RESEARCH COMMUNICATION

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

A Syed Sameer[1,2], Zaffar A Shah[1], Nidda Syeed[1], Safiya Abdullah[1], Roohi Rasool[1], Dil Afroze[1], Mushtaq A Siddiqi[1]*

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 Kasmir, 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

Asian Pacific J Cancer Prev, **11**, 209-213

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 a 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 NQO1cDNA and other C>T base transition mutation at position 609 (Chen et al., 1999). An inactivating SNP involving a base change from cytosine to thymine at base 609 of the NQO1 gene which replaces proline with serine residue at codon 187 in the encoded protein is the most actively studied one. This SNP occurs at a frequency of 50% in the human population, with 10% being homozygous for T alleles (Begleiter et al., 2006). However, these percentages vary in different ethnic groups, with a frequency rate of 2% to 5% in Caucasians and Blacks, and 20% in Asians (Kelsey et al., 1997). The NQO1 TT (Ser/Ser) form of the NQO1 protein has little or no activity as has been demonstrated

*[1]Department of Immunology and Molecular Medicine, [2]Clinical Biochemistry, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India *For Correspondence: mousvi786@gmail.com, vc.tmuk@gamil.com*

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 NQO1enzyme 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 carcinogen-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; Siddiqi, 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.

Data on all CRC patients were obtained from personal interviews with patients and or guardians, medical records and pathology reports. The data collected included sex, age, dwelling, tumor location, Dukes Stage, lymph node status, pesticide exposure, Bleeding PR. All patients and or guardians were informed about the study and their will to participate in this study was taken on predesigned questionnaire (Available on request). The collection and use of tumor and blood samples for this study were previously approved by the appropriate Institutional Ethics Committee.

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 NQO1gene.

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.

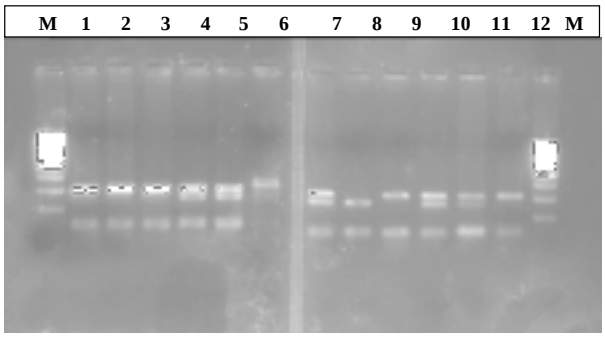

Figure 1. Restriction Fragment Length Polymorphism Analysis of NQO1 Pro187Ser using Hinf1 Enzyme. Hinf1 enzyme cleaves the Variant (Ser/Ser) resulting in the 151bp band while as Wild (Pro/Pro) is represented by 191bp band. Lane M: 100bp ladder; Lanes 1-3,9 & 12 show Wild (Pro/Pro) form; Lanes 4,5,7,10 & 11 show Heterozygous (Pro/Ser) form; and Lane 8 shows Variant (Ser/Ser) form; Lane 6 shows uncleaved amplicon

Table 1. Frequency Distribution Analysis of Selected Demographic and Risk Factors in Colorectal Cancer Cases and Controls

Variable		Cases (n=86)	Controls (n=160)	P Value
Age group	≤50	30 (34.9%)	56 (35.0%)	
	>50	56 (65.1%)	104 (65.0%)	1
Gender	Female	37 (43.0%)	72 (45.0%)	
	Male	49 (67.0%)	88 (55.0%)	0.764
Dwelling	Rural	59 (68.6%)	104 (65.0%)	
	Urban	27 (31.4%)	56 (35.0%)	0.565
Smoking	Never	31 (36.0%)	75 (46.8%)	
status	Ever	55 (64.0%)	85 (53.2%)	0.102
Pesticide	Never	33 (38.4%)	75 (46.8%)	
exposure	Ever	53 (61.6%)	85 (53.2%)	0.200

Table 2. NQO1 Genotype Frequencies in Cases & Controls and Associations with CRC Risk

TP53 Codon 72 Genotype	Cases (n= 86)	Controls (n=160)	OR (95% CI)
Pro/Pro	53 (61.6%)	116 (72.5%)	1.00 (Ref)
Pro/Ser	29 (33.7%)	39 (24.4%)	1.62 (0.91-2.91)
Ser/Ser	4 (4.6%)	5 (3.1%)	1.75 (0.45-6.78)
Pro/Ser+Ser/Ser	33 (38.4%)	44 (27.5%)	1.64 (0.94-2.86)

Statistical analysis

Observed frequencies of genotypes in CRC were compared to con-trols using chi-square or Fisher exact tests when expected frequencies were small. The chi-square test was used to verify whether genotype distributions were in Hardy-Weinberg equilibrium. Statistical significance was set at $P < 0.05$. Statistical analyses were performed with PASW version 18 Software.

