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Effect of High Dose Natural Ionizing Radiation on the Immune System of the Exposed Residents of Ramsar Town, Iran

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ABSTRACT

Iran is one of several countries that has regions of high dose natural ionizing radiation. Two well-known villages in the suburb of Ramsar Town in the Caspian Sea strip, Taleshmahaleh and Chaparsar, have background radiation that is 13 times higher than normal. This radiation is the result of Radium 226 and Radon gas both of which are highly water soluble.

While people living in these regions do not suffer from any major health problems, we decided to study the their immune responses to infection and inflammation in order to determine if their habitat affects their immune defense mechanisms as a way of compensating for their exposure to high dose environmental ionizing radiation.

Our results showed that the total serum antioxidant level in the exposed people was significantly lower than the individuals not exposed to high dose natural ionizing radiation. The exposed individuals also had higher lymphocyte-induced IL-4 and IL-10 production, and lower IL-2 and IFN-γ production. In addition, neutrophil NBT, phagocytosis, and locomotion were higher in the exposed group. In contrast, lymphocyte proliferation in response to PHA was unaffected.

We conclude that the immune system of individuals exposed to high dose ionizing radiation has adapted to its environment by shifting from a Type 1 to a Type 2 response to promote anti-inflammation. This may be because inflammatory Type 1 responses generate more free radicals than Type 2 responses, in addition to the free radicals generated as a result of high environmental radiation. Thus, the serum total antioxidant level in the exposed residents was lower than the unexposed group.

Key words: Antioxidant; FRAP; IL-2; IL-4; IL-10; IFN-γ; Ionizing radiation; NBT; Phagocytosis

INTRODUCTION

Natural radiation exists in all regions of the world, although its levels vary geographically.

Individuals have been exposed to this environmental radiation since the beginning of evolution.[1] Most natural ionizing radiation results from radon 222 and uranium 226 decay.[2,3] It has been reported that chromosomal aberrations and specific cancers are well known macroscopic results of exposure.[4,5] Ionizing radiation collides with molecules in living cells generating clusters of free radicals known as reactive oxygen species (ROS), including

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free radicals (H^+: hydrogen ion, H^-: hydrogen radical, H_2 O_2: hydrogen peroxide, OH^- hydroxyl radical). These free radicals randomly damage cellular constituents including DNA[6-8] and react with almost all structural and functional organic molecules, including proteins and lipids. OH^- induces peroxidation of unsaturated membrane fatty acids, forming peroxyl (ROO^-) and alkoxyl (RO^-) radicals[9] and resulting in a loss of cellular compartmentation that leads to metabolic disturbance. The oxidative attack on proteins[10] can greatly alter their properties and functions, which is why ROS radicals are extremely damaging to living cells. Free radicals are also formed as an indirect consequence of irradiation by phagocytic cells that have become activated during removal of radiation-injured tissues.[11]

Studies strongly suggest that oxidative stress from ionizing radiation exposure can trigger a cascade of events, including altered immune function, cellular transformation, and tissue damage.[12] However, the immune system can resist this oxidative stress by scavenging free radicals and neutralizing them. Superoxide dismutase (SOD) is an oxidoreductase that contains Cu, Fe, or Mn in its active site and catalyzes the dismutation of O^{2-} into O_2 and H_2O_2. Together with catalase and glutathione peroxides, these are the major intracellular enzymes that protect cells and its constituents from oxygen toxicity.[12]

It should be noted that the effect of natural ionizing radiation on living cells is dependent on the level of radiation exposure.[13] During the previous decade, statistically significant evidence has indicated that whole body exposure of humans to low doses of ionizing radiation stimulates immune function, decreases total cancer mortality rates, and increases longevity.[14-17] On the other hand, high dose radiation depresses immune function, increases the incidence of cancer, and induces higher mortality rates.[18-20]

It is well accepted that normal background ionizing radiation is approximately 20 mSv/year worldwide. This is considered low level exposure. However, there are certain regions in the world where background radiations are 10 to 15 times higher than normal. Taleshmahaleh and Chaparsar, two villages in the north part of Iran in the suburb of Ramsar have high background radiation equal to 260 mSv/year.[2,21] Fortunately, inhabitants of these villages do not have a high incidence of cancer or infection.[22,23] We decided to study their immune functions and compare them to inhabitants in other northern villages who are not exposed to high radiation levels in order to determine the mechanism of immune adaptation.

SUBJECTS, MATERIALS AND METHODS

One hundred individuals between 20 and 50 years of age who were residents of one of two villages, Taleshmahaleh or Chaparsar, with high background radiation (exposed group) and two villages with normal background radiation, Sephydtameshk or Daryaposhteh (control group), took part in the study. All participants were informed about the nature, aims, and intention of the study and signed a consent form and questionnaire before providing blood samples. Any individuals suffering from an illness or taking medication were excluded. The questionnaire contained questions about their diet, and any food supplements or special drinks they were taking. It was collected to determine if any participant was regularly ingesting foods that were particularly enriched or were deficient in antioxidants.

