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 g*enotype 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 the 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 >1.5 mGy 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 on the 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].

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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 the gene is our topic of interest, and the present study examines the polymorphism status of the *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 55 kms 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 4–70 mGy/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

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

Sulfation is a 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 has 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 areas 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 the 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, 200 µM 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 1 min 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).

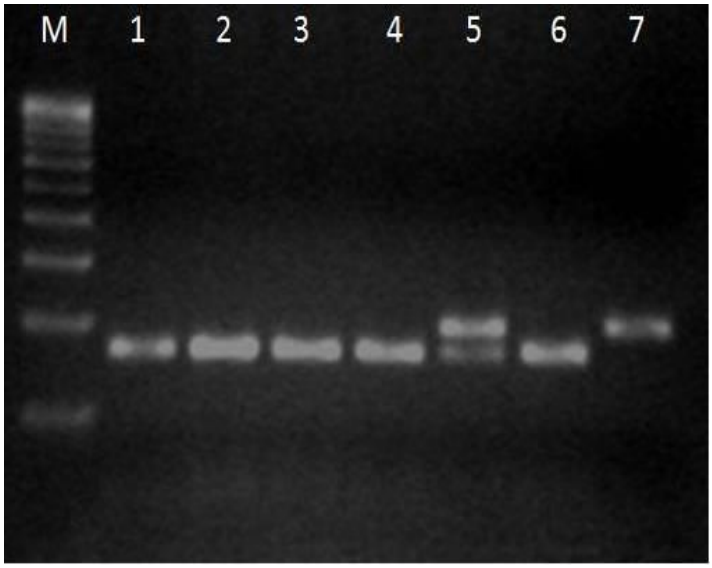

Fig. 1: Ethidium Bromide stained 2.5% agarose gel showing *SULT1A1* Arg^{213}His polymorphism, lane M- 100bp marker; lane 1,2,3,4,6- Homozygous wild, lane 5- Heterozygous variant, lane 7-Homozygous variant

STATISTICAL ANALYSIS

Statistical analysis was performed using the software SPSS version 21 to assess the Group statistics in experimental for mean age. The 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±7.89 year and 38.97±6.83 years 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 was compared using the chi-square test and was found significant (p=<0.05) compared to the control group from the normal radiation area and the difference can only be attributed to radiation exposure. Representative samples of 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	92(83.6)/ 72(72)	G: 0.90	G: 0.83
	GA	15(13.6)/ 23(23)	A: 0.10	A: 0.17
	AA	3(2.7))/ 5(5)		

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	

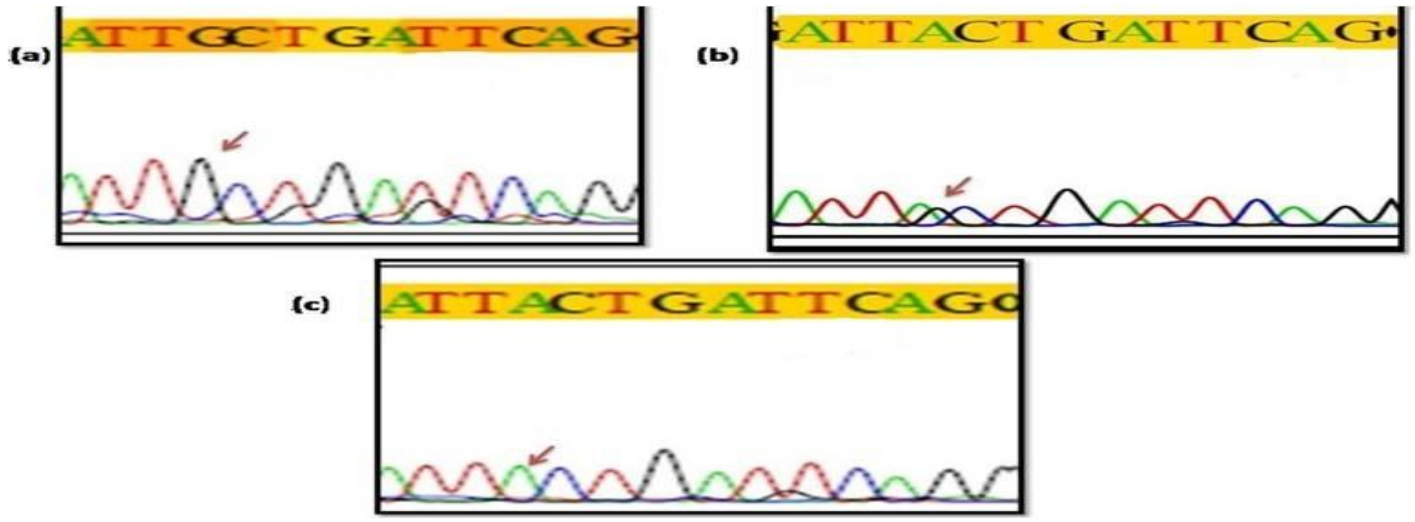

Fig. 2: Electropherogram showing results of nucleotide sequencing analysis of representative samples
(a) Homozygous wild**; (b)** heterozygous variant**; (c)** homozygous variant**.**
Arrows show the location of the nucleotide at which the polymorphism occur

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 was 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 the 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 of the efficient activity of the enzyme in performing its action to avoid the risks associated with its variation in addition to the challenging conditions 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. The 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 cancers 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 was considered to have more activity than its variant form may have participated 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.

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STATEMENT OF ETHICS

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

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