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The Effects of High Level Natural Radiation in Mamuju - Indonesia on the Immune System of Its Residents

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ABSTRACT

The immune system is one of the most significant defenses against environmental insults including natural radiation. The purpose of this preliminary study was to assess the effects of high natural radiation to Mamuju residents, by focusing on immune-related blood cell counts (leukocytes, lymphocytes, monocytes, and granulocytes) and immunoglobulin E (IgE) level. The blood samples were collected from 18 adult residents in a high background radiation area (HBRA) while 18 residents in a normal background radiation area (NBRA) served as a control group. The blood components were measured by using the hematopoietic analyzer, and IgE immune biomarker was measured with ELISA (enzyme-linked immunosorbent assay) according to standard protocols. The data showed that the level of all blood cells, except for monocytes, of residents in HBRA was higher than that of NBRA. Statistical analysis revealed that there was no significant difference (P>0.05) in the blood cell counts and IgE level in both groups and their values were within normal limits. The level of IgE in HBRA was significantly higher than the control area (P≤0.05), as its IgE level in males compared to females in both residents. The relationship between IgE level and age were negative in these residents. From this study, it was concluded that long-term exposure to high radiation may affect the immune system as one of radiation adaptive response.

INTRODUCTION

Natural radioactivity is one of the main sources of radiation exposure. The annual dose of this radiation to the community is very dependent on where they live with the averages of about 2.4 mSv [1]. Several areas in the world, including Mamuju, Indonesia, exhibit higher-than-average natural background radiation that originate from elevated levels of terrestrial radioactivity as well as indoor radon and its decays products [2-4]. Mamuju, located in the West Sulawesi Province, Indonesia, has a natural background radiation of around 10-15 times higher than normal [5].

The Botteng village is one of the anomaly areas in Mamuju District, West Sulawesi, where uranium content is high, mainly upstream of the river. The average dose rate in this area can be up to 2.8 µSv/h as the result of a high level of natural uranium and thorium contents in the rock and the soil, as well as the high concentration of radium-226 and radon gas [6]. Mamuju residents accumulate radiation doses through long-term exposure to low radiation rates, both externally and internally.

Mapping and surveillance were conducted by the Center for Technology of Radiation Safety and Metrology in 2008-2013. Using special measuring devices, it was found that several villages in Mamuju had high natural radiation. In Botteng Village, the average indoor radioactivity for gamma ray was 568.88 nSv/h, whereas for outdoor it was higher (760.95 nSv/h) compared to control area (162.30 nSv/h), so the high background radiation area (HBRA) exhibits indoor radioactivity 3.5 times as high as the control [6].

Studies on the biological effects due to high natural radiation in this area reported no s ignificant differences between the frequency of mononuclei in lymphocyte cells with that of

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normal background radiation areas [7,8]. Another assessment showed no alteration of γ-H2AX levels in lymphocytes of residents living in this area [4,5]. It is predicted that there are indications of the phenomenon of radioadaptive responses to residents in Mamuju which need to be further investigated.

Oxidative stress induced by ionizing radiation exposure may alter immune function. The effects of this high natural dose of ionizing radiation are depressed immunity and dysfunction of this system. Those effects have been clearly demonstrated and confirmed both experimentally and in epidemiological studies [9]. It has been observed in human populations from regions of high background radiation in China that radiation might fortify the immunological ractions and the defense instruments of the human body [10]. In the last few years, several studies have been conducted on the impact of the long term high background radiation exposure on the immune system [11,12].

It has also been known that ionizing radiation impairs hematopoiesis either through reduced bone marrow production or redistribution and apoptosis of mature formed elements of the blood [13]. It is suitable to use peripheral blood cells as bioindicator to estimate damage from ionizing radiation. Of all these cells, lymphocytes have been known to be one of the most radiosensitive mammalian cells. Several recent studies by Shahid *et al*. [14], Gyuleva *et al*. [15], and Surniyantoro *et al*. [16] revealed significant differences in lymphocyte counts between radiation workers and the control population. Such a research on these impacts in the Mamuju area has never been done.

