RESEARCH COMMUNICATION

Lack of Influence of DNA Repair Gene OGG1 Codon 326 Polymorphisms of Gastric Cancer Risk in the Kashmir Valley

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Abstract

Damage to DNA may lead to carcinogenesis but is repaired through activation of pathways involving polymorphic enzymes, including human 8-oxoguanine glycosylase 1 (OGG1). The present study aimed to assess the role of genetic variants of DNA repair gene OGG1 Ser326Cys in susceptibility to gastric cancer in Kashmir valley. A case control study was performed in 303 subjects (108 gastric cancer and 195 healthy controls), all genotyped through the polymerase chain reaction (PCR). Data weres statistically analyzed using the chi-square test and the logistic regression model. The distribution of OGG1 genotypes among controls and gastric cancer cases did not show any significant differences. Although smokers and high salted tea drinkers themselves were at higher risk for gastric cancer (OR=8.975, P=0.0001; OR=14.778, P=0.0001), interaction with OGG1 Ser326Cys did not further modulate the risk. In conclusion, our findings suggest that the OGG1 polymorphism does not influence either gastric cancer risk independently or by interaction with smoking or salted-tea consumption in the Kashmir valley.

Key Words: Oxidative DNA damage - DNA repair genetic polymorphisms - OGG1 gene - gastric cancer - India

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Introduction

Carcinomas of the stomach have diverse incidence patterns and risk factors. Their etiology is incompletely understood, like many other malignancies, but is clearly multifactorial (Brown et al., 2002). One of the proposed mechanisms represents the involvement of oxidative DNA damage which can induce mutations leading to cancers (Fearon et al.,1997; Marnet et al., 2000). Sequence variants in DNA repair genes are assumed to modulate DNA repair capacity and, therefore, are associated with the altered cancer risk.

Among many types of oxidative DNA damage, 8-hydroy-2-deoxyguanine (8-OHdG) is highly mutagenic because of its propensities to mispair with adenine during DNA replication and to cause ultimately GC to TA transversion (Cheng et al., 1992; Hazra et al., 2001). The human 8-oxoguanine glycosylase 1 (OGG1) encoded by the OGGI gene located on chromosome 3 has an activity to remove the directly 8-OHdG from DNA as a part of the base excision repair pathway (Bioteux et al., 2000; Sunaga et al., 2001). The 1245 C>G (Ser326Cys) polymorphism is a well known OGG1 gene polymorphism that results in an amino substitution from serine to cystein in a codon 326. Compared to the OGG1- Ser326 protein, the OGG1-Cys326 protein was shown to be less able to supress spontaneous mutations in an Escherichia coli strain defective in 8-oxoG repair.

Recent studies have suggested that the Ser326Cys OGG1 polymorphism may be associated with an increased risk of lung (Sugimura et al.,1999; Le Marchand et al., 2002), esophageal (Xing et al., 2001), orolaryngeal (Elahi et al., 2002), and prostate cancers (Chen al., 2003). As for the association between gastric cancer, three studies from Japan, Brazilians and China have reported no significantly increased risk with this polymorphism (Shinmura et al., 1998; Hanaoka et al, 2001; Takezaki et al., 2002). Ethnic differences in the frequency of OGG1 Ser326Cys polymorphism have been observed and previous reports also suggest that association of OGG1 Ser326Cys polymorphism with cancer depends on ethnicity and race (Sugimura et al., 1999; Goode et al., 2002).

In the Kashmir Valley, gastric cancer has been reported to exceed 40% of all cancers where incidence is 3-6 times higher than various metropolis cancer registries in India. However, very few reports have associated this malignancy with specific risk factors prevalent in the area (Khuroo et al., 1992). Some of the environment factors including dietary habits like hot noon chai (salted tea), smoked, pickled and preserved foods rich in salt, nitrate and preformed nitrosocompounds have been reported to be associated with an increased risk of gastric cancer in Kashmir valley (Siddiqi et al., 1988). In addition, genetic factors may also be responsible of high incidence of the cancer in the valley. So far, there is no report of association of DNA repair gene polymorphisms with risk of gastric cancer in Kashmir population. Therefore, the main aim

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of the present study was to investigate whether the OGG1 Ser326Cys polymorphism was associated with gastric cancer risk, either independently or in interaction with environmental factors in the Kashmir valley.

