RESEARCH   ARTICLE

SULT1A1 Gene Polymorphism in High Background Radiation Areas (HBRAs) of Kerala, India

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ABSTRACT- Gene polymorphism variation in different ethnic population as well as interindividual difference stresses the importance to establish genotype profile unique to a population. HBRAs are of considerable importance because the inhabitants were continuously exposed to radiation exposure over many generations. Hence the present study aimed to determine the frequency of *SULT1A1* gene polymorphism in 210 female individuals of two nearby areas with high radiation exposure and with normal radiation exposure. Genotyping was carried out by the PCR-RFLP method. *SULT1A1* genotype frequencies revealed 72% Arg/Arg, 23% Arg/His and 5%His/His with His allele frequency of 0.17 in controls and 83.6% Arg/Arg, 13.6% Arg/His and 2.7% His/His with His allele frequency of 0.10 in HBRA inhabitants. The slight difference in frequency distribution of His allele between the two groups can be attributed to radiation exposure and the frequency distributions of the variants were significantly different between the two groups.
Key-words- High Background Radiation Areas (HBRAs), *SULT1A1*, Polymorphism, PCR-RFLP

INTRODUCTION

Areas having the background radiation dose >1.5mGy per year are considered as High Background Radiation Areas (HBRAs)[1]. HBRAs like Guarapari in Brazil, Ramsar in Iran, Yangjiang in China and Kerala in India are study regions of interest to find out the effect of long term exposure to radiation[2]. The studies from the areas regarding the biological and health effect of radiation suggest that the exposure is not harmful for the inhabitants and several epidemiological studies were carried out in these areas. Cytogenetic studies revealed a significant effect of radiation in induction of chromosomal aberrations, but the chromosomal aberrations found out were not known to cause any impact on the cancer incidence rate in the areas as it was assumed that the chromosomal aberrations are itself indicators of radiation carcinogenesis [3].

The lack of the association between chromosomal aberrations and cancer incidence in the HBRAs is clearly indicative of the mechanism adaptive response in the inhabitants to challenge the background radiation exposure in the area [4]. Polymorphism studies of genes are generally carried out for revealing individual susceptibility to various agents including environmental toxins. Most polymorphism studies correlate the association of the various genotypes as a risk factor for carcinogenesis. So far no studies examined the polymorphism of any genes in the HBRAs, as the long term exposure itself attribute to any variation in the wild genotypes of gene is our topic of interest, and the present study examines the polymorphism status of *SULT1A1* gene in the inhabitants of HBRAs of Karunagappally in Kerala and the comparison of the status with control samples of nearby area with normal radiation exposure.

Karunagappally, the monazite bearing coastal belt of Kerala covers a distance of about 55kms and 0.5 km wide, extending from Neendakara (Kollam district) in South to Purakkadu (Alappuzha district) in North. The radioactive component of the area is the monazite soil containing thorium. The radiation level in the area varies from 4-70mGy/year. The area is densely populated which is approximately 1000 years old. Study of the inhabitants of the area showed no evidence of high cancer incidence

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because of the levels of external radiation exposure in the area [5].

Sulfation is an fundamental step to detoxify dietary and therapeutic agents and inactivation of environmental xenobiotics, regulates the activity of endogenous molecules such as thyroid and steroid hormones, catecholamines, neurotransmitters, bile acids, proteoglycans, glycoproteins, and glycolipids, although a large number of carcinogens and mutagens are activated by this process. Sulfotransferases (SULTs) are enzymes that use 3′-phosphoadenosine- 5′-phosphosulfate (PAPS) as the sulfate donor group and are mainly involved in the metabolism of endogenous chemicals including steroids, catecholamines, iodothronines and steroids [6-7]. The *SULT1A1* gene is located on chromosome 16p12.1-p11.2 and a common G-A polymorphism at nucleotide 638 in the coding region resulting in an arginine (Arg) to histidine (His) substitution at codon 213 of *SULT1A1* appears to explain a large portion of the variability in enzyme activity [8]. Individuals with [213]His allele have lower *SULT1A1* activity compared with the carriers of the [213]Arg allele [8].