Sample Preparation

Twenty ml of peripheral blood was drawn and placed into a heparin containing test tube for neutrophil and lymphocyte separation. 5 ml of peripheral blood was collected without anti-coagulant in order to separate the serum. Sera were separated using the standard method for measuring total anti-oxidant levels. White blood cells were used to quantitate neutrophil NBT, neutrophil phagocytosis, neutrophil locomotion, lymphocyte proliferation, and the production of IL-2, IL-4, IL-10, and INF-γ by cultured lymphocytes.

Isolation of Neutrophils and Lymphocytes

Cells were separated by the standard procedure of dextran sedimentation followed by Ficoll-Hypaque centrifugation (24). Mononuclear cells that were mostly comprised of lymphocytes were collected from the interface and neutrophils were collected as the cell pellet. These cells were washed separately with an excess of Hanks balanced salt solution (HBSS) and resuspended in complete RPMI 1640 culture medium prior to running each assay.

Total Antioxidant Assay

The total antioxidant levels in the sera from both groups were assessed using the Ferric Reducing/

Antioxidant Power (FRAP) assay. This assay was performed based on a standard procedure.[25]. In brief, 100µl of serum sample was added to 3ml of FRAP reagent. After 6 min incubation, the optical density was measured at 593 nm and the level of antioxidant was calculated using the standard formula.[25]

IL-2, IL-4, IL-10 and IFN-γ Production

Isolated lymphocytes ($2x10^6$/ml) were suspended in RPMI 1640 medium with 10% FCS and were cultured in the presence of PHA mitogen (1 ug/ml, Sigma, St Louis, USA) for 24 hours at 37ºC in 5% CO_2. Supernatants were harvested and kept at -70ºC until testing. IL-2, IL-4, IL-10, and IFN-γ levels were determined using commercially available ELISA kits according to the manufacturer's protocol.

Lymphocyte Proliferation Assay

Mononuclear cells consisting of >90% lymphocytes were used for this assay. 100µl of a $5x10^6$ cells suspension in complete RPMI 1640 (26) was placed in each well of a 96-well flat bottom microplate and cultured in the presence of mitogen PHA (2 µg/ml, Sigma, St. Louis, USA) for 72 hours at 37ºC in a humidified atmosphere with 5% CO_2. 18 hours prior to the end of the culture period, the cells were pulsed with methyl-H^3-thymidine (1.0 uCi). After 3 days of culture, the cells were harvested and the stimulation indices (SI) were calculated as described previously.[26]

Neutrophil Phagocytosis Assay

The phagocytic activity of neutrophils was assessed after incubating $1x10^6$ cells in MEM containing 50% autologous serum and $2x10^8$ heat killed *Candida albicans* for 15 min at 37ºC. The cells were then centrifuged and thin smears were made from the sediment and stained with Lieshman's stain. The number of candida positive neutrophils in 100 cells provided the phagocytic index (PI). The total number of *Candida albicans* counted within 100 neutrophils divided by 100 provided the mean particle number or avidity index (AI).

Neutrophil Locomotion Assay

$2x10^6$ neutrophils in human serum albumin (Sigma, St. Louis, USA) were allowed to migrate through a cellulose nitrate membrane filter (pore size: 3um, Millipore, Billerica, USA) in response to LPS (20 ug/ml, Sigma, St. Louis, USA). After 90 min incubation at 37ºC, the distance into the filter that was attained by the leading front of cells was measured.[27]

Nitroblue Tetrazolium (NBT) Reduction Assay

The intracellular killing ability of neutrophils was assessed using the NBT test.[28] In brief, neutrophils were incubated at 37ºC for 30 min on a clean glass slide. The slide was then washed gently with cold saline to remove other cell populations. NBT medium (0.2ml of a 0.34% sucrose solution, and 0.2ml 28% NBT) including 0.2ml of inactivated fetal calf serum was added and the cells were further incubated at 37ºC for 30 min. The slide was washed with cold saline and stained with safranin. When NBT is phagocytosed by cells, the intracellular dye converts it into an insoluble blue crystallin form (Formazan crystals). One hundred cells were observed and positive cells with formazan granules were counted.[29]

Statistical Analysis

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS). Comparison of the two groups was carried out using the Mann-Whitney two tailed test. The differences were considered significant when the *p* value was <0.05.

RESULTS

Cytokine Production

Production of cytokines is a necessary step in immune responses and immune regulation. We studied the production of IL-2 and IFN-γ to measure Th1 type responses, and IL-4 and IL-10 to measure Th2 type responses to the mitogen PHA. Results indicated that Type 1 cytokine production was decreased in the radiation exposed individuals, while Type 2 cytokine production was significantly increased (Table 1). This suggests a potential shift in the cytokine profile towards a humoral immune response.

The shift in cytokine production was not of the result of differential lymphocyte proliferation because the stimulation indices in both groups were similar and no significant changes were observed.

Neutrophil Functions

The non-specific innate immune response is the first line of defense against pathogens. Granulocytes, especially neutrophils, are the major innate effector cells.

Table 1. Effect of natural ionizing radiation on peripheral lymphocytes.