Materials and Methods

This case control study comprised histopathologically confirmed cases with gastric cancer (108) and healthy controls (195) from the population of Kashmir valley. All subjects were unrelated ethnic Kashmiri residents, referred from the Departments of Gastroenterology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar from May 2006 to July 2008. The controls were also recruited from Sher-i-Kashmir Institute of Medical Sciences, Srinagar, who come for their routine checkup and were diagnosed as non-severe ailment and no malignancy.

All the individuals were personally interviewed for their age, occupation, demographic features, dietary habits, usage of hot noon chai (salted tea) and smoking habits. Tobacco usage included smoking of cigarette, or hukka (water pipe). Written informed consent was obtained from all participants in the study. The research protocol was approved by the ethical committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow (Ethics Committee Meeting on May 14, 2004). Sample collection, storage and transport were complied with guidelines of the committee. Blood samples were collected in EDTA and the genomic DNA was extracted from peripheral blood leukocytes pellet using the standard salting-out method (Miller SA et al.,1988) The quality and quantity of DNA was checked by gel electrophoresis and spectrophotometry using Nanodrop Analyser (ND-1000) spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE, USA). The ratio of absorbance at 260 and 280 nm of DNA was around 1.7-1.9. The isolated DNA was stored at -70°C.

Genotyping

The OGG1 Ser326Cys polymorphism was genotyped by a polymerase chain reaction (PCR) with confroning two pairs of primers (Ito et al., 2002). PCR products were visualized with ethidium bromide staining. The primer pair F1 and R1 produced the C allele (Ser326) band (252 bp), F2 and R2 produced the G allele (326Cys) band (194 bp) (Figure 1). To validate the results, ten percent of samples from patients and controls including samples of each genotype were re-genotyped by other laboratory personnel. No discrepancy was also found after sequencing randomly selected 5% samples

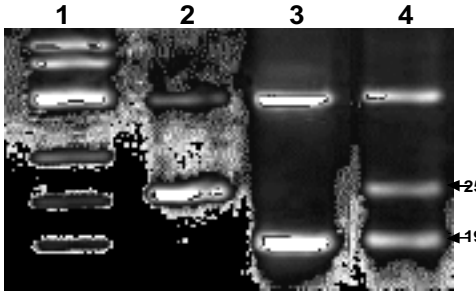

Figure 1. Representative Gel of OGG1Ser326Cys Genotyping. Lane 1: 50bp ladder; Lane 2: CC (Ser326) genotype; Lane 3: GG (326Cys) genotype; Lane 4: CG (Ser326Cys)) genotype

Statistical analysis

Descriptive statistics of patients and controls were presented as mean and SDs for measures, whereas frequencies and percentages were used for categorical measures. The $\chi 2$ goodness-of-fit test was used for any deviation from Hardy-Weinberg equilibrium. Differences in genotype frequencies between cases and controls were estimated by the chi-square ($\chi 2$) test. Binary logistic regression was used to estimate risks as odds ratios (ORs) with 95% confidence intervals (CI) using age and gender as covariates. A P-value of less than 0.05 was considered to indicate a significant difference. All statistical analysis was performed using the SPSS software version 15.0 (SPSS, Chicago, IL, USA)

Results

The mean age of healthy subjects (controls) and gastric cancer patients was 57.98yrs± 12.664 and 55.91yrs±9.728 respectively (t-test P value=ns). Cancer was highly prevalent in males (90/108; 83.3%) than in females (18/108; 16.7%). In patients with gastric cancer most of the cases were with adenocarcinoma (ADC, 79.6%). Smoking habit (Hukka) showed significantly higher risk in gastric cancer patients (OR=8.975; 95%CI=5.156-15.622; P=0.0001). Individuals consumed salted-tea in a range of 2-8 cups per day; and median consumption of tea was 4 cups per day. So we grouped individuals in to ≤4 cups or >4 cups per day and individuals consumed salted tea >4 cups per day were regarded as high salted tea consumers. Higher consumption of salted tea was also found to be associated with increased risk of gastric cancer