Results

A total of 86 colorectal cancer patients and 160 control subjects were included in this study. The patients comprised 49 males and 37 females (M/F ratio = 1.32) and the control subjects consisted of 88 males and 72 females (M/F ratio = 1.2). Mean age in patient and control groups was 52 years. No significant gender- or age-related differences were ob-served between the groups ($P > 0.05$). Further more, out of 86 confirmed cases of CRC, 81 cases were sporadic, 4 were FAP and one case was HNPCC. All but one case were adenocarcinoma and only one was squamous cell carcinoma (SCC) of basal cell type, 59 rural and 27 urban, 36 cases had carcinoma in colon and 50 in rectum and 55 were smokers and 31 non smokers. See Table 1 & Table 2 for further details.

The genotype distribution of C609T SNP of the NQO1 in the healthy control population was similar to a previous report (Beglieter et al., 2006). However, we observed an decreased incidence of NQO1 Pro/Pro but increased incidence of the NQO1 Ser/Ser genotype in patients with colorectal cancer compared with healthy controls [Table 2]. The overall hazard ration of the NQO1 Ser allele in patients with colorectal patients was 1.62 (95% CI = 0.94-2.86) and it was found statistically significant ($p < 0.05$). Overall, both heterozygous CT genotype (Pro/Ser) and the homozygous variant TT genotype (Ser/Ser) was associated with a modestly elevated risk for colorectal cancer {OR = 1.62; 95% CI = 0.91-2.91) and OR = 1.75; 95% CI = 0.45-6.78 respectively}.

Table 3. Association between the NQO1 Gene Polymorphism and Clinico-pathologic Characteristics in Cases (n=86)

Variables	Group I	Group II	OR (95% CI)
Age group ≤50=30 (34.9%) >50 =56 (65.1%)			
Pro/Pro	13	40	1 (Ref)
Pro/Ser	14	15	2.87 (1.09- 7.50)
Ser/Ser	3	1	9.23 (0.88- 96.6)
Pro/Ser+Ser/Ser	17	16	3.27 (1.29- 8.25)
Gender Male=49 (67.0%) Female=37 (43.0%)			
Pro/Pro	28	25	1 (Ref)
Pro/Ser	11	18	0.54 (0.22-1.37)
Ser/Ser	1	3	0.29 (0.02-3.04)
Pro/Ser+Ser/Ser	12	21	0.51 (0.21-1.24)
DwellingRural = 59 (68.6%) Urban = 27 (31.4%)			
Pro/Pro	40	13	1 (Ref)
Pro/Ser	17	12	0.46 (0.17-1.21)
Ser/Ser	2	2	0.32 (0.04-2.54)
Pro/Ser+Ser/Ser	19	14	0.44 (0.17-1.11)
Smoking status Never = 31 (36.0%) Ever = 55 (64.0%)			
Pro/Pro	29	24	1 (Ref)
Pro/Ser	1	28	0.03 (0.00- 0.23)
Ser/Ser	1	3	0.28 (0.03- 2.82)
Pro/Ser+Ser/Ser	2	31	0.05 (0.01- 0.25)
Tumor locationColon = 36 (41.9%) Rectum = 50 (58.1%)			
Pro/Pro	28	25	1 (Ref)
Pro/Ser	5	24	0.18 (0.06- 0.56)
Ser/Ser	3	1	2.67 (0.26- 27.4)
Pro/Ser+Ser/Ser	8	25	0.28 (0.10- 0.74)
Nodal status Involved = 48 (55.8%)Not = 38 (44.2%)			
Pro/Pro	36	17	1 (Ref)
Pro/Ser	9	20	0.21 (0.08- 0.56)
Ser/Ser	3	1	1.42 (0.14- 14.6)
Pro/Ser+Ser/Ser	12	21	0.27 (0.11-0.67)
Tumor grade A+B = 38 (44.2%) C+D = 48 (55.8%)			
Pro/Pro	17	36	1 (Ref)
Pro/Ser	20	9	4.70 (1.77-12.5)
Ser/Ser	1	3	0.70 (0.07- 7.29)
Pro/Ser+Ser/Ser	21	12	3.70 (1.48- 9.24)
Pesticide Exposure Never = 33 (38.4%) Ever = 53 (61.6%)			
Pro/Pro	14	39	1 (Ref)
Pro/Ser	17	12	3.95 (3.94-10.3)
Ser/Ser	2	2	2.78 (0.36- 21.7)
Pro/Ser +Ser/Ser	19	14	3.78 (1.50- 9.50)
Bleeding PR/Constipation Yes = 60 (69.8%) No = 26 (30.2%)			
Pro/Pro	35	18	1 (Ref)
Pro/Ser	22	7	1.62 (0.58-4.49)
Ser/Ser	3	1	1.54 (0.15-15.9)
Pro/Ser+Ser/Ser	25	8	1.61 (0.60-4.27)
Tumor type*Mucinous=33 (38.5%)Non= 52 (60.5%)			
Pro/Pro	20	33	1 (Ref)
Pro/Ser	12	16	1.24 (0.49-3.14)
Ser/Ser	1	3	0.55 (0.05-5.65)
Pro/Ser+ Ser/Ser	13	19	1.13 (0.46-2.77)
p53 statusWild-type = 42 (48.8%)Mutated = 44 (51.2%)			
Pro/Pro	29	24	1 (Ref)
Pro/Ser	11	18	0.51 (0.20-1.27)
Ser/Ser	2	2	0.83 (0.11-6.32)
Pro/Ser + Ser/Ser	13	20	0.54 (0.22-1.30)

