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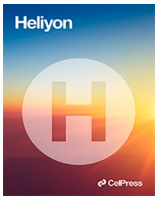

The impact of cyclophosphamide versus high-level of ionizing radiation on the immune response

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ABSTRACT

The immune response to various high doses of Ionizing Radiation (IR) is investigated in this study compared to the non-irradiated group and other immunosuppressive conditions. Thirty rats were divided into six different groups. Group I received no radiation or medications. Groups II, III, IV, and V were subjected to the entire body to 1, 2, 3, and 5 Gray (Gy) of IR. Cyclophosphamide 50 mg/kg was administered intraperitoneally to Group VI. Serum levels of Interleukin-2/1-beta, Tumor Necrosis Factor-alpha (TNF-α), and Interferon-gamma (INF-γ) were measured one day after irradiation. The immunosuppressive effect of cyclophosphamide reduced the majority of the evaluated parameters. When high doses of IR, notably greater than 1Gy, were investigated, all measured parameters increased consistently. Finally, high doses of IR amplify essential pro-inflammatory responses and cannot be used to suppress the immune system in a single dose. More research is needed to clarify immune responses and their therapeutic potential in response to high or low IR doses.

1. Introduction

Ionizing Radiation (IR) was a significant issue in the previous century. The deterministic effects of exposure to a high radiation dose are possible and can occur in various situations. According to the UNSCEAR report, Acute Radiation Syndrome (ARS) develops following an acute whole-body or severe partial-body exposure of more than 1 Gy [1]. Furthermore, accidental, unintended exposure to a single high dose of gamma rays occurred due to an emergency. The essential worry is whether a high dose of IR completely suppresses the immune response.

Cyclophosphamide (CP) is a chemotherapeutic immunosuppressant used to treat cancer. Furthermore, it is widely recognized as an immunosuppressive drug that causes various immune responses [2,3]. This study compared irradiated groups' immune-radiological responses to the non-irradiated group (-ve control) and the group with a CP-induced immune suppressive state (+ve control). Furthermore, the effect of each IR dose above the ARS threshold on the primary essential cytokine implicated in different immunological conditions was established.

Significant regulatory, pro-, and anti-inflammatory cytokines require more research. Interleukin-2 (IL-2) disrupts the function of both pro- and anti-inflammatory T lymphocytes. Other transduction networks are activated by IL-2, which affects transcription and metabolism [4]. TNF-alpha (TNF-α) is a pleiotropic pro-inflammatory cytokine produced by various cells throughout the body [5]. Interferon-gamma (IFN-γ) regulates immunological and inflammatory genes and activates innate immune cells in the immune stress response [6]. Interleukin-1 Beta (IL-1β) is a potent pro-inflammatory cytokine necessary for host defense against infection and injury;

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it's produced and secreted by innate immune cells, monocytes, and macrophages [7].

This study aims to define and evaluate the essential early immune response to different single high doses of IR and compare its effects to those of other standard immune suppressive drugs to illustrate and introduce to the radiological community more facts about high single gamma ray exposure that could occur in various planned or unplanned situations.

2. Material and methods

2.1. Experimental animals

The current study used healthy adult male albino rats six weeks old and weighed between 145 and 155 g as the experimental model. Rats from The National Research Center Egypt's animal house in Cairo were purchased. Rats were cared for and acclimated to the laboratory environment for ten days before the experiment at the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority (NCRRT-EAEA). Experimental procedures were carried out per the general international guidelines for using animals in scientific research. The NCRRT-Research Ethics Committee authorized the experimental methods (protocol serial number: 77 A/21) and complied with its standards and regulations. All ethical animal treatment processes followed the Ethical Animal Research Committee-approved guidelines. All rats were fed standard pellets specific for rats and had free access to clean drinking water.

2.2. Experimental design

This study aims to compare the immunological response to varied high doses of IR to that of the known immunosuppressant cyclophosphamide. As shown below, thirty rats were randomly assigned to one of six groups of five. G-power analysis of the software (version 3.1.9.4) sample size was used to calculate the sample size at $\alpha = 0.05$, power $= 0.8$, Group number $= 6$, and Effect size $= 0.75$.

The control group, Group I, received no radiation or medicines. Groups II, III, IV, and V received ionizing gamma radiation doses of 1, 2, 3, and 5 Gray (Gy) whole-body exposure, respectively. CP intraperitoneal 50 mg/kg for seven days in Group VI (Shruthi, Vijayalaxmi, and Shenoy, 2018). The experimental design shown in the following table. (Table 1).

