ORIGINAL ARTICLE

PHAGOCYTE FUNCTIONS OF HUMAN SUBJECTS LIVING IN HIGH LEVEL OF NATURAL RADIATION AREAS IN IRAN

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Background: Iran is considered as one of the countries which are located in high radiation area. Ramssar contains natural radioactive sources (Uranium-238) that can produce Radium-226 with high solubility and Radon gas with ability to fuse in dirt, soil, and water in its decay chain. Radioactive rays can produce free radicals with a short life time but high energy, which lead to changes structures of protein bindings, hormones activity, lipids oxidation and DNA breakage which can induce autoimmune diseases and other disorders. The aim of this study was to evaluate effects of natural radiation on innate and humoral immune response of those exposed to natural radioactive rays compared to control group. **Methods:** Subjects from high and low natural radiation areas were included in the study. Neutrophil chemotaxis, Nitro-Blue Tetrazolium (NBT), and **a**ntioxidant effects were evaluated for effects of radioactivity on free radicals and innate immunity. Also Cytokines (IL-2, IL-4) levels for humoral immune system of the subjects were measured. **Results:** Total plasma antioxidant level and potency of respiratory burst in individuals who lived in area with high levels of natural radiation decreased significantly compared with, control subjects who lived in the other areas of Ramssar with normal radiation. The level of IL-4 increased in individuals who lived in area with high levels of natural radiation, which could lead to Th2 pattern of immune response whether neutrophils chemotaxis in the groups living in the area with high level of natural radiation didn't change significantly when compared to control. **Conclusion:** Neutrophil respiratory burst activities suggested that NADPH probably reduced due to different reasons including decrease in NADPH oxidase and other enzymes, and mutation of the genes or production of oxidant substances. Neutrophil chemotaxis reduced but this reduction was not statistically significant.

Keywords: Radiation, Antioxidant, IL-2, IL-4, chemotaxis, NBT, free radicals

INTRODUCTION

There are radio-nucleotides in the crust of earth coming into existence of earth and up to now because of them long half life still continues acting which most important are U238, Th232, and K90. Source of these radioactive in human body are internal and external. Internal way includes natural radioactive agents, e.g., Radone, Torone, and radioactive principals exist in live tissue (Rb78, K40).[1] External way includes areas with high level of natural radioactive like Iran, Brazil, India, China etc. Ramsar has highest natural radioactivity in Iran.[2] Yearly effective dose for people of Ramsar is (6 msv),[3] while world mean in low level radioactive areas is 2.4 msv/y.[4] One study shows that B and T cells are susceptible to radioactivity, and it causes cell death, most of them are T suppressor subgroup.[5] Phytohemagglutinin (PHA) could increase stimulation of human pulmonary lymphocyte T cells significantly, increase percent of B cells, and increase DNA synthesis in high level radiation area.[6] An observation on mice showed that gamma irradiation was able to suppress mice immune system but when given vitamin E, proliferation suppressed.[7] Evaluation of the effect of natural radiation on innate immune system and its correlation with antioxidant defence in human leads to produce free radicals (oxidant agents). Up to now, no study has performed on human immune response and level of antioxidant in high natural radiation areas.

In view of these findings, we decided to measure neutrophil function [chemotaxis**,** Nitro-Blue Tetrazolium (NBT)], and plasma antioxidant level for radioactive effect on free radicals and innate immunity, and also Cytokines (IL2, IL4) levels for humoral immune system.

MATERIAL AND METHODS

This study was carried out from Jun 2004 to April 2005. The subjects were included from two areas of Ramssar, (Talesh Mahalleh and Chaparsar) with high level of natural background of radioactivity, and two areas (Darya Poshteh and Sefied Tameshk) with normal level of background radioactivity.

Neutrophil chemotaxis: Ten ml heparinised blood was obtained from each of the 40 subjects and was centrifuged twice at 250 g and twice 140 g. From the prepared neutrophil suspension 400 µl was added to Elisa cup with basal filter and 3 µm Millipore filter, incubated for 3 hours before fixation with isopropanol and haematoxyline and methylene blue staining. The neutrophil chemotaxis was measured in prepared Millipore filters.