*One was SCC

The correlation of NQO1 C609T polymorphic status and the clinicopathologic characteristics was carefully analyzed. It was found that the Ser/Ser variant status was

significantly related to the age group, smoking status, tumor location, nodal status/ higher tumor grade and with exposure to pesticides, but not with other variables (Table 3).

Discussion

Although most polymorphisms are functionally neutral, some affect regulation of gene expression or the function of the coded protein (Costa et al., 2008). But Considering the potential role of NQO1 in modulating various diet and tobacco smoke-related compounds, we assessed the one most common SNP of NQO1 C609T (Pro187Ser) in an ethnic Kashmiri population for the first time since the role of NQO1 polymorphism in relation to colorectal cancer risk has not been reported from this part of the world.

As reported previously in various studies on Kashmiri population (Mir et al., 2005; Sameer et al., 2009 & 2010), it has been proven beyond doubt that our population is exposed to a special set of environmental and dietary risks which include consumption of sun-dried and smoked fish and meat, dried and pickled vegetables, red chilly, Hakh (a leafy vegetable of Brassica family), hot noon chai (salted tea) and Hukka (water pipe) smoke. As previously reported, the etiology and incidence of various GIT cancers in this population has been attributed to a probable exposure to nitroso compounds, amines and nitrates reported to be present in these local food stuffs most of which have been shown to contain important irritant and carcinogens (Siddiqi et al., 1992; Murtaza et al., 2006).

As has been reported previously by two studies on a rat model of colon carcinogenesis, the presence of an NQO1 inactivating polymorphism may represent a genetic factor that predisposes individuals to colorectal cancer (Begleiter et al., 2003; Sivananthan et al., 2005). In this study we analysed 86 colorectal cancer patients in relation to 160 healthy controls in order to examine the role of the C609T SNP in the NQO1 gene in the development of colorectal cancer in Kashmiri population. Cancer patients and healthy control subjects were well-matched for age, gender, ethnic distribution, and tobacco. We found a 1.62-fold greater incidence of the NQO1 CT genotype and 1.75-fold greater incidence of the NQO1 TT genotype in patients with colorectal cancer and compared with the healthy control population (Table 2). This finding is in agreement with several previous studies of the role of the NQO1 base 609 polymorphism in colon cancer risk (Lafuente et al., 2000; Hou et al., 2005; Begleiter et al., 2006; Chao et al., 2006; van der Logt et al., 2006).

