.401-2.312)	0.048*
30	0.747±0.253 (0.322-1.402)	0.926±0.444 (0.280-1.815)	0.125
35	0.599±0.214 (0.249-1.082)	0.688±0.338 (0.164-1.334)	0.330
40	0.420±0.141 (0.172-0.684)	0.421±0.201 (0.101-0.842)	0.991

*All showed a highly significant difference between two dependent means using Paired-t-test at 0.05 level when compared to baseline or compared to previous measurement (P<0.0001).

*Significant difference between two independent means (type of exposure) using Student-t-test at 0.05 level.

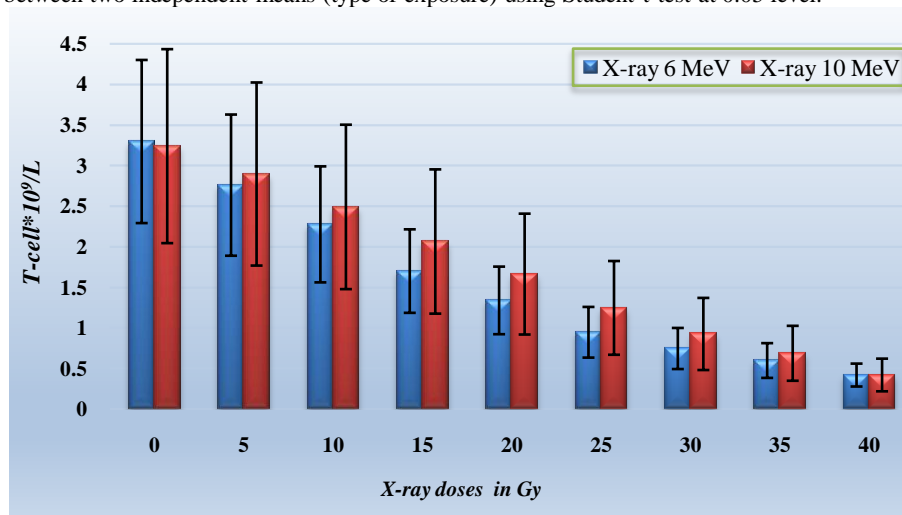
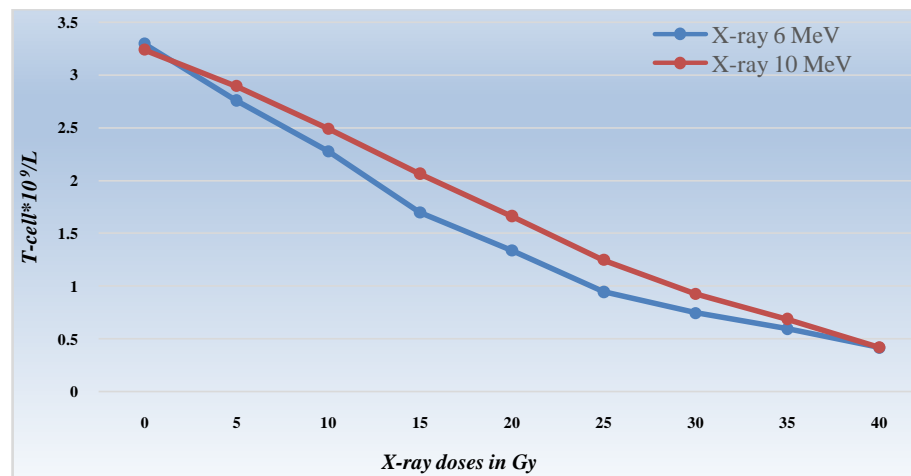
**Figure 7: Graph showing statistical relation between effects of X-ray (6 and 10) MeV doses in T-cell number****Figure 8: Graph showing linear relation between the effect of X-ray (6&10) MeV doses on T-cell number.**

Table 5 showed the inverse relation between X-ray doses and T-cells number. Generally this inverse relation attributed to direct and indirect [79]. X-ray as one of ionizing radiation hit the nuclei cells which caused the damage in DNA chains of the T-cells, where radiation causing fragmentation in the DNA chains in some cases lead randomly cross linking in these chains, and also leads to inhabitation of cell efficiency. An interaction may be affect

the ability of the cell reproduce and thus survive. If enough radiation damage reached to cell, the chromosomes dos not replicate, then the cell may distorted by direct interference with its life,-sustaining system or indirect effect which impact on T-cell and on water molecules in whole blood samples. when radiation interact 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) leads to destruction the cell [80], depending on exposure time (increase exposure time will causes increasing in number affected cells).

At dose 40Gy the effect of two energies found the same, which observed high dropping in Lymphocytes and T-cells. The measured percentage value of reduction T-cell number which effected by X-ray doses depend on:

a-The difference from control (0)Gy which explain in table 6 and plotted in figure 9.

Table 6: shows the T-cell% decreasing with x-ray (6&10)MeV doses (different from control).

