NAD(P)H:quinone oxidoreductase 1 (NQO1) Pro187Ser Polymorphism and Colorectal Cancer Predisposition in the Ethnic Kashmiri Population

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Abstract

The C609T single nucleotide polymorphism of the NAD(P)H:quinone oxidoreductase 1 (NQO1) gene has been identified as an important risk parameter for the susceptibility to colorectal cancer. We here carried out a case-control study and examined the genotype distribution of NQO1 C609T (Pro189Ser) using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, to investigate the possible role of this SNP as a risk factor in colorectal cancer development in Kashmir, India. We investigated the genotype distribution in 86 CRC cases in comparison with 160 healthy subjects and also focused on clinicopathological variables in the CRC cases. The observed genotype frequencies in cases and controls were significantly different [OR=1.64; 95%CI=0.94-2.86]. We also found a significant association between the Ser/Ser variant form with age group, smoking status, tumor location, nodal status/ higher tumor grade and with exposure to pesticides. Therefore, we suggest that the NQO1 C609T SNP is involved either in susceptibility or development of CRC in the ethnic Kashmiri population.

Key Words: Colorectal cancer - NQO1 - polymorphism - RFLP, restriction digestion - Kashmir

Introduction

Colorectal cancer (CRC) is a major cause of mortality and morbidity worldwide being the third most common malignancy in the world (Center et al., 2009). The incidence of this malignancy shows considerable variation among racially or ethnically defined populations in multiracial/ethnic countries. Kashmir has been reported as a high-incidence area for gastrointestinal cancers (Mir et al., 2005; Murtaza et al., 2006). In Kashmir valley colorectal cancer is the third most common, after esophageal and gastric cancers (Sameer et al., 2009).

In 1955, Lars Ernster and Franco Navazio identified an unusual nicotinamide nucleotide-dependent oxidoreductase in rat liver which they named as diphtheria toxin (DT) diaphorase now known as NAD(P)H:quinone oxidoreductase 1 (NQO1) (Chen et al., 2000). NQO1 is located on chromosome 16q22, is 20 kb in length and has 6 exons and 5 introns. NQO1 is a flavoprotein which functions as a homodimer. The physiological dimer has one catalytic site per monomer. Each monomer consists of 273 amino acids. NQO1 is expressed in human epithelial and endothelial tissues and at high levels throughout many human solid tumors. NQO1 is mainly cytosolic enzyme although it has also been localized in smaller amounts to mitochondria, endoplasmic reticulum and nucleus (Ross et al., 2004; Chao et al., 2006).

The enzyme is generally considered as a detoxification enzyme because of its ability to reduce reactive quinones and quinone-imines to less reactive and less toxic hydroquinones by its unique ability to use either NADH or NADPH as reducing cofactors (Siegel et al., 2004). Because of its reducing capability NQO1 prevents the generation of semiquinone free radicals and reactive oxygen species with its unique property of transferring two electrons at a time to quinone, thus protecting cells from oxidative damage (Chen et al., 2000; Winski et al., 2002).

As most genes are subject to genetic variation so is NQO1 gene, so far two important single nucleotide polymorphisms have been discovered in this gene. One C>T base transition at position 465 of the NQO1 cDNA and other C>T base transition mutation at position 609 (Chen et al., 2000). NQO1 is located on chromosome 16q22, is 20 kb in length and has 6 exons and 5 introns. NQO1 is a flavoprotein which functions as a homodimer. The physiological dimer has one catalytic site per monomer. Each monomer consists of 273 amino acids. NQO1 is expressed in human epithelial and endothelial tissues and at high levels throughout many human solid tumors. NQO1 is mainly cytosolic enzyme although it has also been localized in smaller amounts to mitochondria, endoplasmic reticulum and nucleus (Ross et al., 2004; Chao et al., 2006).

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by various studies on both human cell lines and primary human tissues (Siegel D et al., 1999), while as the cells which have heterozygous alleles i.e., CT (Pro/Ser) has approximately half of the NQO1 enzyme activity (Mitrou et al., 2007; Chao et al., 2006).

A number different clinical studies have been carried out on NQO1 genotype, most of which have shown an increased frequency of the NQO1 TT allele in patients with renal (Schulz et al., 1997), urothelial (Schulz et al., 1997), esophageal (Zhang et al., 2003; Sarbia et al., 2003), bladder (Park et al., 2003), breast (Menze et al., 2004), and gastric (Zhang et al., 2003; Sarbia et al., 2003) cancers and both pediatric (Wiemels et al., 1999; Krajinovic et al., 2002) and adult leukemias (Smith et al., 2001; Naoe et al., 2000) compared with healthy controls. These findings suggest that the active form of NQO1 may provide protection from some carcinogenic-induced cancers. However, a limited studies have been carried out on colorectal cancer, but whatever literature available till date suggest that NQO1 CT heterozygous condition might be a risk factor for the predisposition of malignancy (Chao et al., 2006; Begleiter et al., 2006; Mitrou et al., 2007). A recent meta-analysis suggested a statistically significant association of the NQO1 heterozygous genotype (CT) with a moderately elevated risk of developing colorectal cancer (Chao, 2006); while as the homozygous variant genotype (TT) was not associated with colorectal cancer risk. The risk of sporadic colorectal cancer has been linked to exposure to carcinogenic compounds in food, such as heterocyclic amines in fried meat (Felton et al., 1991). As has been hypothesized previously (Mitron et al., 2007) NQO1 activity might influence the risk of colorectal cancer in individuals exposed to NQO1 metabolising compounds as NQO1 expressed in the intestinal tract plays an important role in detoxifying dietary carcinogenic compounds, and the loss of enzyme activity may increase susceptibility to colorectal cancer (Chao et al., 2006).