2.3. Drug

The Baxter Company supplied the CP (Endoxan 1 gm, injection).

2.4. Gamma radiation

The animals were irradiated using the Cesium-137 gamma cell source in the National Center for Radiation Research and Technology, EAEA, Egypt. From Nordion company Canada, model GC40 irradiated chamber with dimension (height 10 cm and diameter 40 cm), dose rate 0.66 rad/Sec (cGy/Sec).

2.5. Enzyme-linked immunosorbent assay (ELISA)

Plate Reader of 2100 Stat Fax AWARENESS TECHNOLOGY INCELISA. Interleukin-2 (IL-2), Tumor necrosis factor-alpha (TNF-α), Interferon-gamma (INF-γ), and Interleukin-1 beta (IL-1β) levels were measured by centrifuging peripheral blood samples to obtain serum. The concentrations of their serum were measured in pg/ml. Blood serum was drawn into a test tube in all study groups, and ELISA was performed according to the manufacturer's instructions to analyze the measured values. The ELISA was carried out following MyBioSource, USA's manufacturer's instructions.

2.6. Statistical analysis

R version 4.1.0 software was used for statistical analysis. The data for each group were not normal according to the Kolmogorov-Smirnov and Shapiro-Wilk tests for normality. The Kruskal-Wallis rank-sum test was used to determine whether there was a statistically significant difference in mean values within the irradiated groups. As a Post-Hoc test, the Dunn test with correction Benjamini-Hochberg method was used to determine the mean difference between groups. The Mann-Whitney test was used to compare the CP and

Table 1
Experimental design.

Groups	Day 0	Day 7 Irradiation (Gy)	Day 8 blood was taken on
I	X	0	√
II	X	1	√
III	X	2	√
IV	X	3	√
V	X	5	√
VI	Cyclophosphamide	X	√

irradiated groups. The Spearman correlation coefficient was used to fit the relationships between the various variables. A significance level of $\alpha = 0.05$ ($P < 0.05$) was chosen for statistical analysis. The mean and standard error (SEM) represents the data.

3. Results

In regards to the immunological response of IL-2 in rat serum, radiation increases the response in a dose-dependent manner. The Kruskal-Wallis H test demonstrated statistically significant differences between the control group (I) and all of the irradiated groups (II–V)) at $\chi^2_{(cv,4)} = 21$ at $\alpha = 0.05$ ($P < 0.001$). When compared to the other groups, the CP group (VI) significantly reduced all serum levels (I–V) at $\alpha = 0.05$ ($P < 0.001$).

Regarding TNF-α immune response in rat serum, radiation raises serum levels. The Kruskal-Wallis H test revealed statistically significant differences between the control group (I) and all of the irradiated groups (II–V)) at $\chi^2_{(cv,4)} = 17$ at $\alpha = 0.05$ ($P < 0.01$). The CP (VI) significantly reduced all serum levels (I–V) compared to the other groups.

The Kruskal-Wallis H test demonstrated statistically significant differences between the control group (I) and all irradiated groups (II–V) in the immunological response to INF-γ in rat serum. At $\chi^2_{(cv,4)} = 18$ at $\alpha = 0.05$ ($P < 0.01$). While the CP group (VI) significantly decreased ($P < 0.05$) serum levels compared to groups III to V, it significantly increased serum levels compared to groups I and II ($P < 0.05$).

Regarding the immunological response of IL-1β in rat serum, the Kruskal-Wallis H test demonstrated statistically significant differences between the control group (I) and all of the irradiated groups (II–V) at $\chi^2_{(cv,4)} = 20$ at $\alpha = 0.05$ ($P < 0.01$). When compared to the other groups, the CP group (VI) significantly reduced all serum levels (I–V) ($P < 0.05$).

Regarding immunological response, the Spearman correlation coefficient suggests a highly significant direct link between IL-2, TNF-α, and INF-γ and radiation doses ($P < 0.0001$). However, IL-1β shows no significant association with radiation doses ($P \geq 0.05$). However, the matrix shows a direct important ($P < 0.05$) link between all of the measured parameters except IL-1β and INF-γ ($P \geq 0.05$).

4. Discussion

It is critical to assess the effects of various high doses of IR to evaluate and precisely define the immune suppressive doses of IR; a control group should be used to compare the immunological responses to multiple IR doses and the induced suppressive immune condition. This research investigates the effects of HD of IR on the immune system. A rat model was used to examine the impact of 0, 1, 2, 3, and 5 Gy irradiation doses (as well as CP as a +ve control) on serum levels of essential cytokines that play a significant role in the immune response. The null hypothesis is accepted when there is no significant difference between groups. This study rejected the null hypothesis regarding the relationship between HD and immunosuppressive treatment in specific tested parameters at $\alpha = 0.05$ ($P < 0.05$).