MATERIALS AND METHODS

Study Population- In the Karunagappally Taluk (Taluk, administrative unit based on area and population), the Regional Cancer Centre (RCC), Trivandrum had set up a Natural Background Radiation Registry (NBRR) system and the Bio-medical Group, BARC has its field laboratory in these area to carry out detailed genetic and epidemiological survey of the entire population. From the data, the study selected a radiation cohort (HBRA) comprising of 4 Panchayats (administrative subunits) with high levels of background radiation. 110 female inhabitants of the area with willingness to participate in the study in the age range of 30-59 years, who were born and brought up there and had been staying there for a minimum period of 30 years were selected for the study. For comparison, 100 women from normal areas of the same ethnical origin were included, exception from the study participants; being not exposed to high background radiation and hence the difference in variation found out can be attributed only to radiation exposure. All of them were healthy at the time of blood sampling. Peripheral blood samples (3-4 ml) were collected after getting informed consent.

DNA extraction and Genotyping

Genomic DNA was isolated from peripheral blood sample using phenol-chloroform method in both study subjects and control samples. *SULT1A1* 19934792 G>A polymorphism was analyzed using the PCR-RFLP method described by Sparks *et al.* [9] with slight modifications. *SULT1A1* specific fragment containing the polymorphism was amplified in a 20μl reaction containing 1X buffer, 200μM deoxynucleotide triphosphates, 10pmol primers, 50-100 ng of genomic DNA and 0.5 U of Taq DNA polymerase. Cycling was as follows: initial denaturation at 94ºC for 5

minutes, followed by 30 cycles of 94ºC for 1 min, 64º C for 1min and 72ºC for 1 min and final extension at 72ºC for 5 minutes. The amplified fragment was digested with HhaI and separated on a 2% agarose gel. The fragment sizes were 168 and 32 base pairs for the wild type allele, 201 base pairs for the variant allele and 201, 168 and 32 base pairs for heterozygous allele (Fig 1).
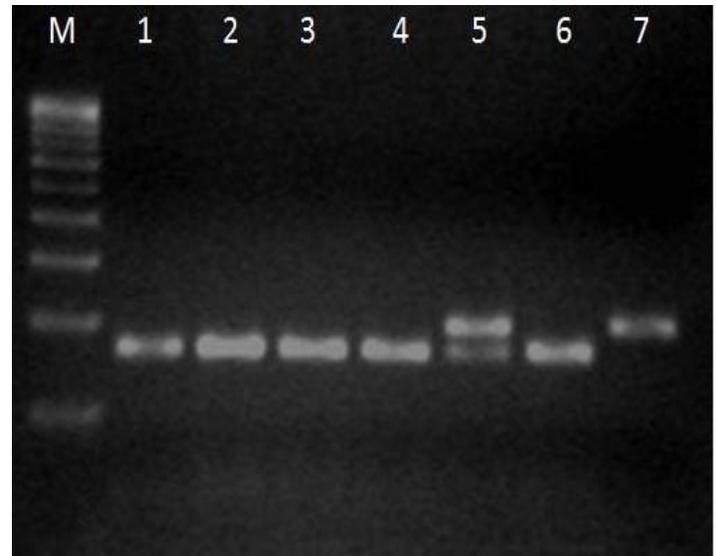
Fig. 1: Ethidium Bromide stained 2.5% agarose gel showing *SULT1A1* Arg[213]His polymorphism, lane M- 100bpmarker; lane 1,2,3,4,6- Homozygous wild, lane 5- Heterozygous variant, lane 7-Homozygous variant

STATISTICAL ANALYSIS

Statistical analysis was performed using the software SPSS version 21 to assess the Group statistics in experimental for mean age. Chi-square test was used for interpretation of results regarding the genotype frequency distribution between study subjects and control. The level of statistical significance was set at the $p < 0.05$ level. Hardy-Weinberg equilibrium was analyzed using the method described by Shi and He [10].