We also found a relation between the increased incidence of NQO1 TT genotype and smoking status of the colorectal patients. This relation was in tune with the previous studies (Hou et al., 2005; Begleiter et al., 2006) where smoking status have been found to be an important risk factor for modulating the role of NQO1 enzymatic activity for predisposition of colorectal cancer. NQO1 protects cells from carcinogens in cigarette smoke by direct scavenging of quinone substrates and inhibiting the formation of CYP1A1-generated metabolites and subsequent binding to DNA (Joseph et al., 1994). NQO1 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.

References

Begleiter A, Hewitt D, Maksymiuk AW, Ross DA, Bird RP (2006). A NAD(P)H:quinone oxidoreductase 1 polymorphism is a risk factor for human colon cancer. *Cancer Epidemiol Biomarkers Prev*, **15**, 2422-6.

Begleiter A, Sivananthan K, Curphey TJ, Bird RP (2003). Induction of NAD(P)H quinone:oxidoreductase1 inhibits carcinogen induced aberrant crypt foci in colons of Sprague-Dawley rats. *Cancer Epidemiol Biomarkers Prev*, **12**, 566-72.

Center MM, Jemal A, Smith A, Ward E (2009). Worldwide variations in colorectal cancer. *CA Cancer J Clin*, 59, 366-378.

Center MM, Jemal A, Ward E (2009). International trends in colorectal cancer incidence rates. *Cancer Epidemiol Biomarkers Prev*, **18**, 1688-694.

Chao C, Zhang Z-F, Berthiller J, Boffetta P, Hashibe M (2006). NAD(P)H:Quinone Oxidoreductase 1 (NQO1) Pro187Ser polymorphism and the risk of lung, bladder, and colorectal cancers: a meta-analysis. Cancer Epidemiol Biomarkers Prev, 15, 979–987

Chen H, Lum A, Seifried A, Wilkens LR Marchand LL (1999). Association of the NAD(P)H:quinone oxidoreductase 609C3T polymorphism with a decreased lung cancer risk.

