



Original Research Article

Effect of Irradiation on Neutrophils Activity using α -particles radiation and Human Blood Samples

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A B S T R A C T

Keywords

Neutrophils activity; α -particles; Irradiation; NBT; Americium.

Neutrophils are the effective arm of innate cellular immune response. This study aimed to determine the effects of irradiation with α - particles on Neutrophils activity using human blood samples *in vitro*. Radiation source used for irradiation was Americium (^{241}Am). The method of Nitro-Blue Tetrazolium (NBT) reduction was utilized to estimate Neutrophils activity by determining the relative number of positive cells. Results: the outcomes of this trial showed that irradiation caused decrease in the relative number of positive Neutrophils for NBT test comparing with controls; indicating that their activity was suppressed. Conclusion: Irradiation with α - radiation suppress Neutrophils phagocytic function, and that may due to early Apoptosis

Introduction

Ionizing Radiation is one of the most effective suppressors of the Immune system. Alpha radiation or α - Ray is the type of radiation under focus in this study. This kind of radiation is of positively charged particles, able to interact with surrounding materials. These particles lose their energy rapidly, cannot penetrate very far into materials. Alpha particles are classified by the International Agency for Response Cancer, IARC as group I carcinogens, they are able to suppress immune system and hit mainly innate and

cellular mediated immunity, Milacic, (2008).

Neutrophils are Granulocytic white blood cells, they are the first line of defense upon nonspecific cellular immunity, and phagocytosis is the process used by these cells to defend human body against invasions, Delves, (2006). This study was done to evaluate the effect of α -particles radiation exposure *in vitro* on human Neutrophils activity.

Materials and Methods

Blood Samples

Five human blood samples of (10 ml) freshly collected, (A, B, C, D and E), these samples were taken from adult males, healthy (no clinical signs), nonsmokers with no history of radiation exposure. Each of the five blood samples were divided at once into two parts using heparinized tubes, one part for test and the other for storage (control) to compare results and eliminate storage effects.

Samples were marked as A1, B1, C1, D1 and E1 as test group samples. And A2, B2, C2, D2 and E2 as control group samples. Both two groups of samples were kept under the same conditions of storage (4°C) as recommended by Lewis, (2006). This study was accomplished between; Apr. 2013 to Jan. 2014.

Irradiation source

Irradiation was done using an alpha radiation emitter source Americium (^{241}Am of 124×10^5 Apm), date of production was 6.22.1977, obtained from Nuclear Physics Laboratory, Physics Department, College of Education, University of Baghdad, and under the supervision of (Al-Ubaidi, K. H. Nuclear physics Proff.).

Assay Method of Irradiation

The irradiation procedure was inspired from, Hu (2006), Falt (2003), Wu (1999) and Amundson (1996). Direct exposure method was used with 2 min. of exposure time to ^{241}Am (total dose of radioactivity was equal to $248 \times 10^5 \text{Bq}$). Control group samples were kept away during irradiation application.

Neutrophils Activity Estimation

Neutrophils activity was estimated using Nitro-Blue Tetrazolium (NBT) reduction test, whereas, the formation of Formazan Particles (dark blue crystals), is of positive reaction indicating for active Neutrophils; and effective oxygen burst pathway during phagocytosis process. This test procedure was carried out according to Al-Hamadany (2011).

Tests of NBT Schedule

- Before Irradiation, NBT test was applied for all samples at once after blood samples collection.
- After Irradiation within one hour for all samples (test and control groups) then stored in refrigerator. After Irradiation with 24 hours
- There was no change in samples color or any turbidity seen along the trial, and before each test, blood samples were mixed gently.

Statistical Analysis

The mean value for NBT positive percentage results was depended for the five samples A1, B1, C1, D1 and E1 as test group value, while the mean value for A2, B2, C2, D2 and E2 was depended as control group value.

Results and Discussion

Before irradiation, all blood samples showed normal results including control tubes, positive percentages for NBT were between (79-68%) with a mean (73%), whereas, each percentage represented the number of positive counted neutrophil cells out of 100. After irradiation within

one hour, NBT mean value for test group retarded to (71.5%) with a range (78-66%), while control group samples still stand normal with no significant difference obtained. The results after 24 hr. since irradiation showed diminished percentages of test group samples and exceeded the lower normal limit for NBT positive percentage, mean value for test group was (54%) with a range (58- 46%). While control group mean value suffered from slight decrease with a mean value (67.5%). The author Mckenzie, (2004), determined the normal value of NBT with no less than 75%.

The scientist Edgar (2006) stated that NBT Test is the best method to detect Neutrophils activity comparing with other techniques. Neutrophils can induce early apoptosis (programed cell death) after exposure to radiation as Hoffbrand (2006) and Mckenzie (2004) stated.

Our results showed that neutrophils activity dropped fast due to irradiation with alpha radiation comparing with controls. Our results are in consistence with those of Buescher (1987), they also studied the effects of radiation on human Neutrophils function using NBT reduction test, but they used gamma radiation and a different dose.

Al-Hamadany, 2011 found that α - radiation resultant from Depleted Uranium internal pollution caused suppression in Neutrophils activity expressed by diminished the relative number of positive cells for NBT reduction.

In addition, our outcomes are in agreement with the results of Heslet, (2012), they found that acute exposure to radiation suppressed potentially cellular defenses of the body and suggested administration of

growth factors to regenerate destroyed immune system cells.

Patients receiving radiotherapy usually suffer from neutropenia and immune system function reduction as stated by Manus, (1997). The point is that the storage of blood induces apoptosis in blood cells, but radiation makes its occurrence faster. This fact was obvious in our trial results since loss function is an indicator for starting apoptosis according to Mckenzie,(2004)and Hammer,(1986). The author Lorimore and his colleagues during 2001 suggested that radiation hits critical genes for the survival of immune cells leading to early death by apoptosis. The scientist François,(2013) and his group studied the effect of radiation and apoptosis induction and found that radiation induces apoptosis in innate immune cells due to clonogenic cell death. These research's results support our results. In conclusion, irradiation with α - radiation suppressed Neutrophils function and that may be related to rapid early apoptosis induction upon storage of blood.

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