RESULTS

The mean age of study participants and controls were 46±7.89year and 38.97±6.83years respectively. Genotype distribution in both subjects and controls follows Hardy-Weinberg equilibrium (Table 1). Genotype distribution of Arg/Arg, Arg/His and His/His in the controls was 72.0% (72/100), 23.0% (23/100) and 5% (5/100) and in the HBRA subjects, it was 83.6 % (92/110), 13.6% (15/110) and 2.7% (3/110) respectively (Table 2). The frequency distribution of the alleles in subjects and controls were compared using the chi-square test and was found significant ($p = <0.05$) compared to the control group from normal radiation area and the difference can only be attributed to radiation exposure. Representative samples for each pattern (homozygous wild, heterozygous variant, homozygous variant) were sequenced (Scigenome, Kerala) (Fig 2).

Table 1: Genotype and allele frequency among study subjects and control

Polymorphism	Genotype	N (%) subjects/ control	Allele frequencies	
			Subjects	**Control**
SULT1A1 213	GG GA AA	92(83.6)/ 72(72) 15(13.6)/ 23(23) 3(2.7))/ 5(5)	G: 0.90 A: 0.10	G: 0.83 A: 0.17

Polymorphisms were in Hardy-Weinberg equilibrium in both controls and study subjects

Table 2: Difference in distribution of genotype among study subjects and controls

		Radiation Exposure				Total	P
		Unexposed		Exposed			
		No	**%**	**No**	**%**		
SULT1A1	**Homozygous Wild**	72	72.0	92	83.6	164	<0.05*
	Heterozygous/ Homozygous variants	28	28.0	18	16.4	46	
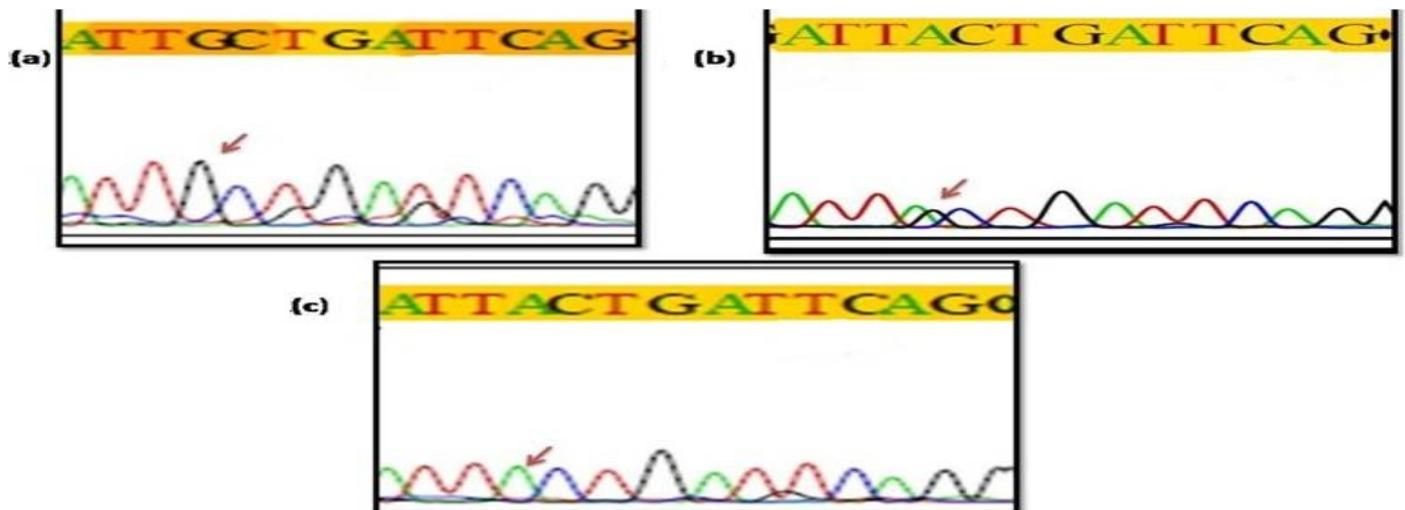
(a) Homozygous wild; (b) heterozygous variant; (c) homozygous variant.
Arrows show the location of the nucleotide at which the polymorphism occur
Fig 2: Electropherogram showing results of nucleotide sequencing analysis of representative samples