Immunoglobulin E (IgE) is a key component of immediate-type allergic reactions. There is limited data on how the immunological responses in humans living in high background radiation areas might stimulate the defense mechanisms and whether this radiation has an antiallergic effect [17,18]. Therefore, this research aimed to assess immunology-related blood cells in the residents who were living in an area where a high concentration of naturally occurring radionuclides was present resulting in long-term low-level doses of radiation. This study emphasized leukocytes, lymphocytes, monocytes, and granulo-cytes cells which play a role in the immune system [19].

EXPERIMENTAL METHODS

Sampling site and subjects

The sampling was conducted in Botteng Village of Mamuju, West Sulawesi, as shown in red in Fig. 1.

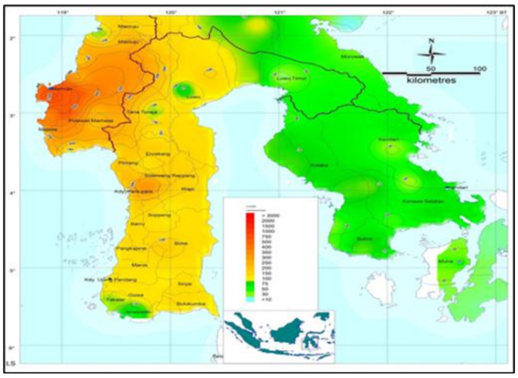


Fig. 1. Natural radiation dose map in Mamuju, West Sulawesi, where the highest natural radiation dose area is shown in red color. Its neighbor rovince (South Sulawesi in yellow color) and the scale of radioactivity (Bq/kg), as well as the location of the island in Indonesia, are also shown.

This study was a case-control study and sample was taken by using simple random sampling. As a preliminary step, only 18 residents of around 1920 people in study area were assessed, where this number was based on the good condition of samples and the ability to test IgE levels.

The blood samples (3 milliliters for each person) of the exposed groups were collected from 18 residents of high background radiation in Botteng Village, Mamuju District, West Sulawesi Province, Indonesia. The blood samples of the control group were obtained from 18 residents in Keang with normal background radiation. The ages of all volunteers ranged from 20 to 79 years for both sexes, and all with no known significant health problems. The information about the aims and intention of the research was notified to all volunteers and they signed a consent form and questionnaire before blood sampling. The protocol had been approved by the Governmental Ethics Committee Review Board of the Indonesian Ministry of Health in Jakarta with the number of LB.02.01/5.2.KE.051/2015. The protocol followed all the recommendations of the Board for research involving human beings.

Immune hematologic (leucocyte, lymphocyte, monocyte, and granulocyte) counting

The collected blood was mixed well and the count of cells was determined by using ABX Micros 60 hematology analyzer (Horiba ABX SAS) with the procedure applied according to the standard protocol provided by the manufacturer. This fully automated hematology analyzer was used for in-vitro diagnostics testing the number of leucocytes, lymphocytes, monocytes, and granulocytes.

Detection of allergen-specific IgE

The counting on serum IgE (a reagent antibody) of HBRA and NBRA residents was performed using the enzyme-linked immunosorbent assay (ELISA). Twenty microliters of sample and standard solution were dispensed into the IgE-based monoclonal antibodies attached to microwells. One hundred microliters of the zero buffer was dispensed into each well, mixed for ten seconds, and incubated at room temperature for half-hour. Any residual test specimen in the well was then washed away and 150 µl goat anti-IgE in the antibody-enzyme (horseradish peroxidase) conjugate reagent was added to each well. Afterward, it was washed with water to remove unbound labeled antibody. A substrate solution of 3,3',5,5'-tetramethylbenzidine was added and incubated for 20 min. A stop solution was added to stop the reaction and then was measured with the ELISA reader at 450 nm [20].

Statistical analysis

The results of the research were analyzed using the Medcalc program. The data normality test was determined by Kolmogorov-Smirnov test on a sample test. The comparison of the two groups was done with T-test.

