Iran. J. Radiat. Res., 2009; 7 (3): 127-133

Effect of ionizing radiation on development process of T-cell population lymphocytes in Chernobyl children

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Background: The aim of preliminary study was determined development process status of T-cell population lymphocytes in Ukrainian children after 22 years from Chernobyl accident for next feasibility study. ***Material and Method:*** 150 participants aged 6 to 16 years are included in three groups: Group I (n=65), 30 to 60 km from center accident at zone 3th, Group II (n=65) 60 to 90 km from same location at zone 4th and control group (n=20) from Kiev, 100 km from same location. Peripheral blood leukocytes from buffy coats were analyzed for T-lymphocytes population such as T-lymphocytes (CD3), T-helper (CD4) and T-cytotoxic (CD8) by roseting method using erythrocytes that conjugated with monoclonal antibody against CD3, CD4, and CD8 receptors; then CD4/CD8 ratio were calculated. ***Results:*** Percentage of CD3 and CD4 in groups II and I decreased significantly in compared to control group at P<0.001. Percent of CD8 decreased significantly in group I compared to control group at P <0.001. CD4/ CD8 ratio decreased significantly in-group I comparison to control group at P=0.02. Leucocytes count in groups II and I have not changed significantly in comparison to control group (P=0.09,P=0.4) but in group II, it was significantly different in comparison to group I at P <0.008. ***Conclusion:*** Our data show that after 2 decade of Chernobyl accident, ionizing radiation may have affected the developmental processes of T-cell population. **Iran. J. Radiat. Res., 2009; 7 (3): 127-133**

Keywords: *Immune status, T-lymphocytes population, Chernobyl's children.*

INTRODUCTION

On April 26 2009, 23 years passed since the world's worst industrial accident. People living in the Ukraine due to Chernobyl fall-out, were exposed to radioactive substances over many years. The total release of radioactive substances was about 14 Ebq. Large areas of Europe were affected to some degree by the Chernobyl releases. An area of more than 200 000 km² in Europe was contaminated with radiocaesium, of which 71% was in Belarus, the Russian Federation and Ukraine [(1)]. The radiation dose a person received depends on a number of factors, including the years over which exposure occurred; age at exposure; the amount of contaminated food and water consumed; the distance and direction from nuclear power plant, and the length of time lived there. The average annual dose and typical dose range worldwide from natural Sources is 2.4 mSv [(2)]. According to measurements and evaluations within the first year after the Chernobyl accident, the external dose rate had decreased by a factor of approximately 30 Sv, mainly due to radioactive decay of the short lived radionuclides [(3)]. During the following decade the external dose rate decreased because of the radioactive decay of ^{134}Cs and ^{137}Cs (half-life = 30 years) and the migration of radio cesium into the soil. Afterwards, the external dose rate was mainly due to ^{137}Cs. In the long term, radiocesium becomes fixed within the soil matrix, and this results in a slow migration into the soil and correspondingly, in a slow decrease of the external dose rate. On the basis of such measurements, it is predicted that, of the total external dose to be accumulated during 70 years following the accident,

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about 30% was accumulated during the first year and about 70% during the first 15 years [3]. The external dose due to Chernobyl deposition accumulated to the present time is 70–75% of the total lifetime dose (70 years) for persons born in 1986 and living all the time in contaminated areas, based on average normalized effective external dose in the intermediate zone (100 km < DISTANCE < 1000 km) of Chernobyl contamination is 14 mSv [4].

Studies of immune status of children affected by the Chernobyl disaster have a special place in the general problem of post-effects carried out under the national program «Children of Chernobyl» long-term monitoring of the immune system in patients irradiated in childhood from exposure to radionuclides as well as (^{131}I, ^{129}I). ^{137}Cs, helped to establish certain regularities in the phasing of development dose changes in the immune system. The results carried out in the first few years after accident, the immune system in children that living in contaminated areas, indicate a blurring expressed, have been statistically significant deviation in the subpopulations of T-and B-lymphocytes in comparison to control group [5].

