Original Article

The Influence of Variations in the DNA Repair (*XRCC1*) Gene on HIV-1/AIDS among Indian Population

(HIV-1/AIDS / DNA damage / *XRCC1* Arg399Gln / repair gene / polymorphism / RFLP)

R. C. SOBTI[1], S. A. MAHDI[1], N. BERHANE[1], S. A. HOSSEINI[1], R. KLER[2], V. KUTTIAT[2], A. WANCHU[2]

[1]Department of Biotechnology, Panjab University, Chandigarh, India
[2]Department of Internal Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Abstract. Genetic polymorphisms in DNA repair genes may influence individual variations in the DNA repair capacity. Polymorphisms in the *XRCC1* gene that cause amino acid substitutions may impair the interaction of its proteins (XRCC1) with the other enzymatic proteins and consequently alter DNA repair function, which may be associated with the risk of HIV-1/AIDS disease. In this study, we aimed to determine the frequency of polymorphisms in *XRCC1* codon 399 in a sample of Indian population with HIV-1/AIDS to evaluate its association with the disease. Polymerase chain reaction and restriction fragment length polymorphism were used to analyse *XRCC1* Arg399Gln polymorphisms in 300 positively diagnosed cases with HIV-1/AIDS and an equal number of negatively diagnosed controls of the matched age. The *XRCC1* homozygous variant genotype Gln399Gln was associated with an increased risk of HIV-1/AIDS disease (OR = 1.8, 95% CI 1.10–2.94), while no association was found with the Arg399Gln genotype. Polymorphisms in the *XRCC1* homozygous variant genotype for the 399Gln allele were associated with the risk of HIV-1/AIDS disease in a sample of North Indian population.

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Corresponding author: R. C. Sobti, 1 Department of Biotechnology, Panjab University, 160014-Chandigarh, India. Phone: 0172-2534085; Fax: 0172-25341022; e-mail: rcsobti@pu.ac.in

Abbreviations: AIDS – acquired immune deficiency syndrome, BER – base excision repair, CI – confidence interval, ELISA – enzyme-linked immunosorbent assay, HIV – human immunodeficiency virus, HR – homologous recombination, IN – integrase, OR – odds ratio, PCR – polymerase chain reaction, PGIMER – Postgraduate Institute for Medical Education and Research, RFLP – restriction fragment length polymorphism, SCE – sister chromatid exchanges, SNP – single-nucleotide polymorphisms, SSB – single-strand breaks, *XRCC1* – X-ray repair cross-complementing group 1 gene.

Introduction

Acquired immune deficiency syndrome (AIDS) is a set of symptoms and infection resulting from the damage to the human immune system caused by human immunodeficiency virus (HIV) (Weiss, 1993). Usually, the HIV infection leads to progressive decline in the functionality and number of $CD4^+$ T lymphocytes, resulting in AIDS development (McCune, 2001). Despite intensive studies, several crucial questions remain to be addressed about the mechanisms through which HIV infection induces T-cell death, and this subject is one of the most controversial issues in AIDS research. McCune (2001) has reported that the loss of CD4 T lymphocytes during HIV-1/AIDS disease occurs either by direct destruction by HIV or through apoptosis. Other studies have shown that the T-cell apoptosis is another mechanism responsible for T-cell depletion in patients infected with HIV (Ameisen et al., 1991; Terai et al., 1991; Hel et al., 2006). De Flora (1996) and his team showed that DNA damage in non-replicating cells may trigger cell apoptosis. However, DNA repair pathways are responsible for maintaining the integrity of the genome in face of environmental insults and DNA replication errors (Lindahl, 2000). Polymorphisms in DNA repair genes can be associated with the difference in their ability to repair DNA damage and then may be a requisite for the risk of many diseases, including cancer. Potentially functional variants of DNA repair genes reside in the regions coding for the integrating domain of repair enzymes. Polymorphisms in this part of a gene may disturb or even abolish the function of its protein product (Goode et al., 2002).

One of the genes that play a role in the repair of DNA damage is X-ray repair cross-complementing group 1 (*XRCC1*), which encodes the portion involved in the repair of single-strand breaks (SSB) and in base excision repair (BER) of damaged bases caused by endogenous and exogenous agents (Shen et al*.,* 1998). Several studies have shown that one of the causes of cell death is DNA damage (Nelson et al., 2002). Three polymorphisms at

codons 194 (Arg-Trp), 280 (Arg-His) and 399 (Arg-Gln) in the *XRCC1* gene have been detected (Shen et al., 1998). In this study, we focused on the codon 399 polymorphism, because two other polymorphisms (codon 194 and 280) reside in functionally insignificant regions (Shen et al., 1998; Thompson et al., 2000). It has been reported that the polymorphisms in *XRCC1* that cause amino acid substitutions may impair the interaction of XRCC1 with the other enzymatic portions and consequently alter DNA repair function (Lunn et al., 1999; Duell et al., 2000; Caldecott, 2003). The present study was undertaken to explore the possible role of the *XRCC1* Arg399Gln exon 10 polymorphism and its significance for HIV-1/AIDS infection in North Indian population.