RESULTS AND DISCUSSION

The research on the immune system was carried out on residents with occupation of mostly farmers, as they were almost continuously exposed to natural radiation from their working place and might also inhale local naturally-occurring radionuclides. The residents who acted as control were from nearby regions of normal background radiation areas in Mamuju with the same physical environmental situation. The results showed that the leukocyte, lymphocyte, and granulocyte levels of blood from HBRA residents were higher than that of NBRA. It is predicted that there is an effect of natural radiation by suppressing the number of these cells. On the other hand monocyte level of blood from HBRA residents was lower than those of NBRA. The statistical results do not show a significant difference (P>0.05) for all the parameters in both areas. However, the average immune parameter values among occupants in areas with normal levels of exposure to natural radiation (NBRA) and high natural radiation (HBRA) are still within normal limits, as detailed in Table 1.

Table 1. The average level of blood cells among residents in areas of normal levels of exposure to natural radiation (NBRA) and high natural radiation (HBRA).

Blood cells (10^3/mm^3)	**Normal value**	**NBRA**	**HBRA**	***P(T<=t)***
		(mean ± STD)		
Leucocyte	4.0 – 10	7.00 ± **1**.17	7.56 ± **2**.55	P = 0.42
Lymphocyte	1.2 – 3.2	2.07 ± 0.54	2.52 ± 0.95	P = 0.15
Monocyte	0.3 – 0.9	0.54 ± 0.25	0.47 ± 0.21	P = 0.35
Granulocyte	1.2 – 6.8	4.39 ± **1**.10	4.75 ± **2**.03	P = 0.94

IgE concentration of HBRA residents, based on statistical analysis, was significantly higher than the control (NBRA). The average IgE concentration in HBRA was 430.72 IU/ml and in NBRA it was 283.59 IU/ml. It was known that the average levels of IgE in the resident of both regions are above normal value (1.5-144 kU/l), according to Rengganis *et al*. [21]. Analysis of IgE with Mann whitney U test showed higher average values in exposed persons compared to controls and statistically significant (P=0.0482). Ten percent of both NBRA and the HBRA samples showed IgE values in normal range. Images of representative IgE concentration in serum of NBRA and HBRA residents are shown in Fig. 2. This is similar with the finding of a significant increased IgE levels in workers due to occupational radiation exposures, indicating a low dose radiation induced imbalance in humoral immunity [22].

Further study on the correlation between leukocytes and lymphocytes in the residents of NBRA and HBRA shows that there is a positive correlation in both locations with a correlation coefficient of 0.0869 for NBRA and 0.5149 for HBRA, as shown in Fig. 3. The correlation value in HBRA is higher than that of NBRA, showing that there are effects of natural radiation on the ratio of lymphocytes to leukocyte.

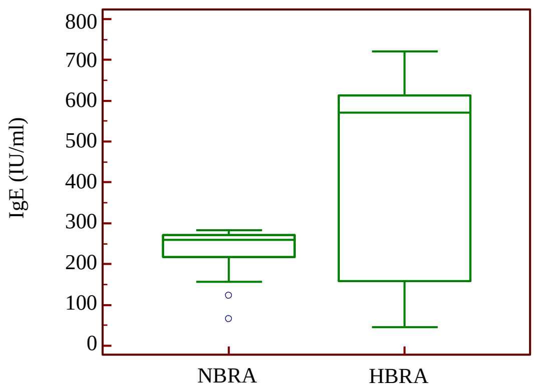
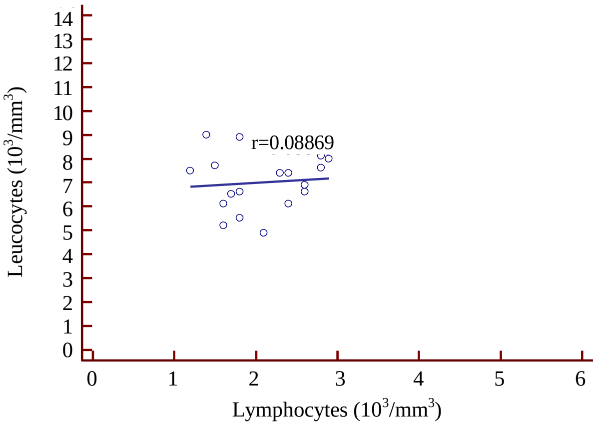
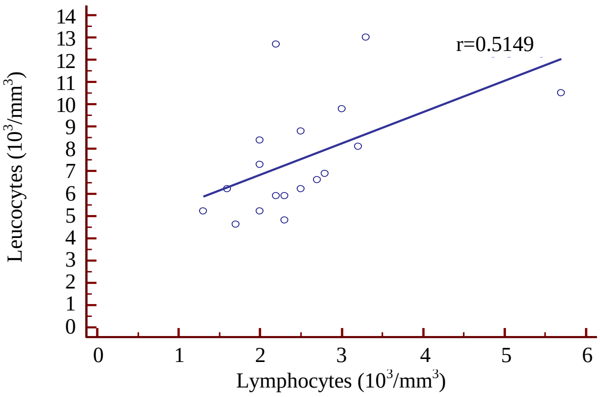