Although the accident occurred two decades ago, controversy still surrounds the real impact of the disaster. Therefore, The aim of preliminary study was determined development process status of T-cell population lymphocytes in Ukrainian children after 22 years from Chernobyl accident for next feasibility study.

MATERIALS AND METHODS

Individual dose measurement

Radioactive fallout resulted in long term contamination of thousands of settlements in Ukraine and some other European countries and in the irradiation of their inhabitants due to both external gamma radiation and internal exposure due to consumption of contaminated food. Radiation exposures to humans from Chernobyl occurred via Ingestion of contaminated food is the main contributors to dose for long time after the accident [6]. Therefore, determined the parameter of the long time transfer of ^{137}Cs in whole body of all subjects was determined by spectrometric assay. The environmental external irradiation was calculated as a blank dose.

Subjects

The present study included 150 children who were aged 6 to 16 years. Children are categorized in three groups: group I (n= 65) from zone 3th (30 to 60 km distance from Chernobyl nuclear power plant), group II (n= 65) from zone 4th (60 to 90 km distance from same location) and 20 healthy participant from Kiev, which is located 100 km from Chernobyl nuclear power plant. The local ethics committee approved the study. All samples were collected in the Ukrainian specializing clinical investigation of radiation defend population (Kiev, Ukraine).

Laboratory assay

In immunological laboratory for determination T-lymphocytes population such as T-lymphocytes (CD3), T-helper (CD4) and T-cytotoxic (CD8) were analyzed by roseting method using erythrocytes that conjugated with monoclonal antibody against of CDs receptors (Granom-Kharkov), 3 ml blood collected by drawing into heparin zed tube (200 µ/ml). Peripheral blood leukocytes was purified by ficoll (density=1.077). Purified lymphocytes were washed twice and diluted by phosphate buffer at pH 7.2-7.4 until total lymphocytes concentration counted 20 cells in Neobar slide. Briefly, in a test tube, 50 µkl monoclonal anti body against CD marker, adhesion to erythrocyte, added to 50 µkl dilation lymphocytes and incubated for 30 minutes at 37° C. The tube was centrifuged at 1000 g for 5 minutes and incubated at +4° C for 1 hour. The percentage of T-lymphocytes, T-helper, and was T-cytotoxic evaluated microscopically using slides

stained by Giemsa stain and then CD4/CD8 ratio were calculated.

Statistics

The values of each parameter in each group were expressed as parameter averages and standard deviation (mean ± sd). It was applied normal distribution, for two samples test and simple linear regression analysis and Pearson correlation coefficient was used to examine regression (R-squared) and correlation (r) between various measurements. Statistical analysis was performed using the Statistica Instat plus v3.36 for windows XP professional (Tulsa-OK 74104, USA). $P<0.05$ was consider significant.

RESULTS

Estimates of doses to individual members of population groups were based on millions of measurements of concentrations of radioactive material in the whole body contents of humans is 0.01 mCi per Kg mass body. The mean value of the long term transfer of ^{137}Cs in control group was estimated 0.04 mCi per Kg. In study groups, the values were 0.05 mCi per Kg in both groups.

The mean age of the subjects in the study groups was 11.2 (range 6-16). The proportions of T-cell population in study groups are presented in table 1. The normal range based on the reference value by the laboratory of CD3 is 50-75%; CD4 is 30-45%; CD8 is 18-35 %; ratio of CD4/CD8 is 1.4-2.0 and leukocyte count is 4.0-9.0. 10^9/l.

The decrease in the leukocyte count observed in the group I, although this value has not been significantly different in comparison to control group ($P=0.09$) but in comparison to group II, it was significantly different at $P<0.008$ (table 2).

Percentage of CD3 and CD4 in groups II and I decreased significantly in compared to control group at ($P<0.001$). Percent of CD8 decreased significantly in group I comparison to control group at $P<0.001$. However, there were no significant differences in the proportions of $CD8^+$ T-cell subsets between the group II and controls. CD4/CD8 ratio decreased significantly in the group I in comparison to control group at ($P=0.02$, $r=0.58$).

