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DNA repair gene *XRCC1* polymorphisms in childhood acute lymphoblastic leukemia

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Abstract

Defective DNA repair has been reported to be a risk factor for various malignancies. Genetic polymorphisms of DNA repair genes are thought to result in different phenotypic features compared to the wild type. Genetic polymorphisms in *XRCC1* gene could, through alteration of protein structure, lead to defective functioning of DNA Polβ, PARP and LIG3 enzymes resulting in defective DNA repair and increased risk of childhood acute lymphoblastic leukemia (ALL). The role of DNA repair gene *XRCC1* in susceptibility to childhood ALL has, however, not been widely studied and no data exists from Indian children. In this pilot study, through the use of PCR and RFLP, further confirmed by DNA sequencing, we have shown an increased risk of ALL among children with *XRCC1* codons 194 and 399 variant genotypes. Among the three variants, only the association between codon 399 variant and risk of ALL appeared to be significant. The risk of ALL was higher in males with codons 194 and 399 polymorphisms than in females. However, no relation was found between the presence of these variant genotypes and treatment outcome.

Keywords: XRCC1; Genetic polymorphism; Leukemia

1. Introduction

It has been known that genes involved in DNA repair are critical in maintaining the integrity of genetic material as well as protection against mutations that could result in cancer. Maintenance of genomic integrity in mammalian cells depends heavily on the presence of efficient DNA repair systems. Reduction in mammalian DNA repair capacity is associated with increasing birth defects, cancer and reduced lifespan [1]. Although mutations have long been identified as early events in carcinogenesis [2], defective DNA repair is also a risk factor for many types of cancer [3–6]. The development of hematologic malignancies—leukemia and lymphoma—in case of Fanconi anemia or ataxia

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telangiectasia is associated with defective DNA repair [7,8]. At least three genetic variants have been identified in the DNA repair gene *XRCC1* gene that result in amino acid changes at codons 194 (Arg→Trp), 280 (Arg→His) and 399 (Arg→Gln), respectively [9]. Codons 194 and 280 are located between the binding domains of DNA Polβ and *PARP* while codon 399 is located within the PARP binding domain in *BRCT-I*. These polymorphisms result in nonconservative amino acid changes, which suggests potential functional relevance. The XRCC1 protein directly associates with DNA Polβ, PARP and LIG3 enzymes in a complex to facilitate base excision repair (BER) pathway [10–12]. DNA Polβ interacts with XRCC1 protein at its N-terminal domain [11] while its C-terminal BRCT-II domain interacts with DNA LIG 3 enzyme [11,13]. While evidence implying mutations as obligatory, but not necessarily sufficient, in carcinogenesis is strong [14], the direct impact of these polymorphisms on the phenotype remains to be studied.

While XRCC1 has been seen to be important in DNA repair and a number of studies have been carried on the role of polymorphic forms of *XRCC1* in susceptibility to cancer, no data exists, to our knowledge, on their role in susceptibility to acute lymphoblastic leukemia (ALL) among Indian children. The *XRCC1* genetic polymorphisms are among the most widely studied DNA repair genes among other populations. In this pilot case-control study, we have looked for any increased risk of development of childhood ALL in Indian children with *XRCC1* codons 194, 280 and 399 gene polymorphisms.

2. Materials and methods

2.1. Study population

A total of 117 patients diagnosed with childhood ALL in the Division of Pediatric Oncology of the Regional Cancer Centre, Thiruvananthapuram, were included in the study. Children coming for treatment for minor ailments at the Department of Child Health of the Medical College Hospital, Thiruvananthapuram with no evidence of cancer and matched for age and sex, were selected as controls ($n=117$). Other inclusion criteria were that subjects be from the Keralite population of South India, maximum clinical data and sufficient biological material be available. The recruited patients comprised of 67.5% males and 32.5% females. All cases and controls were of age ≤ 14 years. The Institutional Review Board and Human Ethics Committee of the Regional Cancer Centre, Thiruvananthapuram, approved the study. Informed consent was obtained from all parents of children included in the study. Five milliliter of venous blood was collected from patients at diagnosis, and controls in heparinized vials. All the samples were processed as early as possible. DNA was extracted from whole blood using the GenomicPrep Blood DNA Isolation kit (Amersham Pharmacia Biotech, Inc., USA).

2.2. Genotyping of XRCC1 by PCR-RFLP

The genomes of codons 194, 280 and 399 were amplified in a PCR as described by Lee et al. [15]. Negative controls in all PCR assays consisted of a similar reaction mixture with the template replaced with sterile water. The primers used were as follows: F: GTT CCG TGT GAA GGA GGA GGA and R: CGA GTC TAG GTC TCA ACC CTA CTC ACT for codon 194 and F2: TTG ACC CCC AGT GGT GCT AA and R2: AGT CTG CTG GCT CTG GGC TGG for codons 280 and 399. The PCR products were visualized using a UV transilluminator after ethidium bromide staining.

The PCR products were digested with specific restriction enzymes for detecting the codons 194, 280 and 399 polymorphisms of the *XRCC1* gene. Ten microliters of the PCR products were digested separately with 10 units of *Pvu II* (for codon 194), *Rsa I* (for codon 280), and *Msp I* (for, codon 399) [New England Biolabs, Beverley, MA, USA] at 37 °C for 1 h. The products were then resolved on 2% agarose gels. DNA molecular weight marker of 100 bp (Bangalore Genei, Bangalore, India) was used to assess the size of the PCR–RFLP products (Fig. 1). RFLP results were confirmed by DNA sequencing of a few PCR products with an automated sequencer [ABI PRISM 100 Version 3.2] (Bangalore Genei, Bangalore, India).