Fig. 2. The mean concentration of IgE in serum from NBRA and HBRA residents.

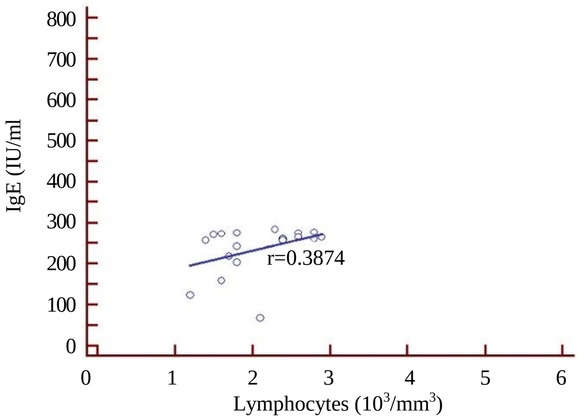


(a)

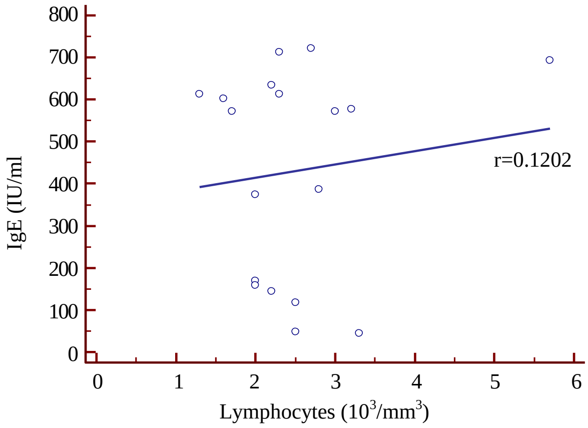


(b)

Fig. 3. The correlation of leucocyte and lymphocyte concentrations in blood from (a) NBRA and (b) HBRA residents.



(a)



(b)

Fig. 4. The correlation of lymphocyte ($10^3/mm^3$) and IgE (IU/ml) concentrations of blood from (a) NBRA and (b) HBRA residents.

The study also considered the correlation between lymphocyte count and IgE. There was a positive correlation between the lymphocytes counts and IgE concentration in both populations, as shown in Fig. 4. The correlation coefficient between the number of lymphocytes with IgE in HBRA was 0.1202, which was lower than NBRA (0.3874). The result above showed that an increase in number of lymphocytes would result in an increase of IgE concentration.

Further assessment of the effect of gender to IgE concentration was also performed. The representative image of IgE concentration in Fig. 5 shows that the mean IgE value of males in HBRA residents (332.45±32.62 IU/ml) was higher than that of females (244.31±37.37 lU/ml). It was similarly found that in NBRA residents the mean IgE concentration was higher in males (192.56±27.0 IU/ml) than in females (137.47±27.53 IU/ml). The IgE concentration in males was significantly higher than females in both populations (P<0.05).

The study on IgE concentration in Western European countries showed the total reference range of IgE as normal in young adults is 95th percentile of the total IgE reference value (148 IU/ml in women, 169 IU/ml in men) [21]. It was also noted that a high concentration of IgE in male is associated with smoking habits [23]. The higher IgE value in males was also shown by studies on non-smoking populations. It is impressive that the hormonal effect on immuno-regulatory explains the existence of sexual dimorphism [24,25].

Considering that aging is characterized by a progressive decline in almost all systems and organ functions including the immune system [25], further analysis was done on the dependence of IgE on age.