DISCUSSION

The main pathways leading to human exposure were external exposure from radionuclides deposited on the ground and the ingestion of contaminated terrestrial food products. Inhalation and ingestion of drinking water, fish and products contaminated with irrigation water were generally minor pathways [6, 7]. Our modeling data show that the urban population was exposed to a lower external dose by contaminated terrestrial food products compared with the rural population living in areas with similar levels of radioactive contamination. This might be because of the better shielding features of urban buildings and different occupational habits, also, as the urban population depends less on local agricultural products and wild foods than the rural population. Impaired immunological function may be related to the risk of diseases and non-cancer mortality. This may occur as a result of radiation induced depression or stimulation of immune system. The immune system is responsible for maintaining the integrity of higher organisms by responding to external agents. The specific response of the immune system is based on the action of T-cells, lymphocytes developed in the thymus. T lymphocytes include at least the following subtypes: cytotoxic T-cells, which respond to cells infected by viruses or tumor cells; T helper cells, which secrete mediators to activate lymphocytes; B cells; macrophages; natural killer cells and the T-cells themselves. Certain phases of the activation process of T lymphocytes in response to recognition of an antigen are known. The binding of the antigen transported by the MHC molecules with the TCR/CD3 complex

Table 1. Laboratory tests measurements in each group of study.

Study group	CD3 %	CD4 %	CD8 %	CD4/ CD8	WBC 10^9/l
Group I (n=65)	43.7±3.8	23.2±3.5	17.4±1.6	1.3±02	5.6±18
Group II (n=65)	54 ± 3.3	29.3±3.3	22±4.3	1.4±04	7.4±1.8
Control (n=20)	61.5±2.6	34.8±2.5	22.9±1.7	1.5±02	6.1±1.8

Table 2. Statistical tests of means against reference constants, regression and correlation in each study group comparison to control group.

Parameters	Group I P-value	Group II P-value
CD3	0.000* R.sq=0.154	0.000 * R.sq=0.175
CD4	0.000* R.sq=0.102	0.000 * R.sq=0.140
CD8	0.000 * R.sq=0.006	0.44 R.sq=0.150
CD4/CD8	0.02 * r=0.58*	0.26 r=0.07
WBC	0.09 R.sq=0.404	0.4 R.sq=0.112

triggers a chain of bio-chemical processes leading to the creation of protein phosphorylation as a final common pathway [8]. These raise the intracellular Ca^{++} concentration and active protein kinases, which in turn leads to the early expression of fas gene and later to the expression c-myc, gamma interferon, interleukins 1 and 2 and transferring, which are essential for T-cell proliferation [9]. Similar mechanisms appear to operate as a consequence of the action of low doses of radiation. Experimental studies characterizing immune response to radiation implicate intracellular calcium and protein kinases C, which cause transcription of the c-fos gene and production of interleukin-2 to activate T-cells [10, 11]. James and Makinodan previously reported that in mice, whole body exposure to gamma rays at dose 0.04 Gy/day for 20 days returned characteristic elevations of splenic T-cell subpopulations to normal and improved the condition of lymphadenopathy and splenomegaly [12]. These mice are known to have mutation within fas gene located on chromosome 19 [13, 14]. Akira *et al.* suggested that the phenomena observed by James and colleague in mice depended on radiation induced apoptosis that occurs specifically in $CD4^-CD8^-$ T-cells that abnormally accumulate, and that a reduction in the number of these cells help these autoimmune conditions [15].