X-ray doses In Gy	T-cell%, decreasing different from control (0)		
	X-ray 6 MeV	X-ray 10 MeV	X6 x X10
	Mean±SD	Mean±SD	
5	-16.00±6.66	-11.08±3.81	0.007*
10	-30.25±8.68	-23.97±5.26	0.009*
15	-47.56±7.88	-37.41±5.68	0.0001*
20	-58.80±5.73	-49.85±5.33	0.0001*
25	-70.74±5.73	-62.54±4.94	0.0001*
30	-76.92±4.99	-72.26±4.20	0.003*
35	-81.71±3.99	-79.68±3.65	0.101
40	-87.09±3.01	-87.54±2.12	0.591

*All showed a highly significant difference between two dependent means using Paired-t-test at 0.05 level when compared to baseline or compared to previous measurement ($P<0.0001$).

*Significant difference between two independent means (type of exposure) using Student-t-test at 0.05 level.

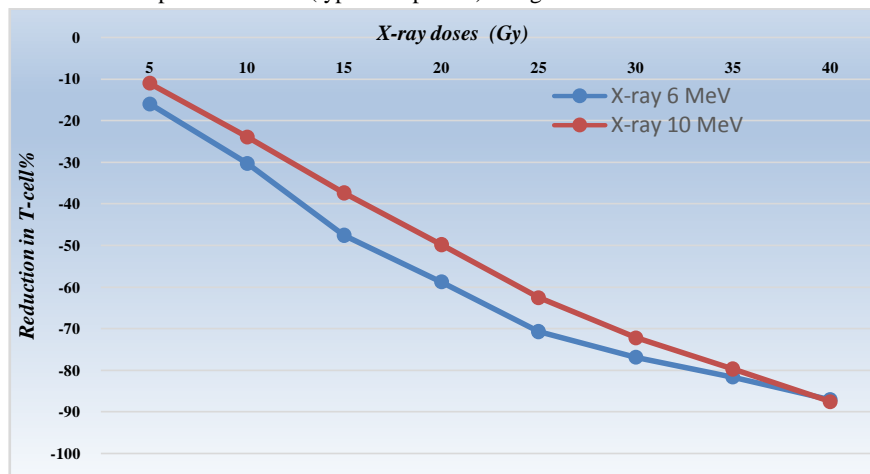


Figure 9: Graph showing T-Cell%, decreasing value (difference from control) with increasing X-ray (6 & 10) MeV dose.

Its observed from the results in table 6, that values, that X-ray doses, counterproductive effected on T-cell% values, means that the increasing in X-ray doses caused

decreases in T-cells%, this declining seems low in the first doses (5&10)Gy at (10)MeV, but high decrement at (40)Gy.

b- The difference from previous, which shows in table 7 and plotted in figure 10.

Table 7: show T-cell% decreasing value different from previous for X-ray

Difference from previous	X-ray 6 MeV	X-ray 10 MeV	X6 x X10
	Mean±SD	Mean±SD	
5	-16.00±6.66	-11.08±3.81	0.007*
10	-17.04±7.15	-14.51±4.33	0.183
15	-24.71±7.49	-17.76±2.74	0.0001*
20	-21.09±5.86	-19.91±3.51	0.443
25	-29.17±7.82	-25.45±3.42	0.059
30	-20.76±10.97	-25.98±4.80	0.058
35	-19.86±12.67	-26.60±9.01	0.060
40	-28.76±10.30	-38.33±6.22	0.001*

*All showed a highly significant difference between two dependent means using Paired-t-test at 0.05 level when compared to baseline or compared to previous measurement ($P<0.0001$). *Significant difference between two independent means (type of exposure) using Student-t-test at 0.05 level.

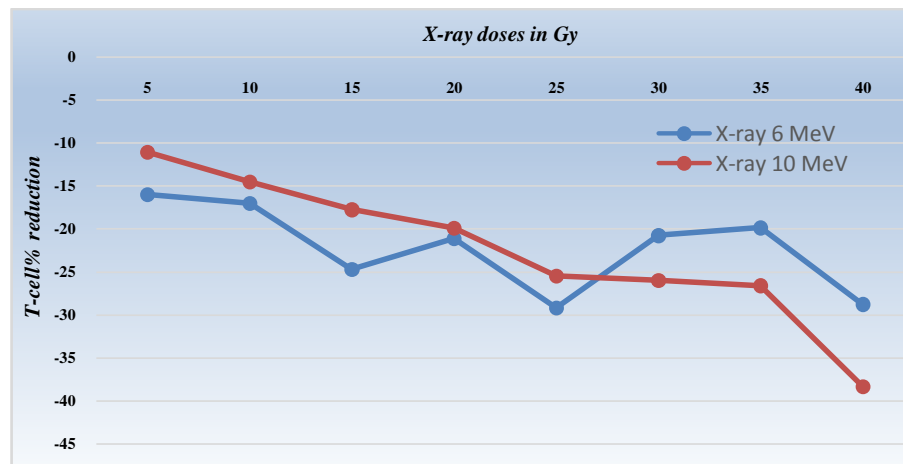


Figure 10: Graph showing T-Cell% decreasing value (difference from priveus) with effect of X-ray (6 & 10) MeV doses

4. Conclusion

- 1) Using irradiation of blood bags to prevent TA-GVHD is practical, safe and frugal technique to get rid of T-cells which are the main effector cells causing TA-GVHD.
- 2) The best dose of X-ray for irradiating blood bags in this research at energy (6) MeV is (25) Gy, while for (10) MeV, is (35) Gy.
- 3) The effect of X-ray with two energies (6&10) MeV on the T-cells at doses (35&40) Gy, are equal.

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