Nitro-Blue Tetrazolium (NBT) test: Fresh heparinised blood 100 µl was added to 100 µl NBT (Nitro-Blue Tetrazolium) solution, to measure respiratory burst in the presence of PMA (Phorbol 12 Myrestate 13 Acetate) as neutrophil stimulator.[8] After 30 minute incubation time, tubes were centrifuged at 250 g. Smears were prepared from the sediments, fixed with methanol and were stained with Giemsa. The stained smears and percentages of neutrophils which reduced and turned yellow NBT to blue Formazan were counted.

Antioxidant measurement test: Two ml of fresh hepirinisied blood was immediately centrifuged, plasma separated and maintained at -30 ºC. Then the plasma samples were harvested and FRAP test was performed. It was complex of ferric three piridil three Azine [(Fe3+TPTZ): Ferric-3 Piridil-3 Azine] which antioxidants of samples reduced to blue dye ferrous (Fe2+). Its reducing intensity was measured with spectrophotometer with 593 ηm.

Cytokines (IL2, IL4) levels: Three ml of sterile heparinised blood was added to CRPMI (complete culture medium), centrifuged at 900 g, separated lymphocytes, washed with RPMI 1640 and centrifuged at 280 g. Lymphocytes suspension was prepared. For preparation of cytokines soup, lymphocytes cultured on CRPMI, and PHA were added. After 72 hours cellular soups were separated. IL-2 and IL4-were measured in cellular soups using Elisa.

Subjects living in area with high levels of radiation and normal subjects were compared using correlation, t-test, Chi-square, and Manu-Whitney test.

RESULTS

The statistical analysis showed that results of immune system evaluation of two subject groups have not been affected by the factor of age and sex. Therefore there was no bias effects regarding the age and sex on statistical analysis of the findings.

Regarding antioxidant activity of subjects sera and potential of respiratory burst and IL-4 level in serum, differences were observed significantly when two groups were compared together. The anti-oxidant activity and potency of respiratory burst were low, and level of IL-4 was higher in the group living in the areas with high level of natural radiation. Neutrophils chemotaxis was declined and level of IL-2 increased in subjects that living in area with high levels of natural radiation compared to control. The level of serum anti-oxidant increased with the increase of exposure time. (Table: 1–3).

Table-1: Results of Man-Whitney statistical analysis of variants, when comparing the control group with the group exposed to high levels of natural radiation

Variables	Exposed subjects			Control			*p*
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	
Age (Year)	37	31	44	32	23	50	0.499
Time period of exposure (Year)	23	13	40	-	-	-	0.000
Antioxidant-levels (µmol)	873	766	1032	967	870	1129	0.009
chemotaxis (µm)	115	94	138	123	97	152	0.322
IL-2 (µg)	159.2	134.8	233.8	155.45	0.873	116.29	0.422
IL-4 (µg)	225.6	151.9	370.675	45.05	26.825	61.875	0.000
NBT complete (%)	50	21	84	99	95	99	0.000
NBT incomplete (%)	20	10	50	0.0	0.0	0.0	0.000
NBT Zero (%)	10	0.0	20	1	1	5	0.000

Table-2: Statistical analysis of sex variant between control group and group exposed to high levels of natural radiation

Variant	Gender	Number	Mean±SD	*p*
Antioxidant	Male	54	1005.96±216.57	0.001
	Female	48	869.22±171.27	
Chemotaxis	Male	58	131.30±35.78	0.004*
	Female	48	112.15±29.88	
IL-2	Male	43	478.52±710.53	0.128
	Female	43	290.42±447.04	
IL-4	Male	43	141.004±138.966	0.213
	Female	43	172.04±144.729	
NBT com	Male	56	78.21±30.476	0.190
	Female	48	70.21±31.276	
NBT in com	Male	56	11.64±22.827	0.102
	Female	48	19.69±26.963	
NBT Zero	Male	56	10.05±17.618	0.750
	Female	48	90.06±13.338	