IR produced Inflammatory responses [8]. The coordinated activation of signaling pathways that regulate the levels of inflammatory

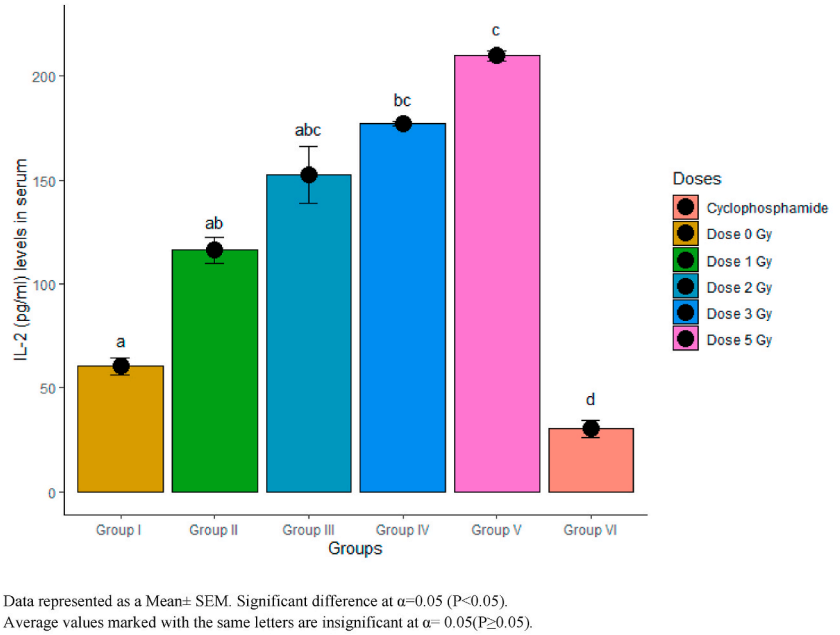


Fig. 1. The rat's serum interleukin-2 (IL-2) immune response to radiation doses (one-day post-exposure) and cyclophosphamide (intraperitoneal 50 mg/kg for seven days).

mediators in tissue-resident cells and blood-derived inflammatory cells is called inflammation. Inflammation is the root cause of many chronic diseases, including cardiovascular disease, bowel disease, diabetes, arthritis, and cancer. Inflammatory response mechanisms are similar regardless of the initial stimulus. When cell surface pattern receptors recognize harmful stimuli, inflammatory pathways are activated, inflammatory markers are secreted, and inflammatory cells are recruited. Inflammation is caused by primary inflammatory stimuli such as cytokines such as IL-1β and TNF-α [9]. TNF-α is an inflammatory cytokine that is involved in a variety of pain models [10].

An HD of IR increased blood levels of the essential cytokines IL-2 as shown in Fig. 1, TNF-α, and IL-1β (Figs. 2 and 4) compared to non-irradiated groups in the current study Furthermore, the high correlation coefficient revealed in Fig. 5's correlation matrix verified this. In contrast, cyclophosphamide decreased all serum levels compared to non-irradiated groups except INF-γ, as shown in Fig. 3. A high IR cannot be treated with an absolute immunosuppressive dose.

IFN-γ is a pluripotent immunomodulatory cytokine that regulates immunological responses [11,12]. Fig. 3 shows that CP significantly increased the INF-γ serum level compared to 0 and 1 Gy irradiation (Group-I and II) ($P < 0.05$). At the same time, there was a significant decrease at $\alpha = 0.05$ ($P < 0.05$) When compared to doses greater than 1 Gy (III, IV, and V).

Finally, CP was associated with a decrease in most immunological parameters tested. High doses of IR (>1 Gy) were not an absolute suppressive dose; HD-IR has synergistic effects with pro-inflammatory cytokines. Pro-inflammatory cytokines produce secondary ROS that can have a significant post-radiation effect. The therapeutic potential of IR motivates new research directions in different immunological conditions.

4.1. Study limitation

The author believes that determining whether doses of less than 1 Gy can be used as immunomodulatory in various immunological diseases requires evaluating the effect of cyclophosphamide as a standard immunomodulatory and low dose of IR below the acute radiation syndrome level (which is below 1 Gy) as side effects of radiation will be minimal below that level.

