

Detection of NAD(P)H: quinone oxidoreductase ⁶⁰⁹C → T polymorphism in blood and archival human tissues using a simple PCR method

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Abstract

NAD(P)H: quinone oxidoreductase (*NQO1*) plays an important role in detoxification of numerous endogenous and foreign compounds. This gene has a single nucleotide polymorphism at site of codon 187 (CCT→TCT). Recently, it has been demonstrated that individuals with T allele may exhibit resistance to quinone based anticancer drugs such as mitomycin C. In the present study, a simple and feasible method was developed for detection of *NQO1* genotype. In this modified procedure, dimethylsulfoxide (DMSO) and Triton X-100 were eliminated, also, PCR cycling conditions were modified to improve the PCR products from blood and formalin-fixed, paraffin-embedded tissues. PCR-RFLP and DNA sequencing analysis carried out on a limited number of blood and archival samples. It is suggested that this procedure convenient for *NQO1* genotyping.

Keywords: *NQO1*, polymorphism, simple PCR-RFLP method

The NAD(P)H: quinone oxidoreductase (*NQO1*) is involved in detoxification of numerous endogenous and foreign compounds (Nebert *et al.*, 2002; Smith *et al.*, 2001; Chen *et al.*, 1999). It has been reported that this gene has a single nucleotide polymorphism (SNP) at site of codon 187 (nucleotide 609) (Kelsey *et al.*,

1997 and Moran *et al.*, 1999). It has been shown that homozygous individuals having T allele exhibit negligible *NQO1* enzyme activity ((Phillips *et al.*, 2004). Lack of *NQO1* activity might increase the risk of certain types of toxicity and cancer (Smith *et al.*, 2001; Chen *et al.*, 1999). A Pro (CCT) to Ser (TCT) substitution at codon 187 of the *NQO1* gene was found to be associated with esophageal cancer (Zhang *et al.*, 2003). In addition, has been indicated that polymorphism at *NQO1* associated with susceptibility to several malignancies (Zhang *et al.*, 2003; Choi *et al.*, 2003; Sabria *et al.*, 2003; Hamajima *et al.*, 2002; Rothman *et al.*, 1997 and Chen *et al.*, 1999).

It has been shown that individuals with T allele may exhibit resistance to quinone based anticancer drugs such as mitomycin C (Fleming *et al.*, 2002). Hence, *NQO1* allelotyping can be useful before chemotherapy. Recently, Phillips *et al.* (2004) described a new PCR amplification method using nested primer to detect *NQO1* genotype for analyzing genome DNA isolated from formalin-fixed paraffin embedded human tissues. Ozawa *et al.* (1999) also showed that genome DNA isolated from blood can be amplified using dimethylsulfoxide (DMSO) and Triton X-100 to detect *NQO1* genotype. In this study we modified and simplified the method for assessment of *NQO1* C609T genotype in healthy individuals and archival cancer tissues.

For this purpose, first archival cancerous human tissues were collected. Then blood samples from healthy individuals (n=11) were collected for this

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study. An informed consent was signed from normal individuals volunteers followed by completion of a structured questionnaire. Three milliliters of venous blood was drawn from each individual, blood sample was immediately frozen and transferred to laboratory for analysis. Genomic DNA was extracted from the frozen blood and kept at -20°C for further use. The samples were anonymized and then PCR and RFLP were performed. The study was approved by the National Institute for Genetic Engineering and Biotechnology (NIGEB) and the local ethical Committee on human experimentation. Genomic DNA from the 3 formalin-fixed, paraffin-embedded esophageal carcinoma epithelial and 2 colon adenocarcinoma, with high neoplastic cellularity, were extracted as described by Biramijamal *et al.* (2001). The *NQO1* C609T genotyping was performed by PCR, a 318-bp PCR fragment was amplified from DNA isolated from whole blood and esophageal and colon tissues using the primers as described by Ozawa *et al.* (1999). PCR was performed in a 50 μl volume containing 100 ng DNA template, 5 μl 10x PCR-buffer, MgCl_2 1.5 mM, 1.25U/ μl *Taq* DNA polymerase (Cinnagen, Tehran-Iran), 0.2 mM dNTPs and 10 pmol sense primer (5'-ATTCTCTAGTGTGCCTGAG-3') and anti-sense primer (5'-AATCCTGCCTGGAAGTTTAG-3'), initial denaturation for 5 min at 95°C was followed by 35 cycles at 95°C for 1 min, at 57°C for 1 min, and at 72°C for 45 sec. The PCR products were subsequently digested with 15 units of *HinfI* (Fermentas, Lithuania) for 3h at 37°C and separated on a 3% or 4% agarose gel (Fig. 1A and 1B). PCR products with homozygous genotype (C/C) was not cut, but PCR products with heterozygous genotype (C/T) was cleaved to yield one or two fragments (154 and 164 bp) on either 3% or 4% agarose gel respectively. Selected PCR products were purified with PCR product purification Kit (Roche, Germany) and sequenced directly by Big DyeTM fluorescent dye dideoxy sequencing and microcapillary electrophoresis using an ABI 310 Genetic Analyzer according to the supplier's instructions (Applied Biosystems International). In addition, the samples were reanalyzed by retrieving genomic DNA stocks, performing new PCR amplifications, and resequencing of new PCR amplification products. It was found that this procedure is simple and feasible for detection of *NQO1* genotyping.