Figure 6 shows the scatterplot obtained that suggests a definite relationship between IgE concentration and age, with the increasing age tending to be associated with a lower level of IgE in NBRA residents but not in HBRA residents. There was a very weak correlation for both cases.

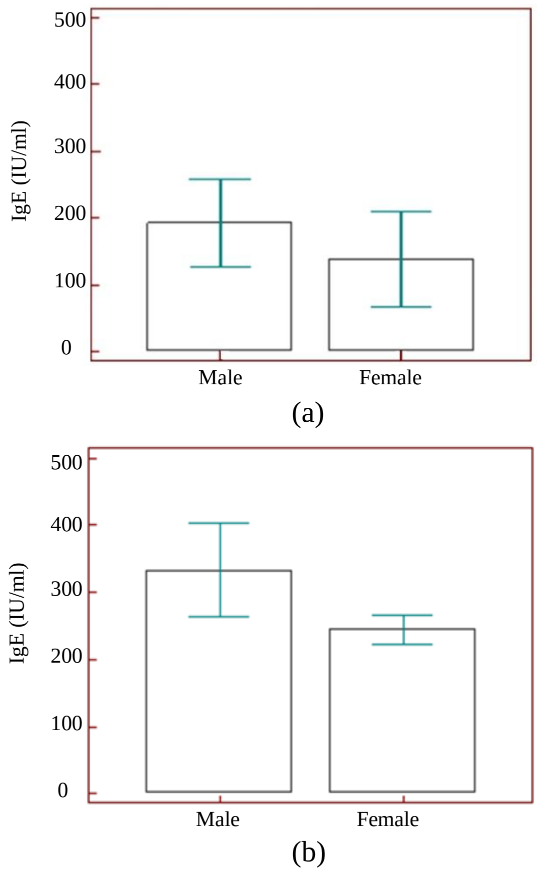


Fig. 5. Mean IgE concentration (IU/ml) in male and female (a) NBRA and (b) HBRA residents.

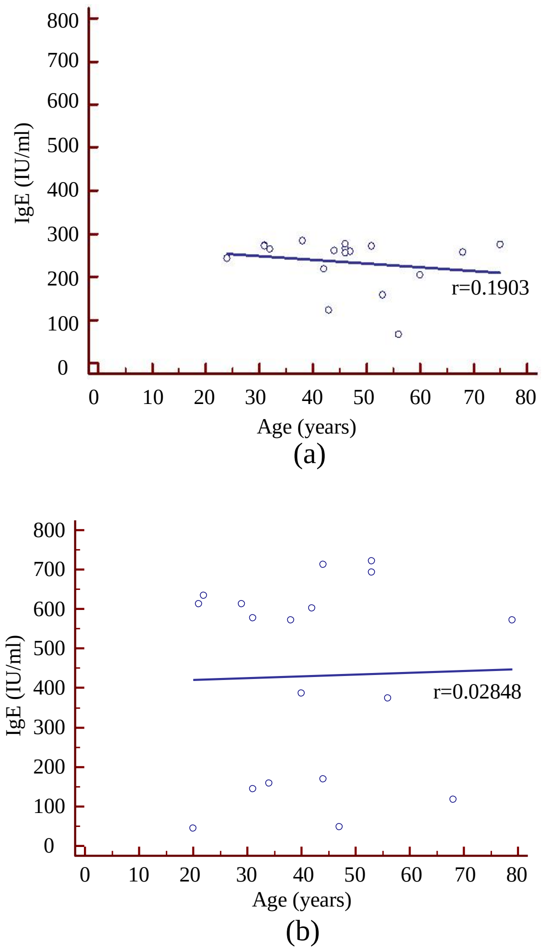


Fig. 6. Linear regressions between IgE concentration (IU/ml) and age (years) of the (a) NBRA and (b) HBRA residents.

The HBRA inhabitants were found under the long-term pressure of exposure to low-level doses of ionizing radiation. At lower levels of exposure, detrimental impacts are not promptly seen; however, there is a chance of their late appearance in humans.