Table 1. Characteristics of Gastric Cancer Patients and Healthy Controls of Kashmir Valley

Variables	Cancer (n=108)	Controls (n=195)	OR* (95%CI)	P value
Age (Mean±SD)	55.9±9.7	58.0±12.7		
Sex Male	90 (83.3)	139 (71.3)		
Female	18 (16.7)	56 (28.7)		
Histology				
Adenocarcinoma	86 (79.6)	--		
SCC	22 (20.4)	--		
Smoking#(Hukka)	48 (67.6)	38 (20.5)	8.9 (5.2-15.6)	0.0001
Salted tea intake#				
(≤4 cups daily)	31 (30.7)	159 (85.9)		
(>4 cups daily)	70 (69.3)	26 (14.1)	14.8 (8.0-27.2)	0.0001

*Age and gender adjusted odds ratio; Data are No. (%); SCC, squamous cell carcinoma; #Data missing

Table 2. Frequency Distribution of OGG1 Genotypes in Gastric Cancer Patients and Controls

Genotypes	Controls	Cancer	OR*(95%CI)	P-value
Ser/Ser	94 (48.2)	50 (46.3)	1 Reference	
Ser/Cys	89 (45.6)	51 (47.2)	1.05 (0.64-1.72)	0.851
Cys/Cys	12 (6.2)	7 (6.5)	1.32 (0.47-3.66)	0.600

*Age and gender adjusted odds ratio; Data are No. (%)

Table 3. Associations of Genotypes with Tumor Histopathology and Risk of Gastric Cancer

Genotypes	Controls	GSCC[1] 22	OR* (95%CI)	P-value	GADC[2] 86	OR* (95%CI)	P-value
Ser/Ser	94 (48.2%)	14 (63.6%)	1 (Reference)		36 (41.9%)	1 (Reference)	
Ser/Cys	89 (45.6%)	8 (36.4%)	0.977 (0.428-2.229)	0.955	43 (50.0%)	1.232 (0.721-2.107)	0.445
Cys/Cys	12 (6.2%)	0 (0.0%)	0.999 (0)		7 (8.1%)	1.856 (0.654-5.265)	0.245

[1]Gastric squamous cell carcinoma; [2]Gastric adenocarcinoma; *Age and gender adjusted odds ratio

Table 4. Interaction of Genotypes with the Smoking Habit

Genotype	Controls[#] (N=185)		Gastric cancer[#] (N=107)			
	Smoker 38	Non-smoker 147	Smoker 75	Non-smoker 32	OR* (95%CI)	P-Value
Ser/Ser	21 (55.3%)	68 (46.3%)	35 (46.7%)	15 (46.9%)	1 (Reference)	
Ser/Cys	16 (42.1%)	71 (48.3%)	39 (52.0%)	11 (34.4%)	1.467 (0.661-3.258)	0.346
Cys/Cys	1 (2.6%)	8 (5.4%)	1 (1.3%)	6 (18.8%)	0.689 (0.035-13.68)	0.807

*Age and gender adjusted odds ratio; OR calculated for smokers only; [#]Data missing in some subjects

Table 5. Interaction of Genotypes with Salted Tea Consumption

Genotype	Controls[#] (N=185)		Gastric cancer[#] (N=101)			
	Cups/day<4	Cups/day≥4	Cups/day<4	Cups/day≥4	OR*(95%CI)	P-Value
Ser/Ser	77 (48.4%)	10 (38.5%)	14 (45.2%)	32 (45.7%)	1 (Reference)	
Ser/Cys	72 (45.3%)	16 (61.5%)	15 (48.4%)	33 (47.1%)	0.977 (0.428-2.229)	0.955
Cys/Cys	10 (6.3%)	0 (0.0%)	2 (6.5%)	5 (7.1%)	1.280 (0.220-7.455)	0.784

*Age and gender adjusted odds ratio; [#]Data missing in some subjects.

