Alterations of PSA, CA15.3, CA125, Cyfra21-1, CEA, CA19.9, AFP and Tag72 tumor markers in human blood serum due to long term exposure to high levels of natural background radiation in Ramsar, Iran

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ABSTRACT

***Background*:** Ramsar (Mazandran province) is known for its extremely high levels of natural background radiation. Although no excess cancer rate is reported in these areas by epidemiological studies, the study of tumor markers in the inhabitants of these areas may shed some light on the impact of high levels of background radiation on cancer induction. ***Materials and Methods*:** The level of background gamma radiation as well as indoor radon was determined using RDS-110 and CR-39 dosimeters. Thirty five individuals from a high background radiation area (HBRA) and 53 individuals from a normal background radiation area (NBRA) were randomly selected to participate in the study. Commercial ELISA kits (sandwich type ELISA tests) were used to measure the serum levels of PSA, CA15.3, CA125, Cyfra21-1, CEA, CA19.9, AFP and Tag72 tumor markers. ***Results*:** Among the eight biomarkers investigated, the means of PSA, CA15.3, CA125, CA19.9 and AFP concentrations between the HBRAs and NBRAs were not significantly different. However, Cyfra21, CEA and Tag72 in HBRA group revealed statistically significant increases compared to those of NBRA group ($P<0.05$). Furthermore, a statistically significant correlation between the external gamma dose as well as indoor radon level and the concentration of CEA ($P<0.001$), Cyfra-21($P<0.001$) and TAG 72 ($P<0.001$ and 0.01 respectively) biomarkers were observed. ***Conclusion*:** Chronic exposure to high background radiation induces significant alterations in Cyfra21, CEA and Tag72 levels. We believe that studies with other relevant tumor markers might overcome the limitations of epidemiological studies on cancer incidence in high background radiation areas.

Keywords: *Ramsar, tumor markers, high background radiation areas (HBRAs), gamma radiation, Radon.*

INTRODUCTION

Ionizing radiation is known as a classical physical carcinogen. Tumor markers, substances which are produced by cancer or by other cells of the body in response to cancer, can be found in the blood, urine, stool, tumor tissue, other tissues or bodily fluids of some cancer patients. Tumor markers can be used in both detection and management of some types of cancer. Although the presence of cancer may be linked with an elevated level of a tumor marker, this elevation alone is not an efficient tool for cancer diagnosis. In this light, in cancer diagnosis, assessment of the level of tumor markers is usually combined with other medical tests such as biopsy. New tumor markers, gene expression analysis and proteomics are among the current alternatives of early tumor detection [1, 2].

High levels of natural background radiation, resulting in exposures far exceeding the world average or even exceeding the current dose limits set by ICRP for occupational exposures, are found in Ramsar, Iran; Guarapari, Brazil; Kerala, India; and Yangjiang, China [3]. The high background radiation in the hot areas of Ramsar, Iran is primarily due to Ra-226 and its decay products, which have been brought to the surface by the waters of hot springs. There are more than 9 hot springs with different concentrations of Ra-226 in these areas which are used by tourists and residents as spas. Due to extraordinary levels of natural background radiation in these areas which in some cases reaches levels 55-200 times higher than normal background areas [4, 5], some experts suggested that dwellings having such extraordinary levels of natural radiation need urgent remedial actions. In spite of this, the majority of inhabitants still live in their unchanged ancestral dwellings.

Previously no evidence was shown for hematological or immunological differences or any cytogenetic abnormalities in lymphocytes from people living in the Ramsar area [6]. It was reported that lymphocytes from this population showed a significant adaptive response when they were exposed to a challenging high dose radiation [6]. To date, researchers have tried to investigate if there is a correlation between elevated levels of background radiation with cancer incidence [7]. In this light, numerous epidemiological studies in these regions have been conducted so far and while some studies indicated lower cancer mortality among the inhabitants living in high background radiation areas (HBRAs) [8-10], it was discussed that these studies are complicated by many confounding factors. On the other hand, the population in high background radiation areas such as Ramsar is insufficiently large to obtain statistical significance.