Material and Methods

Subjects

All patients included in this study visited the Internal Medicine Immunodeficiency Clinic of the Postgraduate Institute of Medical Education and Research (PGIMER) Chandigarh, India. Information regarding age, sex, mode of transmission, medical history and occupation was gathered from the subjects in a structured form. Peripheral blood samples (5 ml) of 300 positively diagnosed patients and an equal number of controls, negatively diagnosed, were collected from the same geographic area after they had been confirmed to be seronegative for HIV-1/AIDS by the enzyme-linked immunosorbent assay (ELISA) test. According to World Health Organization (WHO) staging guideline of HIV-1/AIDS and physician's observations, the subjects were classified into four stages; the mean of CD4 count was used for comparative purposes. DNA was isolated from peripheral blood by using the standard phenol-chloroform procedure and stored at -20 °C. DNA samples were amplified to detect the polymorphisms in *XRCC1* Arg399Gln codon 399 to explore its influence on HIV-1/AIDS infection among North Indian population.

Genotyping

XRCC1 polymorphisms were determined by PCR restriction fragment length polymorphism (PCR-RFLP) assay (Duell et al., 2000). For exon 10 codon 399, primers were 5'-CC CC AA GT AC AG CC AG GT C-3' and 5'-TG TC CC GC TC CT CT CA GT AG-3' (Sigma, St. Louis, MO). The mix of 50 µl of the PCR reactions containing 0.1 µl of DNA, 5 mmol/l dNTPs, 250 nM of each primer and 1 U of Taq polymerase was added to PCR buffer containing 10 mmol/l Tris-HCL, 1.5 mmol/l $MgCl_2$ and 50 mmol/l KCl. PCR was performed under the following conditions: denaturation at 94 °C for 4 min followed by 35 cycles of 30 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C and then for 10 min at 72 °C. Products of 242-bp length were then visualized on 2% agarose gel, digested with 5 U of *Msp*I restriction enzyme and incubated at 37 °C overnight, and analysed on 2 % agarose gel. Individuals homozygous for 399Arg displayed 94 and 148 bp fragments. The Arg399Glu heterozygous individuals displayed 94, 148 and 242 bp fragments, and homozygous variants for 399Glu showed only the 242 bp fragment.

Results

Subject characteristics

The study included 300 HIV-1/AIDS cases and an equal number of negative controls from the North Indian population. The distribution of mean age, gender, standard deviation, and P values have been summarized in Tables 1 and 2. In terms of mean age and gender distribution, there was no statistically significant difference among cases and controls.

Analysis of the Arg399Gln polymorphism in the XRCC1 gene

The genotype and allele frequencies for the polymorphism analysed between cases and controls is given in Tables 3 and 4. Allelic frequencies were in Hardy-Weinberg equilibrium. The frequencies of wild-type homozygous for 399Arg, heterozygous, and variant homozygous for 399Gln were 37, 42 and 21 %, respectively, in the case group; the values were different from those in the controls (44.3, 41.7, and 14.0, respectively). With regard to the allele frequency of 399Arg and 399Gln, 58 and 42 % were found in the case group compared to 65.1 and 34.9 % in the controls ($P < 0.05$). Our findings demonstrated that the individuals with the Gln/Gln genotype had an elevated risk of HIV-1/AIDS as compared to those with Arg/Gln (OR = 1.8; 95% CI 1.10 –2.94; P = 0.01) genotype. The present study also showed that the 399Gln allele had borderline increased risk for HIV-1/AIDS (OR = 1.35, 95% CI 1.07–1.72, P = 0.01) and as the frequency of 399Gln alleles increased, the risk for the disease increased, too. No significantly increased risk of HIV-1/AIDS for heterozygous individuals for the 399Arg allele (OR = 1.21; 95% CI 0.84–1.75; P = 0.33) was observed. Concerning HIV-1/AIDS stages, along with the *XRCC1* Arg399Gln genotype frequencies results it was found