(OR=14.778; 95%CI=8.020-27.231; P-value=0.0001) (Table 1). None of patients or controls reported consumption of alcohol, so interaction of alcohol intake with genetic variations could not be analyzed.

OGG1 Ser326Cys frequency distribution

The distribution of OGG1 genotypes among controls were (Ser/Ser, 48.2%; Ser/Cys, 45.6% and Cys/Cys 6.2%) and in gastric cancer cases, the frequency distribution of three genotypes were Ser/ Ser, 46.3%; Ser/Cys, 47.2% and Cys/Cys, 6.5%. The corresponding heterozygous and homozygous variant frequency did not show any significant differences when compared to controls between these two groups (Table 2).

Interaction of OGG1 genotypes with tumor histopathology

When tumor histopathologies were analyzed, but none OGG1 genotype was found to be associated with particular histology of gastric cancer (Table 3).

Interaction of OGG1 genotypes with smoking habit and high salted tea consumption

On analyzing the interaction of genotypes with tobacco habit, smokers with OGG1 Cys/Cys genotype did not show any significant association with risk for cancer (Table 4). Also the interaction of genotypes with high salted tea intake showed no modulation of risk for developing gastric cancer (Table 5).

Discussion

Damage to an individual's DNA occurs by various mechanisms, either as a by-product of normal cellular metabolism such as reactive oxygen species (ROS), or as a result of exposure to environmental and biological mutagens. DNA damage that is not repaired leads to apoptosis or mutation, which in turn may cause carcinogenesis. There are several systems, both at the cellular and molecular level, in order to respond to this damage. At the molecular level, several pathways exist that recognize and repair DNA damage, each operating on specific types of damage. OGG1Ser326Cys is among the most widely studied DNA repair gene polymorphisms in various cancers.

In present study, a similar frequency of OGG1 Ser326Cys polymorphism was observed in the groups of healthy controls and gastric cancer patients and no association could be established. Three previous studies from China, Japan and Brazil also did not observe significant associations between the OGG1Ser326Cys polymorphism and risk of gastric cancer (Shinmura et al., 1998; Hanaoka et al., 2001; Takezaki et al., 2002). These observations suggest that OGG1Ser326Cys polymorphism may not influence gastric cancer susceptibility in different populations with varied incidence of cancer.

In addition to possible exposure to well known risk factors for gastric cancers like smoking and beverages, the people of the valley have many unique social, cultural and dietary features which are different from rest of world. Salted tea used by people is prepared by using baking soda (sodium bicarbonate) along with common salt (sodium chloride) and boiled for few hours before consuming. It has been suspected that the salts might cause thermal injury to gastric epithelium. Several previous studies have attributed high incidence of gastric cancers in Kashmir to considerable amount of nitroso compounds in raw foodstuffs (Khuroo et al., 1992, Siddiqi et al., 1992). The possible endogenous formation of N-nitroso compounds in strongly acidic conditions of salted tea as well as smoking may be critical risk factors for high occurrence of gastric cancers in Kashmir valley (Ivankovic et al., 1998). In the present study, consumption of large amounts of salted tea was significantly associated with

increased risk for gastric cancer (OR=16.338; P=0.0001). Also our results show significant association of smoking (Hukka) with gastric cancer (OR=16.338; P=0.0001). However, in gene environmental interaction, we did not find any association of OGG1Ser326Cys with smoking and salted tea consumption. In future, genome-wide association studies should be carried out to find out other genes involved in the cancer susceptibility and interactions with environmental factors.

In conclusion, the present case control study found that OGG1 Ser326Cys polymorphism was not found to be associated with gastric risk in Kashmiri population. Although the OGG1 Ser326Cys polymorphism is reported to be a risk factor in some cancers, it does not appear to play a major role in gastric cancer development in Kashmir valley.

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