Prostate-specific antigen (PSA) is a glycoprotein produced by the epithelium of prostate. CA125, CA19-9 and CA15-3 are among tumor associated antigens or cancer antigens. CYFRA 21 -1 is a cytokeratin 19 fragment which can be found in serum of patients with cancer. Alpha-feto protein (AFP) and carcino embryonic antigen (CEA) are among oncofetal antigens. Tumor Associated Glycoprotein 72 (TAG 72) is also known as the 72-3 antigen [11].

The health outcomes of the long term exposure to high levels of natural radiation in HBRAs of Ramsar has been reported previously [5, 12-22]. In this study we tested the effect of high natural background radiation on the expression of some relevant tumor markers.

MATERIALS AND METHODS

External gamma dose measurements

The exact levels of gamma background radiation were measured in HBRAs and NBRAs using a calibrated RDS-110 (RADOS Technology, Finland) survey meter. This survey meter was mounted on a tripod approximately 1 meter above the ground.

Radon concentration

After the tests were explained to the participants and their informed consent was taken, CR-39 dosimeters were installed in their houses to measure the indoor level of radon gas. After three months of exposure to indoor radon, the detectors were collected and sent back to the

dosimetry laboratory of the National Radiation Protection Department (NRPD), Iranian Nuclear Regulatory Authority (INRA).

Study participants

Considering the previous study carried out by Mortazavi *et al.* [42], and to get the highest possible statistical power, 35 individuals from a high background radiation area (HBRA) and 53 individuals from an adjacent normal background radiation area (NBRA), were randomly selected to participate in the study. There was an attempt to select subjects with similar basic health indicators. The blood sampling performed in this study was approved by the ethical committee of Shiraz university of Medical Sciences.

Blood sampling and the tests

After explaining the purpose of the study to the residents in these areas and obtaining their informed consent, blood samples were taken. After separation of the sera, the blood samples underwent analysis through commercial ELISA kits to measure the serum levels of tumor markers. The sandwich type ELISA test was used in this study [23].

Data analysis

Collected data were analyzed using SPSS software (Version 19). Student's t-test and Mann-Whitney statistical tests were used for testing the equality of the means in the groups. *P value* <0.05 was considered as significant.

RESULTS

According to table 1, mean external gamma levels in the houses of the participants from HBRAs and a nearby NBRA showed a statistically significant difference (P<0.001). On the other hand as shown in table 2, there was also a statistically significant difference between the mean radon concentrations in the houses of the participants from HBRAs and a nearby NBRA (P<0.001). When the normality of variables was checked using Kolmogrov-Smirnov test, it was revealed that CA153, CA125, CA199, CEA concentrations and Age followed a normal distribution. However, external Gamma level, indoor radon concentration, PSA concentration, AFP concentration, Cyfra21 concentration and TAG72 concentration did not follow the normal distribution. In this light, *t-test* and Mann-Whitney test were used to determine the equality of the means of the variables with normal and non-normal distribution in the two groups (HBRAs and NBRAs), respectively.

Based on the results of *t-test*, only the means of CEA concentration between the two areas were significantly different (p=0.016). However, the means of other variables were not significantly different. Furthermore, based on the results of Mann-Whitney test, the means of external gamma radiation (P<0.0001), indoor radon level (P<0.0001) and Cyfra-21 concentration (P=0.024) for the two groups were significantly different. However, the difference in the means of PSA concentration and AFP concentration for the two areas was not statistically significant. Moving to the issue of the correlation between external gamma dose or indoor radon level and the eight biomarkers studied in the residents of HBRAs of Ramsar, there were statistically significant correlations between the external gamma dose and the concentration of CEA (P<0.001), Cyfra-21 (P<0.001) and TAG 72 (P<0.001) biomarkers. There were also statistically significant correlations between the indoor radon level and the concentration of CEA (P<0.001), Cyfra-21 (P<0.001) and TAG 72 (P<0.01) biomarkers.

