

Cytokine Status in Ukrainian Children with Irritable Bowel Syndrome Residing in a Radioactive Contaminated Area

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ABSTRACT

Background: The effect of low dose radiation on immune system is shown. Ionizing radiation can affect cytokine production and polarization of T helper cells. **Objective:** The current study focused on ionizing radiation in Ukrainian children residing in a contaminated area with clinical irritable bowel syndrome. **Method:** Our study included 75 rural children population aged 4-18 yrs, who lived in a contaminated area exposed to natural environmental radiation with clinical irritable bowel syndrome (categorized in three groups) and 20 rural children participants aged 5-15 yrs who were living in areas with similar levels of radioactive contamination without clinical irritable bowel syndrome as control group. Internal radiation activity was measured by gamma-ray spectrometry. Serum levels of IL-4 and IFN- γ were measured by enzyme linked immunosorbent assay. **Results:** A trend towards increased levels of IL-4 was observed in children with clinical irritable bowel syndrome. In these children, IFN- γ levels were lower than that of the control group. **Conclusion:** The IBS symptoms in Ukrainian children residing in a contaminated area may have stemmed from Th1 to Th2 immune deviation and differential expression of IL-4 and IFN- γ .

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INTRODUCTION

The adverse biological effects of low natural radiation on immune system are well known from studies involving people related to Chernobyl disaster. Irritable bowel syndrome (IBS) is commonly observed in Ukrainian children and related to ionizing radiation (1). This has led to a low grade inflammatory state which causes gut sensory motor dysfunction resulting in IBS symptoms (2) and has led to the hypothesis that cytokines may lead this resulting in IBS symptoms, it may be represented that polymorphisms cytokine genes resulting in higher level of pro-inflammatory and lower level of anti-inflammatory cytokines may be a risk factor for IBS or may make the response different from others (3).

In Ukrainian children residing in a contaminated area, the humoral immune status has activated and related to low dose radiation and related mostly to changes in the amount of peripheral T-lymphocyte population (4) to suggest an imbalance toward to Th2 profile induced by radiation exposure. The prevalence of a radiation induced Th2 response has been verified in experimental studies, for example the spleens of mice irradiated with different doses of gamma ray produced less IFN- γ and more IL-5 than the untreated group (5).

In present study, we examined the effect of natural ionizing radiation in Ukrainian children residing in a contaminated area with clinical syndrome of irritable bowel syndrome which induced Th2 response and caused an increase IL-4 productions.

MATERIALS AND METHODS

Subjects. The research has been conducted on 95 rural participants aged 4 to 18 who lived in a contaminated area at a distance of 60 to 90 km from Chernobyl Nuclear Power Plant exposed to natural environmental radiation with clinical symptom of IBS such as pain or discomfort in the abdomen and an altered bowel habit without any inflammation. Children fall under four groups: Group I: 21 participants aged 4-9 with clinical symptom of IBS, Group II: 26 participants aged 10-13 with clinical symptom of IBS, Group III: 28 participants aged 14-18 with clinical symptom of IBS, Group IV: 20 participants aged 5-15 without clinical symptom of IBS as control group. All the patients were undergoing an outpatient radiation treatment program at Ukraine-based specialized hospitals and were directed to our hospital for health care monitoring from March to June 2009 (Kiev, Ukraine). Clinical diagnosis of IBS was based on the Rome III criteria. The local ethics committee has approved this research. Parents were willing to allow their children to take part in medical research.

Radioactivity. The internal whole body radioactivity due to ^{137}Cs was measured by gamma spectrometry with scintillation detector (Gamma plus, Moscow, Russia). The micro curie was used to express the activity of ^{137}Cs in a period of time and then convert to Becquerel (Bq) per kg of body mass (4).

Immune Correlates. Subpopulation of lymphocytes including T lymphocytes (CD4) were analyzed by rosetting method using erythrocytes conjugated with monoclonal antibody against CD4 receptor (Granom-Ukraine). Serum levels of IL-4 and IFN- γ were measured by double antibody sandwich ELISA according to the manufacturer's protocol (Polingnost, Russia). The optical density of each well was determined within 30 min using a microplate reader (450 nm).

The values of each parameter in each group were expressed as parameter averages and standard deviation (mean \pm SD). Normal distribution was applied for paired-samples T test.

Table 1. Comparison of cytokines concentrations, T cell subpopulations and internal radiation activity in study groups.

	Internal Radiation Activity (Bq)	IL-4 (pg/ml)	IFN- γ (pg/ml)	T cell Subpopulation (CD4)
Group I (n=21)	1.86 \pm 0.51	4.6 \pm 1.4	49.7 \pm 1.1	28 \pm 6.0
Group II (n=28)	1.85 \pm 0.57	5.1 \pm 1.8	49.5 \pm 1.4	25 \pm 4.5
Group III (n=21)	2.01 \pm 0.56	5.9 \pm 0.9	48.5 \pm 2.3	27.9 \pm 6.1
Group IV (n=21)	1.80 \pm 0.21	3.3 \pm 0.7	50.8 \pm 0.2	32.3 \pm 2.5

Statistical Analysis. One-way analysis of variance (ANOVA) was used to compare the mean preload values among the groups. Statistical analysis was done using SPSS 12 for Windows XP Professional. $p < 0.05$ was considered significant.

RESULTS

The mean age of the participants in group I was 6.4 \pm 1.8, in group II was 11.4 \pm 1.0, in group III was 14.0 \pm 1.0, and in control group was 10.2 \pm 3.3 yrs.

The mean value of the internal dose of ¹³⁷Cs in control group was estimated to be 1.80 \pm 0.21 Bq.106. In study groups, the values were 1.86 \pm 0.51, 1.85 \pm 0.57, 2.01 \pm 0.56 Bq.106 in groups I, II and III.