Residents	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-10 (OD)	INF-γ (OD)	Proliferation (SI)	P value*
High Dose Area	399±221	220±28	0.26±0.04	0.12±0.04	3.7±0.8	<0.05
Control	760±275	145±34	0.17±0.03	0.17±0.05	3.9±0.7	-

* Mean of the two groups of exposed and controls were used.

As a result, we primarily compared their functions in the exposed and control groups with regard to locomotion, phagocytosis, and the respiratory burst required for pathogen killing (Table 2). As shown, all neutrophil functions were increased in individuals exposed to high background radiation as compared individuals in the control group.

Total Serum Antioxidant Level

Total serum antioxidant levels in individuals exposed to the high dose ionizing radiation were significantly lower than those in residents exposed to normal background radiation (Table 3). This may be due to higher free radical generation in this group.

According to the questionnaires, there were no major differences in antioxidant enriched food intake between the exposed and control groups. Thus, the decrease in antioxidant level may be the result of scavenging free radicals generated by higher environmental ionizing radiation or a shift in the cytokine production pattern.

DISCUSSION

Immune surveillance is one of the most important defense mechanisms against cancer and infection. Most carcinogens are immunosuppressants and ionizing radiation can also be suppressive, especially at high doses.[30,31] There are many regions in the world that have high levels of background ionizing radiation.[1] Among these areas, some villages in the suburb of Ramsar, Iran, Taleshmahaleh and Chaparsar, show the highest level of natural radiation.[2] Surprisingly, however, the inhabitants of these villages do not show a higher incidence of cancer or infections.[23] Several reports indicate that low dose ionizing radiation from a natural source[32] or in professional radiation workers[33] may stimulate the immune system and potentiate its effector function, a phenomenon called "Radiation Hormesis".[14] Low dose radiation not only stimulates the immune system but also repairs DNA breakage, transforms free oxygen radicals, and increases longevity.

However, the harmful effect of high dose ionizing radiation is well established.[4,34] One of the damaging effects of high dose radiation is the generation of free radicals that can cause tissue damage and DNA breakage resulting to malignancies.[1] Since our observations in Taleshmahaleh and Chaparsar were inconsistent with this observation, we studied the immune functions of individuals from these regions in order to elucidate potential mechanism(s) by which these high dose radiation exposed residents have adapted to their environment.

Table 2. Effect of natural ionizing radiation on peripheral neutrophils.

Residents	NBT (%)	Phagocytosis (pI)	Locomotion (*u*m)	P value*
High Dose Area	84±13	92±6	124±16	<0.05
Control	65±12	80±5.8	84±8.5	-

* Mean of the two groups of exposed and controls were used.

Table 3. Effect of natural ionizing radiation on serum antioxidant.

Residents	Total antioxidant (*u*mole)	P value*
High Dose Area	686±170	<0.05
Control	1187 ±199	-

* Mean of the two groups of exposed and controls were used.

We found that residents in both Taleshmahaleh and Chaparsar had lower total serum antioxidant levels in comparison with residents in areas with normal radiation background. This reduction was probably due to the utilization of antioxidants that could scavenge the higher free radical concentrations in the exposed individuals. The data collected from the food intake questionnaire showed no major differences in diets between the exposed and control groups. However, we did notice that all residents of the Caspian strip consume a high amount of fresh vegetables, fish, garlic, and citrus, all food that is enriched in antioxidants. The high antioxidant intake probably compensates for higher free radical formation in the radiation exposed group and protects them from the detrimental effects of these molecules.

This study also revealed a shift in the cytokine production pattern from Th1 to Th2 cytokines in residents exposed to high dose radiation (Table 1) without significant proliferative response of the lymphocytes. This finding was inconsistent with Godekmerdan et al who studied effects of low dose ionizing radiation on X-ray workers and found lower number of $CD4^+$ T-cells and weaker humoral immune response.[35] This inconsistency probably is due to the nature of the ionizing radiation and duration of the exposure.

Similarly, in contrast to Hrycek *et al* who reported high level of IL-2 and IL-4 production by peripheral blood lymphocytes in the X-ray equipments operators,[36] we found lower IL-2 and INF-γ and higher IL-4 and IL-10 production in the exposed group. The latter finding is consistent with reports of Ibuki and Goto[37] who studied the effect of low dose γ-radiation on mice and showed higher production of inflammatory cytokines of the irradiated mice. Thus, we conclude that the shift in the cytokine production pattern from Th1 to Th2 in the residents exposed to high level ionizing radiation suggests that the immune system in the affected individuals has adapted itself to respond to inflammatory or tissue damage that might occur from high dose ionizing radiation. This notion explains why we observed higher neutrophils functions in the exposed group in compare to that of control individuals (Table 2).

Although the data reported here suggest that the immune system may have adaptation mechanism(s) for coping with high dose radiation exposure that may explain why we do not observe a higher incidence of cancer and/or infections in affected individuals, further analysis is required. The role of IL-2 and IgE production, a closer evaluation of the cellular immune response, and measurement of the direct free radical content in affected residents is necessary in order to elucidate the complete mechanism of immune adaptation.

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