*Comparing the two groups was not affected by sex factor
*The levels of plasma total antioxidant and levels of chemotaxis were significantly higher in man than woman regardless of two studied groups

Table-3: The results of comparing exposed to high levels of natural radiation with another variant

Variant	Coefficient	*p*
IL-2	-0.177	0.267
IL-4	-0.158	0.311
Chemotaxis	-0.065	0.649
NBT com	-0.161	0.258
Antioxidant	-0.364	0.014

Anti oxidant level significantly increased with exposure to high level of natural radiation

DISCUSSION

Radioactive rays produce free short life-time and high energy radicals which lead to changes in immune system. The results showed that in the subjects from Ramssar (Chaparsa and Talesh Mahalleh), serum antioxidant level decreased, but its enhancing had direct correlation with exposure time (p<0.009). Mitsunobu *et al*[9] described that humans who use warm mineral water containing high levels of radon (product of Uranium-238 and Radon-236) had increased level of catalase (an

anti-oxidant enzyme) and super oxide Dismutase (an anti-oxidant inhibitor), but the level of glutathione with anti-oxidant activity had no significant changes.[9] It is noted that in the present study plasma antioxidant potency measured by FRAP (a procedure which can evaluate levels of food's anti-oxidants such as Vitamin C, Vitamin E, Beta-Caroten and other Carotinoids, did not have capability to assess the anti-oxidant enzymes such as Single Oxide Dismotase (SOD) and Catalase. Therefore evaluation of SOD and Catalase enzyme needs to be studied further. Since the level of endogenous enzymatic antioxidant activity may tend to increase in subjects who are living in high radiation area, resulting in reduction in deleterious effect of radiation and decline plasma antioxidant, it also needs to be studied.

In the present study the level of IL-4 in exposed to high levels of radiation increased significantly ($p<0.009$), however the IL-2 remained unchanged ($p<0.422$). Another study conducted in this area of Iran showed that the level of serum IgE in the individuals living in the area with high level of natural radiation, increases significantly.[4] Another study[10] reported that blood lymphocytes exposed to gamma radiation increases IL-4 production, however increase in IL-5 level (a Th2 cytokine), which is needed for IgE switching. It could be suggested that radiation affects cytokine production profile, and could shift Th1 response to Th2. This result can lead us to future studies conclude profile of cytokine production and Th1/Th2 balance in individuals who living in the area with high level of radiation. The findings of the present study also indicate that the level of the neutrophils chemotaxis in the group living in the area with high level of natural radiation had no significant changes when compared to control group. In both groups, the levels of chemotaxis of the neutrophils had showed normal range. Since, in other studies similar results had been obtained, we suggest that natural radioactive radiation does not affect phagocytosis of neutrophils. Other parameter studied in the present investigation, neutrophil respiratory burst in both groups using NBT test indicated that the potency of respiratory burst in individuals living in area with high levels of natural radiation significantly decreased which is not beneficial for innate immunity. Shune[11] showed that the low doses of X-radiation (IGY), causes a significant reduction in respiratory burst of activated macrophages (IU). In spite of radiation producing low effect on respiratory burst induction[11] it could be concluded that exposure to higher radiation may cause no further responses to higher stimulation of neutrophils, perhaps if it was more stimulated respiratory burst activation in neutrophils such a way by PMA in NBT test could not affect in this condition. However, possible genetic mutations of the sub-units of NADPH-oxidase and other respiratory burst cascade enzymes could result in glaucomatous disease and susceptibility to catalase-positive bacterial infections. This leads us to further clinical and genetic studies.

CONCLUSION

Radioactivity could stimulate cells to produce free-radicals (oxidants) resulting in a decrease in total plasma antioxidant level. However, in prolonged exposure to higher radiation, a compensatory response may occur which affects potency of respiratory burst and profile of cytokine production.

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