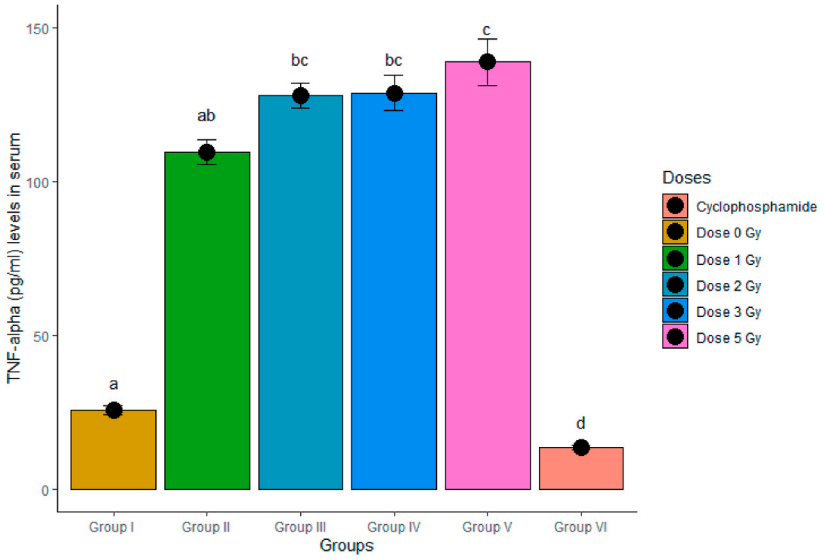
The author declarations

Ethical approval

The procedures used in the experiment were approved by the NCRRT-Research Ethics Committee and complied with its guidelines and rules.

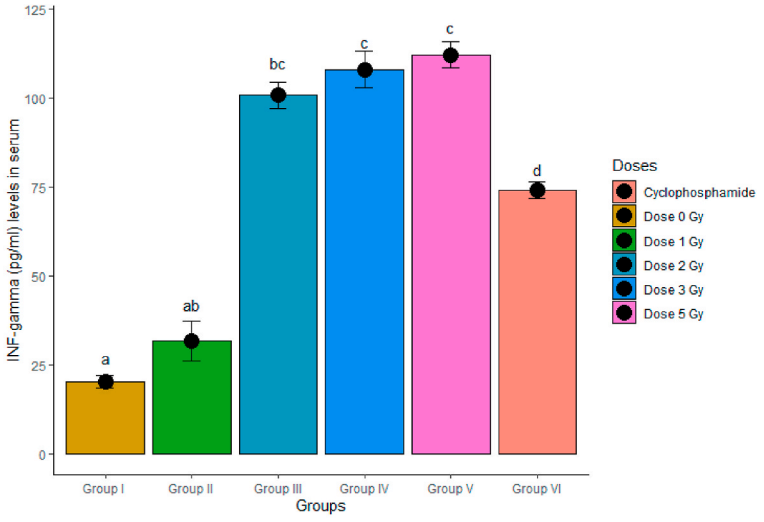
Consent for publication

I consent to publication.



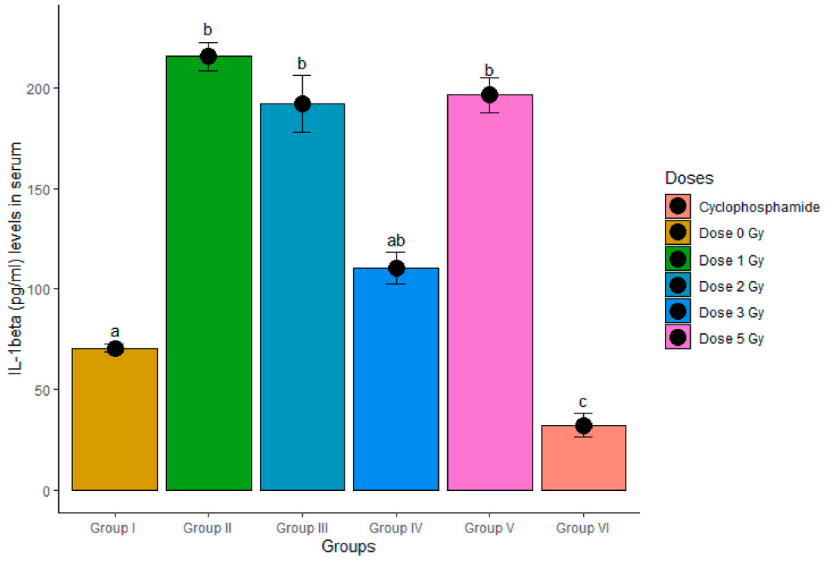
Data represented as a Mean± SEM. Significant difference at α=0.05 (P<0.05).
Average values marked with the same letters are insignificant at α= 0.05(P≥0.05).