Results of the regression analysis for estimating the effect of external gamma level or indoor radon concentration on the CEA, Cyfra-21 and TAG 72 biomarkers showed that external gamma level significantly altered the CEA (regression Coefficient = .045 , P-Value<0.0001)

Table 1. Mean (±SD) external gamma and radon levels in the houses of the participants from HBRAs and a nearby NBRA.

Area / Exposure	HBRA (#Sample size)	NBRA (#Sample size)	Significance (P-Value)
Gamma (μSv/h)	18.33±12.57 (53)	0.410±0.32(53)	<0.0001
Radon (Bq/m^3)	1179.23±929.78(30)	115.68±52.02 (45)	<0.0001

and Cyfra-21 (regression Coefficient = .056, P-Value<0.0001) levels. Furthermore, indoor radon level significantly altered the CEA (regression Coefficient = 0.001, P-Value<0.0001) and Cyfra-21(regression Coefficient = 0.001, P-Value<0.0001) biomarker levels.

DISCUSSION

There have been many epidemiological studies in high background radiation areas of Guarapari in Brazil [24], Kerala in India [9, 25], Yangjiang in China [26], and Ramsar in Iran [27]. It is evident that the exposure of the inhabitants of these areas far exceeds the world average. However, studies seeking to correlate high levels of background radiation with cancer incidence found no evidence for higher cancer mortality in inhabitants living in these famous HBRAs. The existence of numerous confounding factors has made these studies very complicated. On the other hand, in case of HBRAs of Ramsar, the population in these areas (1000 – 2000 individuals) is not sufficiently large to obtain statistical significance. In this light, the study of tumor markers in the inhabitants of these areas can shed some light on the issue of correlation of high levels of background radiation with cancer. The findings of this study showed a statistically significant correlation between the external gamma dose and the concentration of CEA (P<0.001), Cyfra-21 (P<0.001) and TAG 72 (P<0.001) biomarkers. There were also statistically significant correlations between the indoor radon level and the concentration of CEA (P<0.001), Cyfra-21 (P<0.001) and TAG 72 (P<0.01) biomarkers.

Mortazavi *et al.* have previously investigated the hematological, immunological differences as well as cytogenetic abnormalities in lymphocytes of the individuals living in the high background radiation areas in Ramsar, Iran. They showed that the lymphocytes from the inhabitants of HBRAs of Ramsar revealed an adaptive response when challenged in vitro with high dose radiation [6]. In a small-*scale study performed in 2005, Mortazavi et al.* the overall cancer mortality, lung cancer mortality and neonatal death rate of different districts in the years from 2000 to 2001 were collected. The radon prone houses were located in a district named Ramak. Their study showed that the highest lung cancer mortality rate was in Galesh Mahaleeh, where the radon levels were normal. On the other hand, the lowest lung cancer mortality rate was in Ramak, where the highest concentrations of radon in the dwellings were found [10].

Moving to other HBRAs, insignificant lower cancer mortality in people living in Yangjiang, China has been reported (28). A more recent study showed that the mortality due to leukemia or other cancers was not related to cumulative radiation dose. In a similar manner, the mortality of non-cancer disease was not related to cumulative radiation dose. These investigators concluded that the cumulative natural radiation dose was not related to the mortality due to cancer or all non-cancer diseases among the inhabitants of HBRAs of Yangjiang [26]. Similarly, in Kerala, India, no increase in cancer incidence is reported based on anecdotal evidence [8]. Nair *et al.* in 644[3] performed a complete study and found

Table 2. Mean (±SD) concentrations of the tumor markers in the blood serum of the participants from HBRAs and a nearby NBRA.

Area / Biomarker	HBRA* (# Sample size)	NBRA** (# Sample size)	Significance (P-Value)
PSA (micro gr/l)	1.23± 1.56 (35)	1.14±1.36 (53)	0.86
CA153 (U/ml)	9.30±1.53 (35)	9.08±1.55 (53)	0.57
CA125 (U/ml)	8.47±1.72 (35)	8.34±1.65 (53)	0.63
CA199 (U/ml)	9.12±4.01 (35)	7.93±4.34 (53)	0.19
CEA (micro gr/l)	1.79±1.22 (35)	1.20±1.02 (53)	0.016
AFP (micro gr/l)	2.64±2.64(35)	3.07±3.14 (53)	0.78
Cyfra (ngr/ml)	1.57± 1.25 (35)	0.90±0.92 (53)	0.024
TAG 72 (U/ml)	0.45±0.64 (35)	0.70±0.66 (53)	0.002

an excess cancer rate excluding leukemia of -0.13/Gy in the region [9].