Based on the reference value by the laboratory the normal range of IL-4 was 2.1 \pm 1.0 pg/ml; and IFN- γ was 51.8 \pm 8 pg/ml.

Table 2. Pairwise comparison of Cytokine levels and T cell subsets between groups.

	IL-4	IFN- γ	CD4
Pair I and IV	0.001 ¹	0.004 ²	0.000 ¹
Pair II and IV	0.001 ¹	0.005 ²	0.000 ¹
Pair III and IV	0.000 ¹	0.001 ¹	0.000 ¹

¹P Values of 0.001 are considered statistically different.

² P Values of 0.05 are considered statistically different.

Table 1 shows the results of the analyses of T lymphocyte phenotypes. The normal range based on the reference value by the laboratory count of CD4 is 30%-45%. All participants had significantly lower numbers of total T lymphocytes. The study group had significantly lower proportions of CD4⁺ lymphocytes compared with the control group at $p < 0.001$.

Table 3. Multigroup comparison of immunological parameters between groups.

		IL-4	IFN- γ	CD4
I	Between groups	0.947	0.258	0.574
II	Within group	0.815	0.674	0.646
III	Total	0.112	0.188	0.796

P values of 0.05 are considered statistically different (ANOVA).

The mean level of IL-4 was significantly higher in children with clinical symptoms of IBS when compared with the group of children without clinically symptom of IBS. In contrast, children with clinical symptoms of IBS had significantly lower proportions of level of IFN- γ (Tables 1-2).

No significant difference between and within the children with clinical symptoms of IBS was observed in the proportions of IL-4, IFN- γ and CD4 (Table 3).

DISCUSSION

Irritable bowel syndrome (IBS) is commonly observed in Ukrainian children related to Chernobyl disaster (1). Previous studies have shown that internal radiation activity of whole body in rural population is higher than urban population. This arises from 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 rural population (1,4,6). In these studies the levels of radiation that Chernobyl populations were exposed to have been shown to affect the immune system. In addition some evidence suggests that IBS is affected by the immune system (7-9). In this study we investigated irradiation in children with clinical symptoms of IBS may be inclined to produce Th2 cytokine response than to children without clinical symptoms of IBS.

Interaction between cytokines and their receptors leads to the activation of multiple signaling molecules, including the family of "signal transducer and activator of transcription" (STAT) proteins. Different STAT proteins are capable of regulating the activity of common signaling pathways used by many cytokines. It has been reported that mice with disrupted STAT gene have impaired IL-12 responsiveness of NK and T cell, a lack of Th1 responsiveness which enhanced Th2 function (10). Gamma radiation has been shown to reduce STAT-1 phosphorylation. In agreement, mRNA levels for IL-5 were only slightly increased by gamma radiation compared with non irradiation samples, suggesting that ionizing radiation induce a polarized Th2 response by interfering with STAT signals, thereby causing suppression of Th1 response (5). On the

other hand, after low dose radiation, the change observed might contribute to a shift in favor of Th1 differentiation (11).

Role of T-cell in the pathogenesis of IBS syndrome is clear now, Ohman showed that IBS patients have an increased frequency of activated T-cells, the increased level of T-cell activation is consistent with the hypothesis of low-grade immune activation in IBS and may also be involved in symptom generation in IBS (12). Van Der Veek suggests IBS subgroups are warranted to establish definite associations with tumor necrosis factor-alpha and IL-10 gene polymorphisms (9).

Different studies indicate that ionizing radiation causes augmentation of Th2 cytokine production, for example IL-4, IL-5 and IL-10 (13,5). In the present study, we investigated whether increased levels of IL-4 were associated with alteration in cytokine release from Th2 cell type in agreement with several other authors (14,15). This may cause enhancing Th2 immune responses (15-16) and suppression of Th1 while decreasing in level of IFN- γ . We suggested that radiation exposure from the Chernobyl accident appear to be related mostly to changes in the amount of peripheral T-lymphocyte population and may have affected change in the immune status in Ukrainian children (1). Children in our study have impairment of cell mediated immunity associated with increase in B cell component and a rise in immunoglobulin levels (4) indicates that the mechanisms responsible for increased immunoglobulin synthesis are somewhat disturbed, which may be associated with the effect of ionizing radiation on B lymphocytes and Th2. The influence may be associated with the expression of the adhesion particles involved in the communication of those cells during IgG synthesis, in the production of Th2 cytokines at that stage of the humoral response as well as IL-4, or may result from the direct effect on gene expression and IgG synthesis. As stated earlier, B lymphocytes are more sensitive to ionizing radiation than T lymphocytes (17,18). In addition, Th2 lymphocytes responsible for class switching of produced immunoglobulins are more sensitive to radiation than Th1 population, whose role is mainly associated with cellular-type response (19). In our study we showed that ionizing radiation may change innate immune system (4) suggesting that an imbalance toward a Th2 profile induced by radiation exposure. It seems that soluble lymphokines derived from T-cells whose main bioactivity is to stimulate the activated T-cell to reproduce continually, and proliferate are affected. In agreement with our results, recent research has demonstrated that higher levels of pro-inflammatory and lower level of anti-inflammatory cytokines may be a risk factor for IBS or may make the response different from others. Santhosh et al. showed that there are polymorphism differences in cytokine genes between patients with IBS and healthy controls (3).

However, the roles of cytokines in IBS have not been fully elucidated, this study led us to suggest that the IBS symptoms in Ukrainian children residing in a contaminated area may have stemmed from Th1 to Th2 immune deviation and differential expression of IL-4, IFN- γ .

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