DISCUSSION

SULT1A1 gene is mainly involved in the metabolism of carcinogens, both endogenous and exogenous, and also plays important role in the detoxification mechanism [11]. Since the HBRA inhabitants are exposed to a wide range of carcinogens, as radiation is considered to be a carcinogenic agent and also through the consumables exposed to radiation. Thus, it is evident to postulate that carcinogen-metabolizing enzymes may play a role in environmental carcinogenesis and their activities may mediate susceptibility to various risks among exposed individuals [12]. Hence the study of *SULT1A1* polymorphism in the inhabitants has its own importance in

an environment with radiation exposure. The female participation in the study assures the variation due to radiation risk and other risk factors can be excluded because the selection of the participants was based on a detailed interview and those with any confounding factors like tobacco chewing, alcohol consumption, medical interventions etc were avoided.

The *SULT1A1*Arg[213]His genotype frequencies of the present study revealed a significant deviation, when compared with the control population of the same ethnic origin. The polymorphism occurs in relatively high but various frequencies in different ethnic populations, but the difference in the study can only be attributed to radiation exposure. Studies have revealed that the variant allele is

associated with lower sulfotransferase activity [13] hence the increased frequency of the homozygous wild genotype in the inhabitants is suggestive of the fact the environment itself favors the effective form of the *SULT1A1* gene in its wild form for the efficient activity of the enzyme in performing its action to avoid the risks associated with its variation in addition to the challenging condition in the area. The control population also had the wild genotypes in common, but the frequency is less compared to the HBRA subjects and the variant frequency is more in the control group than the inhabitants of HBRA. Association of the polymorphism with many cancer studies revealed significant risk in association with the *SULT1A1* AA variants [14], hence the presence of the *SULT1A1* GG in the inhabitants is supportive of the fact the distribution offers a protective effect for the inhabitants.

SULT1A1 allele and genotype frequency vary markedly with ethnicity. Among Africans and African-Americans, the A allele frequency was shown to be about 0.27 and in Caucasian populations, the A allele frequency was reported to be about 0.30 [15]. In the present study with 100 healthy controls, we found that the A allele frequency was 0.17 and in the HBRA inhabitants, it was 0.10, which are less than those among Caucasians and Africans. The slight difference in the A allele frequency between the study subjects and controls can be attributed to the radiation exposure in the area and in the inhabitants; the low frequency may favor a protective effect against the risk of its variant allele association with various cancer in many studies. The ethnic difference in the *SULT1A1* allele frequencies might act as a genetic factor influencing the cancer profiles among different populations[14]. Even though radiation is considered as a carcinogenic agent, the cancer incidence in these areas with elevated level of radiation is not high, is suggestive of the additive effect of various gene in its active form, hence *SULT1A1* gene GG allele, which is considered to be have more activity than its variant form may have participation in the additive effect of many genes to provide an adaptive response shown by the inhabitants in many studies from HBRAs.

CONCLUSIONS
The result indicates a distinct polymorphism at the *SULT1A1* loci among the HBRA inhabitants and controls of the same ethnic origin. The small sample size in the present study restricts to reach a firm conclusion; our study provides an estimate of the frequency of *SULT1A1* alleles in the HBRA of Kerala, India. Hence similar studies from other HBRAs of the world or large sample size will helpful in assessing the complex interrelationship between the genetic variants of various genes in various pathways and adaptive response, if any.