Therefore, we carried out a case-control study in our population to determine if this NQO1 polymorphism is associated with an altered risk of developing colorectal cancer as many dietary habits (Sameer et al., 2010, Mir, 2005; Murtaza et al, 2006; Siddiqa, 1992 ) of our population have already been established as risk factors for the development of GIT cancers. We also investigated whether there was a link between these risk factors and the NQO1 genotype and colorectal cancer predisposition.

Materials and Methods

Study population

This study included 86 consecutive primary colorectal cancer patients. All CRC patients were recruited from Department of Surgery, Sher-I-Kashmir Institute of Medical Science from March 2008 to August 2009. Tumor types and stages were determined by two experienced pathologists. Blood samples of 160 age and sex matched cases with no signs of any malignancy were collected for controls. The mean age of both patient and control groups was 52 years old, and 56 of patients and 104 of controls were >50 years old or older. See Table 1 for details.

DNA extraction & Polymerase chain reaction-restriction fragment length polymorphism

DNA extraction was performed using any one of the previously described techniques. Previously reported primers: Forward primer 5'-TCC TCA GAG TGG CAT TCT GC-3' and Reverse primer 5'- TCT CCT CAT CCT GTA CCT CT-3' (Park et al., 2003) were used for the amplification of the 230bp target region within the NQO1 gene.

PCR was carried out in a final volume of 25 μL containing 50 ng genomic DNA template, 1X PCR buffer (Biotools) with 2 mM MgCl2, 0.4 μM of each primer (Genescript), 50 μM dNTPs (Biotools), and 0.5 U DNA polymerase (Biotools). For PCR amplification, the standard program was used as follows: one initial denaturation step at 94°C for 7 min, followed by 35 denaturation cycles of 30 s at 94°C, 30 s of annealing at 58°C, and 30 s of extension at 72°C, fol-lowed by a final elongation cycle at 72°C for 5 min. For RFLP, the PCR product of NQO1 Pro187Ser was digested with Hinf1 (2 U at 37°C for 16 h) (Fermentas). In case of NQO1 Pro187Ser polymorphism Pro/Pro wild was identified by 191 bp band while Ser/Ser variant was identified by 151 bp band and heterozygous Pro/Ser variant displayed two bands (191 bp and 151bp) (Figure 1).

DNA fragments were electrophoresed through a 2-3% agarose gel for resolution. The genotypes of >20% of the samples were double blindly reassessed to confirm the results by two independent researchers. A positive control for each polymorphism was used for 50% of samples.

Figure 1. Restriction Fragment Length Polymorphism Analysis of NQO1 Pro187Ser using Hinf1 Enzyme. Hinf1 enzyme cleaves the Variant (Ser/Ser) resulting in the 151bp band while as Wild (Pro/Pro) is represented by 191bp band. Lane M: 100bp ladder; Lanes 1-3 & 9 & 12 show Wild (Pro/Pro) form; Lanes 4,5,7,10 & 11 show Heterozygous (Pro/Ser) form; and Lane 8 shows Variant (Ser/Ser) form; Lane 6 shows uncleaved amplicon.
The correlation of NQO1 C609T polymorphic status and the clinicopathologic characteristics was carefully analyzed. It was found that the Ser/Ser variant status was associated with a modestly elevated risk for colorectal cancer (OR = 1.62; 95% CI = 0.91-2.91) and OR = 1.75 (0.94-3.14) respectively. The overall hazard ratio of the NQO1 Ser allele in colorectal cancer compared with healthy controls was 56 (65.1%).
ser187 allele has been linked to decreased NQO1 enzyme activity (Parkinson et al., 2003; Siegel et al., 2004). Thus, the presence of variant NQO1 leads to decreased detoxification ability which in turn may result in greater doses in smokers of B[a]P reactive metabolites at the cellular level, yielding increased risks for associated diseases which serve as the platform for the development of malignancy (Hou et al., 2005).

In addition to the already reported clinic-pathological risk factors, we also found a significant association of the NQO1 Ser allele with age group, smoking status, tumor location, nodal status/ higher tumor grade and with exposure to pesticides, identifying all of them as the potential risk factors for colorectal cancer.

Hence, in this study which has been carried for the first time in the Kashmir valley, we observed a significant correlation between the Ser/Ser variant NQO1 status with various clinicopathological variables in this ethnic Kashmiri population. Although these correlation need to be authenticated in large sample study in future, so as to help in better discernment of racial differences and in determining aggressiveness of colorectal cancer.

Acknowledgements

The authors gratefully acknowledge the Sher-I-Kashmir Institute of Medical Sciences, Kashmir for providing funds for this research work. We would like to express our gratitude to Miss Mahoor S. Nanda of Department of Biosciences, Jamia Millia Islamia, New Delhi, INDIA for her technical support. The authors also gratefully acknowledge the technical staff of Department of General Surgery for helping in the procurement of tumor tissue samples from Operation Theater. We also thank the anonymous pathologists of Department of Pathology for the histopathological assessment of the tumor tissues.

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