Identification of arsenic susceptibility by using the micronucleus assay and Single Nucleotide Polymorphisms (SNP)

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ABSTRACT: About 30 million people in the State of West Bengal, India, are exposed to very high amount of arsenic in their drinking water. Although a large number of individuals are exposed to arsenic through drinking water, but only 10–12% individuals showed arsenic induced specific skin lesions. For this reason, it is believed that genetic variations among the arsenic exposed individuals might be responsible for this arsenic susceptibility and carcinogenicity. We have tried to identify the arsenic susceptible individuals by assessing the genetic damage as measured by micronucleus assay in human lymphocytes and Single Nucleotide Polymorphisms (SNP) in both arsenic induced skin symptomatic and asymptomatic individuals exposed to similar arsenic contaminated water.

1 INTRODUCTION

About 150 million people are exposed to arsenic through different sources throughout the World. In West Bengal the groundwater of 9 districts out of 16 districts have very high concentrations of arsenic which people are drinking for more than 2 decades. Skin lesions are the hallmark sign of arsenic toxicity. Hence, we attempted a combinatorial approach of assessing single nucleotide polymorphisms (SNPs) in the p53 (tumor suppressive) and ERCC2 (DNA repair) gene with genetic damage as measured by micronuclei formation in lymphocytes, to predict the risk of skin lesions and skin cancer upon arsenic exposure.

2 METHODS

2.1 Selection of study subjects

Recruitment of the arsenic exposed individuals were done from three highly arsenic-affected districts of West Bengal i.e. North 24 Parganas, Nadia and Murshidabad. The control subjects were chosen from East Midnapur district of West Bengal with little or no history of arsenic exposure. All the study subjects were age and sex matched with similar socio-economic status. The selection criteria were followed as described in Beau *et al.* (2004). Samples were collected from those subjects who provided informed consent to participate in the study. This study was conducted in accord with the Helsinki II Declaration and approved by the Institutional Ethics Committee of CSIR-Indian Institute of

Chemical Biology. Drinking water and urine were collected for exposure assessment by Atomic Absorption Spectrometer and blood samples were used to isolate DNA and Micronucleus assay.

2.2 Genotyping and Micronucleus assay from lymphocytes

DNA extraction from blood was carried out and PCR was done using primers that flanked the p53 codon 72 Arg/Pro polymorphic locus and ERCC2 codon 751 Lysine/glutamine and then subjected to restriction digestion to carry out genotyping of the study samples. Lymphocyte culture was done according to Basu *et al.* (2004). Micronucleus assay was done according to established protocol and Giemsa stained, scored for MN under the microscope (Basu *et al.*, 2004).

3 RESULTS AND DISCUSSION

3.1 Genetic variations and susceptibility in Arsenic exposed population

PCR, sequence-BLAST and RFLP analysis were used to identify the key SNPs in DNA-repair and tumor suppressor pathways have been associated with increased incidence of arsenic-induced skin lesions. Table 1 tabulates the SNPs that may be responsible towards arsenic induced skin lesions and premalignant form of skin lesions in the population exposed to arsenic through drinking water (Banerjee *et al.*, 2007; De Chaudhuri *et al.*, 2006).

Table 1. SNPs in different genes studied in this population.

Gene of interest	Polymorphism	Genotype	Risk/protection OR [95% C.I.]
<i>p53</i>	Arg72Pro (G > C)	GG	1.99 [1.28–3.08]
<i>ERCC2</i>	Lys751Gln (A > C)	AA	4.8 [2.78–8.3]

Figure 1. Comparison of the genetic damage (MN) in the risk genotype (RR) and non-risk genotype (PR/PP) in both the study groups; $p < 0.01$.

Figure 2. Comparison of the genetic damage (MN) in the risk genotype (AA) and non-risk genotype (AC/CC) in both the study groups; $p < 0.01$.

3.2 Association of genetic damage with genetic variants

Frequency of MN/1000 cells in the individuals exhibiting hyperkeratosis and those devoid of any arsenic-specific skin lesion in the risk genotype has been depicted in Figure 1 and Figure 2.

4 CONCLUSIONS

In this study, we speculate that, may be, it is the contribution of the *p53* Arg/Arg and *ERCC2* Lys/Lys genotype individuals that is responsible for increased MN frequency in the arsenic induced skin lesion group. Hence a combined evaluation of the SNPs and genetic damage as measured by MN assay might be a good predictor of the occurrence of skin lesions.

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