It is well known that radiation may modulate the immune system that provides the first line of defense for environmental hazards and the maintenance of disease-free homeostasis. This modulation is complex, multifactorial, and dependent on radiation dose/quality and immune cell type. Ionizing radiation at high doses has ben recognized as destroying cells of the immune system, particularly lymphocytes, and has the potential to suppress protective immunity. The results above provide evidence that exposure to natural radiation in HBRA may induce some changes in the immune system.

Many pieces of evidence showed that blood cells produced in the bone marrow are very susceptible to radiation damage. In this study, lymphocytes are a type of leukocyte that has an important role in adaptive response and in the immune system. Due to its high sensitivity to radiation, this cell type is commonly used to assess the effects of radiation. Other cells called monocytes also have an important role in the adaptive immunity by presenting antigens [26].

The immune response depends on the capacity taken by white blood cells including leukocytes, monocytes, lymphocytes, and granulocytes. Ionizing radiation was reported to decrease this hematopoietic system [27,28]. The severity of this effect depends on dose and dose rate [28]. Our study, as seen in Table 1, recognized that radiation-exposed blood biomarkers were more affected as compared to the unexposed people. Several studies have shown the impact of low radiation levels on peripheral blood but did not find it statistically significant. Study by Shahid *et al*. reported the same results that the lymphocyte level was higher than control, whereas the neutrophil level was lower than control, both of which were statistically significant [14]. The study in Ramsar population indicated no statistically significant differences between the two groups of participants on all these hematological parameters [2].

In-vitro and *in-vivo* studies have confirmed that the effects of low-dose exposure on immunity depend on several factors that cover immune cell level, microenvironment, and its interactions [29]. It is also depend on radiation dose/quality and immune cell type [10]. Leukocytes, granulocytes, monocytes, and lymphocytes are blood cells that have a nucleus that is susceptible to damage due to radiation exposure.

The correlation between chronic low radiation doses and clinical signs in exposed humans is determined by damage accumulated mainly in fast-recovery cell systems such as hematopoietic tissue especially lymphocytes and granulocytes [19]. The results of our research showed that living in an HBRA induces some alterations in the immune system. In spite of the fact that increasing IgE was appeared to adequate to appear upgraded defense system, it is obvious that elevated dosages of background radiation are not immunosuppressive. In several studies, high natural radiation showed an increase in IgE in the population [2].

Results obtained in Mamuju are almost similar with the results of study about the immune level of the residents from the HBRA in Ramsar, Iran [11]. This study reported that high natural radiation was not immunosuppressive. However, another study revealed a tendency of strengthening in cell-mediated immune functions among the HBRA residents in China [12]. The changes in IgE are not known as risks that lead to adverse health effects.

Here a positive correlation between the lymphocytes counts and IgE concentration was seen. It is logical due to the fact that the proliferation of lymphocytes is closely related to serum IgE levels, where it is predicted that activated helper T (Th) cells participate in the induction of IgE synthesis [30]. Our results further confirm this phenomenon.

The finding in a current research is that increasing age tend to be associated with a decrease of IgE level of residents of both HBRAs and NBRAs. Thus, this study illustrates that there is a discrepancy between the changes in IgE levels with age. However, IgE in the serum of HBRA and NBRA residents tends to decrease along with increasing age. This is similar to the results of a study that revealed that total IgE levels decreased with age [30].

Continuous or long-term radiation exposure can affect human tissue development, especially hematopoietic cells. Further research is needed to clarify immunoglobulin alterations other than IgE induction by natural radiation in Mamuju by assessing other inflammatory cytokine levels which could be altered with cumulative doses of natural radiation, by refereed to others [11,31], or CD8(+) T cells alteration either by low dose rate of X-ray irradiation [32] or by natural radiation [11]. Even though this study was conducted by a very limited sample number, but it could provide an impact of radiation on IgE production.

CONCLUSION

It was concluded that high exposure to natural radiation significantly affected the immune system of the HBRA residents. IgE in the serum of HBRA residents was higher than that of NBRA residents, and it is thought to be part of the adaptive response process of people living in the area with long-term exposure to high natural radiation.

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AUTHOR CONTRIBUTION

Darlina and Mukh Syaifudin are the main contributor of this paper. All authors read and approved the final version of the paper.

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