Cancer Res, **59**, 3045-8.
Chen S, Wu K, Knox R (2000). Structure-function studies of DT-diaphorase (NQO1) and NRH:quinone oxidoreductase (NQO2). *Free Rad Biol Med*, **29**, 276-84.
Felton JS, Knize MG (1991). Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food. *Mutat Res*, **259**, 205-17.
Hou L, Chatterjee N, Huanh W-Y, et al (2005). CYP1A1 Val462 and NQO1 Ser187 polymorphisms, cigarette use, and risk for colorectal adenoma. *Carcinogenesis*, **26**, 1122-8.
Joseph P. Jaiswal AK (1994). NAD(P)H:quinone oxidoreductase1 (DT diaphorase) specifically prevents the formation of benzo[a]pyrene quinone--DNA adducts generated by cytochrome P4501A1 and P450 reductase. *Proc Natl Acad Sci USA*, **91**, 8413-7.
Kelsey KT, Ross D, Traver RD, et al (1997). Ethnic variation in the prevalence of a common NAD(P)H quinone oxidoreductase polymorphism and its implications for anti-cancer chemotherapy. *Br J Cancer*, **76**, 852-4.
Krajinovic M, Sinnett H, Richer C, Labuda D, Sinnett D (2002). Role of NQO1, MPO and CYP2E1 genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. *Int J Cancer*, **97**, 230-6.
Lafuente MJ, Casterad X, Trias M, et al (2000). NAD(P)H: quinone oxidoreductasedependent risk for colorectal cancer and its association with the presence of K-ras mutations in tumors. *Carcinogenesis*, **21**, 1813-9.
Menze H-J, Sarmanova J, Soucek P, et al (2004). Association of NQO1 polymorphism with spontaneous breast cancer in two independent populations. *Br J Cancer*, **90**, 1989-94.
Mir MM, Dar NA, Gochhait S, Zargar SA, et al (2005). p53 Mutation profile of squamous cell carcinomas of the esophagus in Kashmir (India): a high-incidence area. *Int J Cancer*, **116**, 62-8.
Mitrou PN, Watson MA, Loktionov AS, et al (2007). Role of NQO1C609T and EPHX1 gene polymorphisms in the association of smoking and alcohol with sporadic distal colorectal adenomas: results from the UKFSS Study. *Carcinogenesis*, **28**, 875-82.
Murtaza I, Mushtaq D, Margoob MA, Dutt A, et al (2006). A study on p53 gene alterations in esophageal squamous cell carcinoma and their correlation to common dietary risk factors among population of the Kashmir valley. *World J Gastroenterol*, **12**, 4033-7.
Naoe T, Takeyama K, Yokozawa T, et al (2000). Analysis of genetic polymorphism in NQO1, GST-M1, GST-T1, and CYP3A4in 469 Japanese patients with therapy-related leukemia/myelodysplastic syndrome and de novo acute myeloid leukemia. *Clin Cancer Res*, **6**, 4091-5.
Park S-J, Zhao H, Spitz MR, Grossman HB, Wu X (2003). An association between NQO1 genetic polymorphism and risk of bladder cancer. *Mutat Res*, **536**, 131-7.
Parkinson A (2003). Biotransformation of Xenobiotics. In Klaussen,C. (ed.), Casarett and Doull's Toxicology: The Basic Science of Poisons. McGraw--Hill, New York, pp. 133-237.
Ross D (2004). NQO1. Atlas Genet Cytogenet Oncol Haematol. . URL : http://AtlasGeneticsOncology .org / Genes / NQO1ID375.html
Sameer AS, Rehman S, Pandith AA, et al (2009). Molecular gate keepers succumb to gene aberrations in colorectal cancer in Kashmiri population, revealing a high incidence area. *Saudi J Gastroenterol*, **15**, 244-52.
Sameer AS, Shah ZA, Syeed N, et al (2010). TP53 Pro47Ser and Arg72Pro polymorphisms and colorectal cancer predisposition in the ethnic Kashmiri population. *Genet Mol Res*, **9**, 651-60.
Sarbia M, Bitzer M, Siegel D, et al (2003). Association between NAD(P)H:quinine oxidoreductase 1 (NQ01) inactivating C609T polymorphism and adenocarcinoma of the upper gastrointestinal tract. *Int J Cancer*, **107**, 381-6.
Schulz WA, Krummeck A, Rosinger I, et al (1997). Increased frequency of a nullallele for NAD(P)H:quinone oxidoreductase in patients with urological malignancies. *Pharmacogenetics*, 7, 235-9.
Siddiqi M, Kumar R, Fazili Z, Spiegelhalder B, et al (1992). Increased exposure to dietary amines and nitrate in a population at high risk of esophageal and gastric cancer in Kashmir (India). *Carcinogenesis*, **13**, 1331-5.
Siegel D, Gustafson DL, Dehn DL, et al (2004). NAD(P)H: quinone oxidoreductase 1: role as a superoxide scavenger. *Mol Pharmacol*, **65**, 1238-47.
Siegel D, McGuinness SM, Winski SL, Ross D (1999). Genotypephenotype relationships in studies of a polymorphism in NAD(P)H:quinone oxidoreductase 1. *Pharmacogenetics*, **9**, 113-21.
Sivananthan K, Bird RP, Maksymiuk AW, Lefas G, Begleiter A (2005). Effect of post-initiation induction of NQO1 by oltipraz on AOM induced colon tumor formation in Sprague-Dawley rats. *Proc Am Assoc Cancer Res*, **46**, 581.
Smith MT, Wang Y, Kane E, et al (2001). Low NAD(P)H: quinone oxidoreductase 1 activity is associated with increased risk of acute leukemia in adults. *Blood*, **97**, 1422-6.
Van der Logt EMJ, Bergevoet SM, Roelofs HMJ, et al (2006). Role of epoxide hydrolase, NAD(P)H:quinone oxidoreductase, cytochrome P450 2E1 or alcohol dehydrogenase genotypes in susceptibility to colon cancer. *Mutat Res*, **593**, 39-49.
Wiemels JL, Pagnamenta A, Taylor GM, et al (1999). A lack of a functional NAD(P)H:quinone oxidoreductase allele is selectively associated with pediatric leukemias that have MLL fusions. *Cancer Res*, **59**, 4095-9.
Winski SL, Koutalos Y, Bentley DL, Ross D (2002). Subcellular localization of NAD(P)H: quinone oxidoreductase 1 inhuman cancer cells. *Cancer Res*, **62**, 1420-4.
Zhang J-H, Li Y, Wang R, et al (2003). NQO1 C609T polymorphism associated with esophageal cancer and gastric cardiac carcinoma in North China. *World J Gastroenterol*, **9**, 1390-3.