Low doses can be defined as those less than 0.35 Sv to the whole body. Donald provided useful risk estimated for dose as low as 0.05-0.1 Sv, which are not overestimated by linear risk cancer estimates computed from the wider dose ranges 0.2 or 0.4 Sv [16]. The effects of radiation on the immune system generally intensify with the amount of dose received. Researchers know less about the effects of low dose radiation on the immune system than about the effects of high-dose radiation. Liu and colleagues discussed immunological changes in mice exposed to single doses of X-ray in the range of 0.025 to 0.075 Gy and by continuous exposure to gamma rays with a cumulative dose of 0.065 Gy [17]. There is currently no medical evidence that cell mutations in exposed persons will cause immune system diseases, only in 1994, a team of Japanese scientists reported finding

regarding increased risk for autoimmune hypothyroidism among people exposed during the atomic bombing of Nagasaki in World War II [18]. Apparently, this is the first report of detecting a significant increase in an autoimmune disease among people who survived the atomic bombings. In addition, it has been shown that, there is a higher than usual rate of immune system diseases, particularly autoimmune diseases, in people that exposed to Hanford's releases [19]. Several ecological and epidemiological studies have examined the association between radiation exposure and leukemia. Parkin *et al.* compared acute leukemia incidence rate before and after Chernobyl accident. They concluded that excess in leukemia rate was more pronounced in rates that were most affected by Chernobyl related ionizing radiation exposure [20].

In our study, leucocytes count in groups II and I have not significantly in comparison to control group (P=0.09,P=0.4) but in group II, it is significantly different in comparison to group I at P <0.008 (table 2). This data approved that in group I, reduced leukocyte count related to low dose radiation, because doses were high enough to cause change count white blood cell in 3th zone after two decade in some individuals. Many previous studies, evaluated that leukocyte count, were relatively lower for the X-ray exposed individuals [21]. Arenas suggested, the number of leukocytes was reduced by different doses of radiation tested; but the highest inhibition was observed which is maximal at 0.3 Gy [22].

Previous studies of blood cells from atomic bomb survivors have shown that frequencies of chromosome aberrations and somatic mutations are elevated in heavily exposed survivors and that T-cell functions and the number of mature T-cells were decreased in the survivors who were exposed to radiation as adults [23, 24]. Previous studies around Chernobyl accident relating to immune system have yielded conflicting results. Galizekao found that Russian children after two years of accident did not show abnormality in level of T-lymphocytes, but showed a minor increase in B- lymphocytes [25]. Many studies reported an initial decrease in $CD3^+$ and $CD4^+$ Cells. Yarlin *et al.* reported a decrease in $CD3^+$ cells in groups exposed to 0.1-0.5 Gy and other exposed to 0.5-9 Gy, however there was a decrease in T-helper cells only in lower dose group and a decrease in $CD8^+$ cells only in the higher dose group [26]. In other study of workers in the 30 km zone, Titova *et al.* reported a decrease in both $CD4^+$ and $CD8^+$ [27], and Kurjane *et al.* reported that doses between 0.01-0.5 Gy reduced $CD3^+$,$CD4^+$ and $CD8^+$ T-cells [28]. However Kuzmenok *et al.* did not find any change in the level these cell population 11-14 years after accident [29].

In the present study, we investigated whether decreases in $CD3^+$ and $CD4^+$ T-cells were associated to increased radiation dose. Among T-cell subsets, the proportion of $CD3^+$ and $CD4^+$ T-cell subsets was decreased significantly in groups II and I (table 2, figures 1 and 2); this tendency was apparent for the $CD8^+$ T-cell subset in group I that could lead to poor proliferation control. However, there were no significant differences in the proportions of $CD8^+$ T-cell subsets between the group II and controls (figure 3). In the group I, a significant ($P < .05$) decrease in $CD4^+$ T-cells of peripheral blood, with a concomitant decrease in $CD8^+$ T-cells, was observed, resulting in a marked difference in the $CD4^+$: $CD8^+$ ratio in the immune status. Although, this status in the group II was characterized by a value of $CD4^+$: $CD8^+$ ratio near the control group with $CD8^+$ T-cell predominance (P=0.26, r=0.07). The stromal $CD4^+$: $CD8^+$ ratio was significantly ($P= 0.02$, r=0.58) lower in the group I compared with the control, suggesting a possible clinical importance to this phenomenon (figure 4). In agreement with our results a decrease in T-cell count and immunoglobulins was previously reported [30, 31]. Bazyka *et al.* have shown that, late period after the acute radiation exposure in Chernobyl radiation emergency workers is characterized by decreased $CD8^+$ suppressor cell function. Low T-suppressors and NK-