We believe that these studies can overcome the limitations of epidemiological studies on cancer incidence in high background radiation areas. As discussed recently by Sohrabi, findings of studies in high background radiation areas such as Ramsar, propose protection of the inhabitants of such areas. He states that due to high human exposures of the public in these areas, an effective remedial action program to protect public should be of immediate concern [3]. In this light, study of tumor markers help policy makers to decide if they should approve a series of rules to decrease the irradiation of the residents of high background radiation areas of Ramsar.

Conflict of Interest:
None Declared

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REFERENCES

1. Rapkiewicz AV, Espina V, Petricoin EF, 3rd, Liotta LA (2004) Biomarkers of ovarian tumours. *Eur J Cancer,* ***40(17):*** *2604-12.*
2. Dupont J, Tanwar MK, Thaler HT, Fleisher M, Kauff N, Hensley ML, *et al.* (?)Early detection and prognosis of ovarian cancer using serum YKL-40. *J Clin Oncol,* ***22(16):*** *3330-9.*
3. Sohrabi M (2013) World high background natural radiation areas: Need to protect public from radiation exposure. *Radiation Measurements,* ***50(0):****166-71.*
4. Mortazavi SMJ and Mozdarani H (2012) Is it time to shed some light on the black box of health policies regarding the inhabitants of the high background radiation areas of Ramsar? *International Journal of Radiation Research,* ***10 (3):****111-6.*
5. Mortazavi SMJ and Mozdarani H (2013) Non-linear phenomena in biological findings of the residents of high background radiation areas of Ramsar. *International Journal of Radiation Research,* ***11:*** *3-9.*
6. Ghiassi-Nejad M, Mortazavi SMJ, Cameron JR, Niroomand-Rad A, Karam PA (2002) Very high background radiation areas of Ramsar, Iran: Preliminary biological studies. *Health Physics,* ***82(1):****87-93*.
7. Hendry JH, Simon SL, Wojcik A, Sohrabi M, Burkart W, Cardis E, *et al.* (2009) Human exposure to high natural background radiation: what can it teach us about radiation risks? *J Radiol Prot,* ***29(2A):*** *A29-42.*
8. Nair MK, Nambi KS, Amma NS, Gangadharan P, Jayalekshmi P, Jayadevan S, *et al.* (1999) Population study in the high natural background radiation area in Kerala, India. *Radiat Res,* ***152(6 Suppl):*** *S145-8.*
9. Nair RR, Rajan B, Akiba S, Jayalekshmi P, Nair MK, Gangadharan P, *et al.* (2009) Background radiation and cancer incidence in Kerala, India-Karanagappally cohort study. *Health Phys,* ***96(1):*** *55-66.*
10. Mortazavi SMJ, Ghiassi-Nejad M, Rezaiean M (2005) Cancer risk due to exposure to high levels of natural radon in the inhabitants of Ramsar, Iran. *International Congress Series,* ***1276:*** *436-7.*
11. Nair RR and Johnson JK (2008) A dictionary to tumor markers and the methods of estimation. *Advanced Biotech,* ***24:*** *22-32.*
12. Mortazavi S, Ghiassi Nejad M, Beitollahi M (2001) Very High Background Radiation Areas (VHBRAs) of Ramsar: Do We Need any Regulations to Protect the Inhabitants. Proceedings of the 34th midyear meeting, Radiation Safety and ALARA Considerations for the 21st Century, California, USA, *177-82.*
13. Mortazavi S, Ghiassi-Nejad M, Ikushima T, Assaie R, Heidary A, Varzegar R, *et al.* (2003) Are the inhabitants of high background radiation areas of ramsar more radioresistant? Scope of the problem and the need for future studies. *Iranian Journal of Radiology,* ***1****: 37-44.*
14. Mortazavi S (2005) Ramsar Hot Springs: How Safe is to Live in an Environment with High Level of Natural Radiation. *Nippon Genshiryoku Kenkyujo JAERI, Conf 194-204.*
15. Mortazavi S, Shabestani-Monfared A, Ghiassi-Nejad M, Mozdarani H (2005) Radioadaptive responses induced in human lymphocytes of the inhabitants of high level natural radiation areas in ramsar, Iran. *Asian Journal of Experimental Sciences,* ***19:****19-39.*
16. Mortazavi SMJ, Abbasi A, Asadi R, Hemmati A (2005) The need for considering social, economic, and psychological factors in warning the general public from the possible risks due to residing in HLNRAs. *International Congress Series,* ***1276:*** *440-1.*
17. Mortazavi SMJ, Ghiassi-Nejad M, Rezaiean M (2005) Cancer risk due to exposure to high levels of natural radon in the inhabitants of Ramsar, Iran. In: Sugahara T, Morishima H, Sohrabi M, Sasaki Y, Hayata I, Akiba S, *editors, p. 436-7.*
18. Mortazavi SMJ and Karam PA (2005) Apparent lack of radiation susceptibility among residents of the high background radiation area in Ramsar, Iran: can we relax our standards? In: Simopoulos ES, editor. *Radioactivity in the Environment,* ***7:*** *1141-7.*