Table 1. Number of cases and controls, mean age and standard deviation

Age (years)	Cases (N = 300)	Controls (N = 300)	P value
Mean ± SD	35.23 ± 8.04	36.17 ±10.49	0.179

Table 2. Gender distribution of cases and controls used in this study

Gender	Cases (N = 300)	Controls (N = 300)	P value
Female	107 (35.7)	105 (35.0)	0.93
Male	193 (64.3)	195 (65.0)	

Table 3. Distribution of genotype frequency of the XRCC1 ***A****rg399****G****ln polymorphism in HIV-1/AIDS patients and healthy controls*

XRCC1	Cases (N = 300)	Controls (N = 300)	OR (95% CI)	P value
Arg/**A**rg	111 (37.0)	133 (44.3)	Reference	
Arg/**G**ln	126 (42.0)	125 (41.7)	1.21 (0.84-1.75)	0.33
Gln/**G**ln	63 (21.0)	42 (14.0)	1.80 (1.10-2.94)	0.01*

Table 4. Distribution of allele frequency of the XRCC1 ***A****rg399****G****ln polymorphism in HIV-1/AIDS patients and healthy controls*

XRCC1	Cases	Controls	OR (95% CI)	P value
Arg	348 (58.0)	391 (65.1)	1.35 (1.07-1.72)	0.01*
Gln	252 (42.0)	209 (34.9)		

that 59.8 % of cases with stages III and IV of the disease were heterozygous for Arg399Gln and variant homozygous for Gln399Gln genotypes, whereas 40.2 % of the cases were wild-type homozygous for Arg399Arg.

Discussion

In this study the homozygous variant genotype of *XRCC1* Gln399Gln was found to be more frequently associated with an increased risk of HIV-1/AIDS. Previous studies showed an association between the Gln399Gln genotype and an increased risk for various cancers (Shen et al., 2000; Divine et al., 2001). The results on the *XRCC1* 399Gln allele frequencies in the present study are consistent with those described by Parak et al. (2002), Yu et al. (2003) and Rybicki et al. (2004), who found that the 399Gln allele is associated with an increased risk of cancer. In addition, these conclusions corresponded well with the finding that the subjects with the 399Gln allele had a higher number of chromosomal breaks per cell than those with other genotypes (Wang et al., 2003). The present results may be supported by the fact that the life cycle of retroviruses including HIV distinguishes itself from that of other viruses by undergoing the process of reverse transcription and insertion of its genome into that of the host. This latest step is carried out by viral integrase (IN) in concert with host proteins, most likely as part of the various DNA repair machineries. A number of physical and/or functional interactions have been described between the viral constituents involved in the integration process and cellular elements that may act as cofactors for catalysis or gap repair (Turlure et al., 2004). Mechanisms that have been proposed to be involved in DNA repair and DNA metabolism during retroviral integration include base excision repair (BER) and homologous recombination (HR) (Brin et al., 2000, Yoder et al., 2000). To the best of our knowledge, there is no published report regarding the association between *XRCC1* Arg399Gln polymorphisms and HIV-1/AIDS. It is, therefore, not easy to give sufficient and accurate explanation of our findings of the *XRCC1* variant genotype associated with increased risk of the HIV-1/AIDS disease. Further functional studies of *XRCC1* polymorphisms are necessary to give a clear picture about the role of *XRCC1.* With regard to the relation between the development of the disease and *XRCC1* Arg399Gln polymorphism, a significant association was found between the *XRCC1* homozygous variant genotype Gln399Gln and the stages of disease by comparison of the genotype frequency with AIDS stages. The present observations have suggested that the variant homozygous genotype (Gln399Gln*)* may play a role in the developmental stages of disease through its indirect effect on apoptosis, and subsequent development of the disease. A credible explanation for the present suggestions may be supported by studies of Duell et al. (2000), who demonstrated that the individuals with homozygous variant genotype (Gln399Gln*)* had a higher frequency of sister chromatid exchanges (SCE; marker of DNA damage) in their blood lymphocytes than those with heterozygous or homozygous wild-type genotypes. Other studies have linked the DNA damage with apoptosis. Nelson et al. (2002) have stressed that the increased DNA damage levels might give rise to enhanced damaged-related apoptosis in individual cells. Further functional studies of *XRCC1* polymorphisms including larger numbers of patients are needed and necessary to confirm our findings.

In conclusion, the present study is the first to explore the significance of the *XRCC1* DNA repair gene polymorphism in HIV-1/AIDS patients and has suggested that the 399Gln allele is associated with an increased risk of HIV-1/AIDS disease in North Indian population. Further studies, however, are needed to elucidate the precise process.

Acknowledgments

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