cell counts in the promotion of malignant proliferation reactions have to be estimated with the comparative analysis of onco gene expression and the role of apoptosis [32]. In conclusion these results strongly suggest that previous radiation exposure altered the composition of T-cells in the peripheral blood of children exposed due to Chernobyl accident. This might imply the possibility of the effect of the release of ionizing radiation after 23 years to the developmental processes of T-cells.

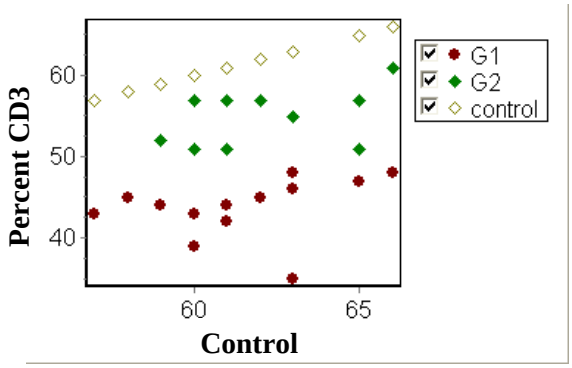

Figure 1. Percent of CD3 phenotype in study groups compare to control group.

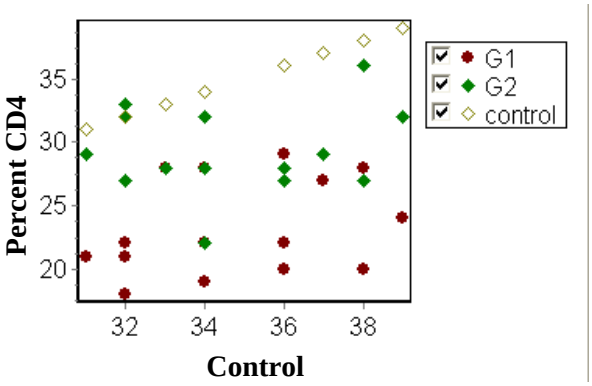

Figure 2. Percent of CD3 phenotype in study groups compare to control group.

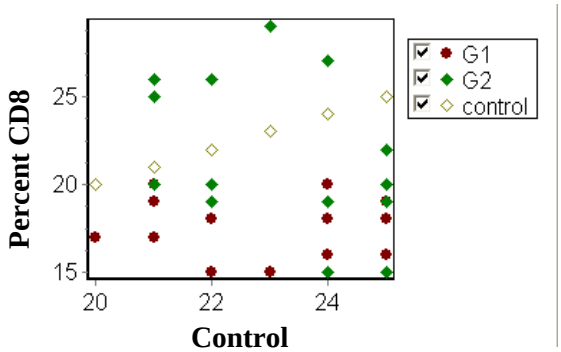

Figure 3. Percent of CD8 phenotype in study group compare to control group.

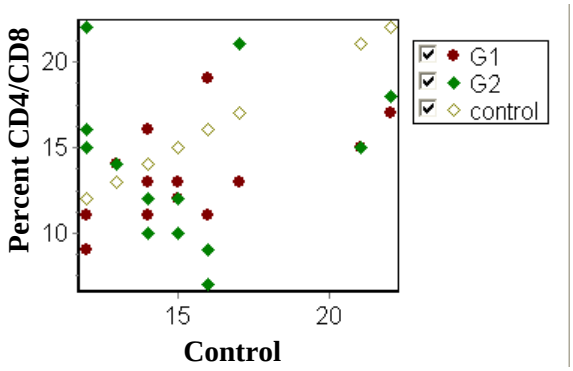

Figure 4. Percent of CD4/CD8 ratio in study groups compare to control group.

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