19. Mortazavi SMJ, Ghiassi-Nejad M, Karam PA, Ikushima T, Niroomand-Rad A, Cameron JR (2006) Cancer incidence in areas with elevated levels of natural radiation. *International Journal of Low Radiation,* ***2:*** *20-7.*
20. Mortazavi SMJ, Mosleh-Shirazi MA, Mehdizadeh S, Rouintan MS, Ebrahimi J, Tamaddon M, *et al.* (2010) Short-term radon inhalation induces significant survival adaptive response in Balb/c mice. *International Journal of Low Radiation,* ***7:*** *98-109.*
21. Mortazavi SMJ and Mozdarani H (2012) Is it time to shed some light on the black box of health policies regarding the inhabitants of the high background radiation areas of Ramsar? Iranian Journal of Radiation Research.;10(3-4):111-6.
22. Mortazavi SMJ, Niroomand-Rad A, Mozdarani H, Roshan-Shomal P, Razavi-Toosi SMT, Zarghani H (2012) Short-term exposure to high levels of natural external gamma radiation does not induce survival adaptive response. *International Journal of Radiation Research,* ***10:*** *165-70.*
23. Lai RS, Hsu HK, Lu JY, Ger LP, Lai NS. (1996) CYFRA 21-1 enzyme-linked immunosorbent assay. Evaluation as a tumor marker in non-small cell lung cancer. *Chest,* ***109:*** *995-1000.*
24. de Affonseca MS, Alves RN, Junior JT, de Almeida CE (2002) The influence of urbanization on natural radiation levels in anomalous areas. *Journal of Environmental Radioactivity,* ***63:*** *65-75.*
25. Raghu Ram Nair K, Akiba S, Binu VS, Jayalekshmi P, Gangadharan P, Krishnan Nair M, *et al.* (2005) Individual dose estimation—our experience with the Karunagappally study in Kerala, India. *International Congress Series,* ***1276:*** *41-5.*
26. Tao Z, Akiba S, Zha Y, Sun Q, Zou J, Li J, *et al.* (2012) Cancer and non-cancer mortality among Inhabitants in the high background radiation area of Yangjiang, China (1979-1998). *Health Phys,* ***102:*** *173-81.*
27. Sohrabi M and Babapouran M (2005) New public dose assessment from internal and external exposures in low- and elevated-level natural radiation areas of Ramsar, Iran. *International Congress Series,* ***1276:****169-74.*
28. Tao Z, Zha Y, Akiba S, Sun Q, Zou J, Li J, *et al.* (2000) Cancer mortality in the high background radiation areas of Yangjiang, China during the period between 1979 and 1995. *J Radiat Res,* ***41:*** *31-41.*