Fig. 2. The immunological response of Tumour Necrosis Factor-alpha (TNF-α) in rat serum to Radiation doses one day after exposure and cyclophosphamide (intraperitoneal 50 mg/kg for seven days).



Data represented as a Mean± SEM. Significant difference at α=0.05 (P<0.05).
Average values marked with the same letters are insignificant at α= 0.05(P≥0.05).

Fig. 3. The immunological response of Interferon-gamma (INF-γ) in rat serum to radiation doses (one-day post-exposure) and cyclophosphamide (intraperitoneal 50 mg/kg for seven days).



Data represented as a Mean± SEM. Significant difference at α=0.05 (P<0.05).
Average values marked with the same letters are insignificant at α= 0.05(P≥0.05).

Fig. 4. The immunological response of Interleukin-1beta (IL-1β) in rat serum to radiation doses (one-day post-exposure) and cyclophosphamide (intraperitoneal 50 mg/kg for seven days).

Author contribution statement

Soha Mahmoud Hussien: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

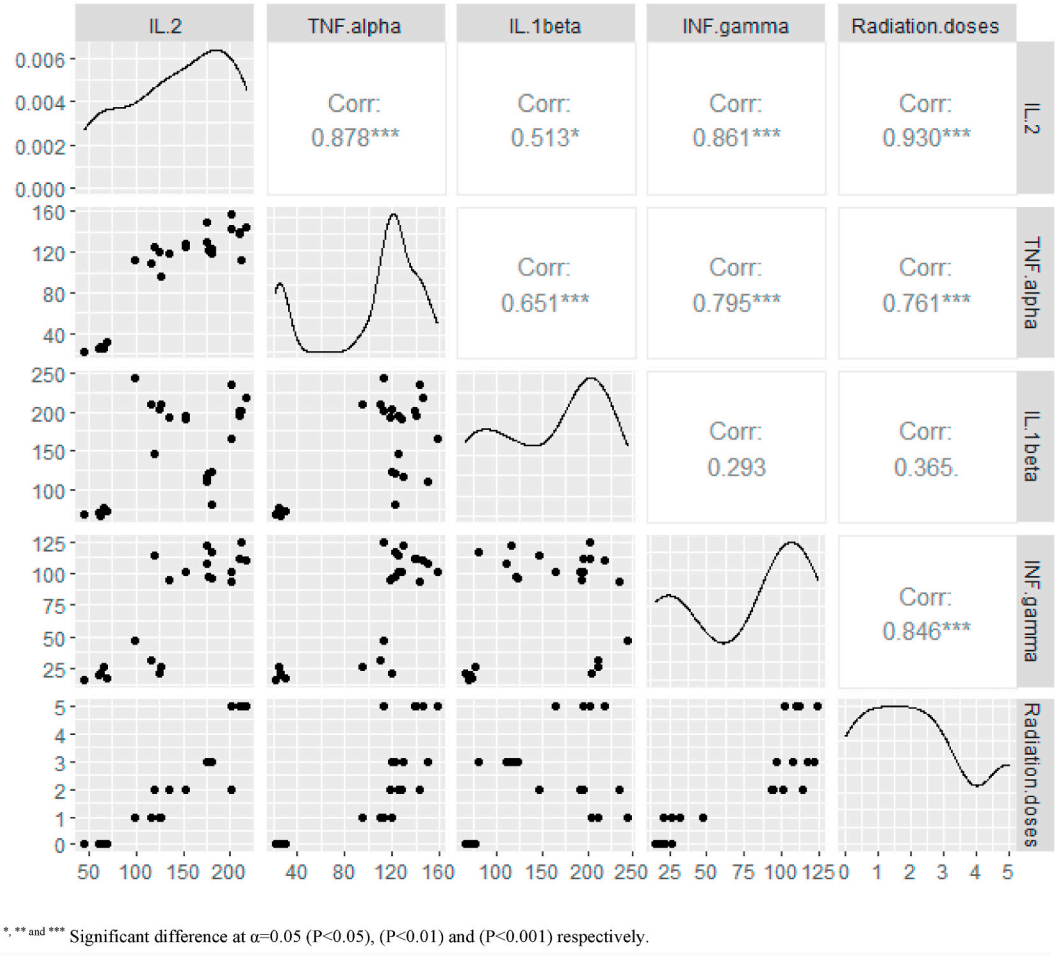


Fig. 5. Spearman correlation coefficient matrix of all variables analyzed with all radiation doses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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