ACKNOWLEDGMENT

STATEMENT OF ETHICS
The participants gave written informed consent. This study was approved by Institutional Human Ethics Committee.

REFERENCES
[1] Das B. Genetic studies on human population residing in High Level Natural Radiation Areas of Kerala coast. BARC News letter. 2010; 28-37.

[2] Mohanty AK, Sengupta D, Das SK, Saha SK, Van KV. Natural radioactivity and radiation exposure in the high background area at Chhatrapur beach placer deposit of Orissa, India. Journal of environmental radioactivity. 2004; 75(1):15-33.

[3] Bonassi S, Hagmar L, Strömberg U, Montagud AH, Tinnerberg H, Forni A, Heikkilä P, Wanders S, Wilhardt P, Hansteen IL, Knudsen LE. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. Cancer Research. 2000; 60(6):1619-25.

[4] Mohammadi S, Taghavi-Dehaghani M, Gharaati MR, Masoomi R, Ghiassi-Nejad M. Adaptive response of blood lymphocytes of inhabitants residing in high background radiation areas of ramsar-micronuclei, apoptosis and comet assays. Journal of radiation research. 2006; 47(3+ 4):279-85.

[5] Nair MK, Nambi KS, Amma NS, Gangadharan P, Jayalekshmi P, Jayadevan S, Cherian V, Reghuram KN. Population study in the high natural background radiation area in Kerala, India. Radiation research. 1999; 152(6s):S145-8.

[6] Glatt H. Sulfation and sulfotransferases 4: bioactivation of mutagens via sulfation. The FASEB Journal. 1997; 11(5):314-21.

[7] Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C, Raftogianis RB. Sulfation and sulfotransferases 1: Sulfotransferase molecular biology: cDNAs and genes. The FASEB Journal. 1997; 11(1):3-14.

[8] Raftogianis RB, Wood TC, Otterness DM, Van Loon JA, Weinshilboum RM. Phenol Sulfotransferase Pharmacogenetics in Humans: Association of CommonSULT1A1Alleles with TS PST Phenotype. Biochemical and biophysical research communications. 1997; 239(1):298-304.

[9] Sparks R, Ulrich CM, Bigler J, Tworoger SS, Yasui Y, Rajan KB, Porter P, Stanczyk FZ, Ballard-Barbash R, Yuan X, Lin MG. UDP-glucuronosyltransferase and sulfotransferase polymorphisms, sex hormone concentrations, and tumor receptor status in breast cancer patients. Breast Cancer Research. 2004; 6(5):R488.

[10] Shi Y, He L. Shesis, A Powerful Software Platform For Analyses Of Linkage Disequilibrium, Haplotype Construction, And Genetic Association At Polymorphism Loci. American Journal of Medical Genetics-A. 2009; 138(1):143.

[11] Falany CN. Enzymology of human cytosolic sulfotransferases. The FASEB Journal. 1997; 11(4):206-16.

[12] Bartsch H, Hietanen E. The role of individual susceptibility in cancer burden related to environmental exposure. Environmental health perspectives. 1996; 104(Suppl 3):569.

[13] Nagar S, Walther S, Blanchard RL. Sulfotransferase (SULT) 1A1 polymorphic variants* 1,* 2, and* 3 are associated with altered enzymatic activity, cellular phenotype, and protein degradation. Molecular pharmacology. 2006; 69(6):2084-92.

[14] Liang G, Miao X, Zhou Y, Tan W, Lin D. A functional polymorphism in the *SULT1A1* gene (G638A) is associated with risk of lung cancer in relation to tobacco smoking. Carcinogenesis. 2004; 25(5):773-8.

[15] Coughtrie MW, Gilissen RA, Benedicte SH, Richard C, Fryer AA, Jones PW, Bamber DE. Phenol sulphotransferase *SULT1A1* polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations. Biochemical Journal. 1999; 337(1):45-9.