The results showed that of the 3 esophageal squamous cell carcinoma, two samples were heterozygous and one sample was homozygous wild genotype, (Fig. 1B). The colon samples were homozygous wild genotype and heterozygous. Of the 11 blood samples from healthy individuals, 5 samples showed C allele, one sample with T allele, and 5 samples were heterozygous genotype (C/T), (Fig. 1A). The results of some samples were confirmed by direct DNA sequencing, (Fig. 2). The results showed that this modified method for PCR is suitable and feasible for analyzing the *NQO1* genotype. The suitability of PCR products for analyzing the *NQO1* genotype was further confirmed by direct DNA sequencing and digestion by *HinfI* restriction enzyme. Recently, Phillips *et al.* (2004) described

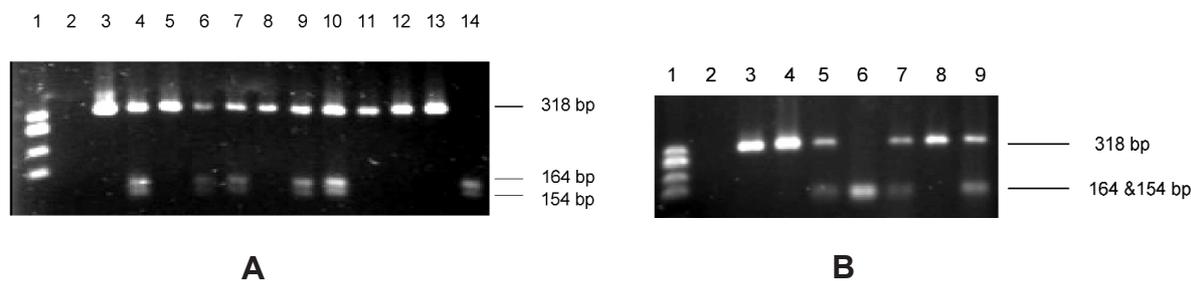


Figure 1: A PCR assay to detect genetic polymorphism at the *NQO1* locus. A 318 bp region of genomic DNA flanking exon 6 was amplified using primers NQO1-F (5'-ATT CTC TAG TGT GCC TGA G-3') and NQO1-R (5'-AAT CCT GCC TGG AAG TTT AG-3'). (A) The PCR product was then digested by *HinfI* and run on 4% agarose gel. Lane 1, 72-1353 bp molecular marker; Lane 2, negative control (PCR reaction without using genomic DNA to control analysis and possibility of contamination); Lane 3, PCR product of genomic DNA from esophageal cancerous tissue sample; Lanes 5, 8, 11-13, homozygous genotype (C/C); Lanes 4, 6, 7, 9, 10, heterozygous genotype (C/T); Lane 14, homozygous genotype (T/T). (B) A PCR assay to detect genetic polymorphism at the *NQO1* locus. The PCR product was then digested with *HinfI* and run on 3% agarose gel. Lane 1, 72-1353 bp molecular marker; Lane 2, negative control (PCR reaction without using genomic DNA to control analysis and possibility of contamination); Lane 3, PCR product of genomic DNA from colon cancerous tissue sample; Lane 4, homozygous genotype (C/C) for colon cancer sample; Lane 5, heterozygous genotype (C/T) for blood sample; Lane 6, homozygous genotype (T/T) for blood sample; Lanes 7, 9 heterozygous genotype (C/T) for esophageal cancer samples; Lane 8, homozygous wild genotype (C/C) for blood sample.

a new PCR amplification method using nested primer but we present in this study a simple method without using nested primer. Also, DNA isolated from blood was successfully amplified after elimination of DMSO and Triton X-100 using new cycling conditions.

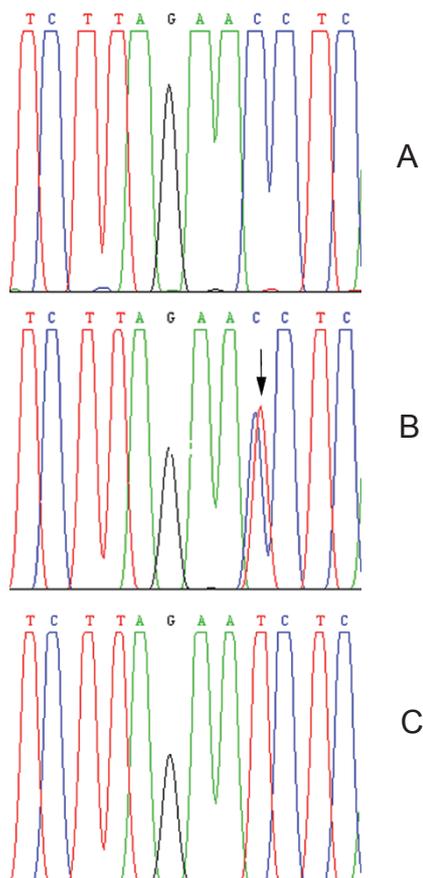


Figure 2: Electropherogram of DNA sequencing (5' → 3') showing a single base substitution (C → T) polymorphism at nucleotide 609 of *NQO1* gene. (A) homozygous wild genotype (C/C). (B) heterozygous genotype (C/T). (C) homozygous genotype (T/T).

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