

## DNA repair enzyme polymorphisms and oxidative stress in a Turkish population with gastric carcinoma

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**Abstract** Although the developmental stages of gastric carcinoma are still not clear, the constantly generated reactive oxygen and nitrogen species (ROS/RNS) may contribute to the process of carcinogenesis by interacting with DNA. 8-oxoguanine DNA glycosylase-1 (*OGGI*) is an enzyme involved in base excision repair of 8-oxoguanine that is one of the premutagenic lesions generated by ROS in DNA. The bulky adducts, are recognized and repaired by nucleotid excision repair (NER) enzymes, including xeroderma pigmentosum C and D (*XPC*, *XPD*). Eligible 106 gastric cancer patients and 116 cancer-free individuals constituted the study and control groups, respectively. Association between *OGGI* Ser326Cys, *XPC* Lys939Gln, *XPD* Lys751Gln polymorphisms and the susceptibility to the cancer and the oxidative stress status were evaluated. DNA was extracted from peripheral blood cells and genotypes were determined by using PCR–RFLP. Serum nitric oxide, albumin concentrations, total antioxidant status and *Helicobacter pylori* IgG were determined. Serum albumin and nitric oxide of cancer patients were lower than that of the controls ( $P < 0.05$ ). None of the evaluated polymorphisms

or *Helicobacter pylori* IgG seropositivity associated with increased risk of gastric cancer, despite of the increased oxidative stress in cancer patients.

**Keywords** Gastric cancer · *OGGI* Ser326Cys polymorphism · *XPC* Lys939Gln polymorphism · *XPD* Lys751Gln polymorphism · *Helicobacter pylori* · Oxidative stress

### Introduction

Despite of the steady decline in the incidence of gastric cancer over the last decades, overall 5-year survival rate is still 15–20%, as gastric cancer can mostly be diagnosed at an advanced stage. Therefore, the identification and control of risk factors might be effective for reducing the burden of these tumours [1–3]. During the cellular metabolism as well as from extra cellular processes, several reactive oxygen and nitrogen species (ROS/RNS) that challenge the DNA integrity are produced [4]. Prolonged and excessive generation of intracellular ROS as well as RNS like nitric oxide (NO), decreased availability of major and predominant circulating antioxidants in plasma, like albumin, and altered balance in total antioxidant status (TAS) of the organism are assumed to contribute to the development of carcinogenesis by inducing oxidative DNA damage [3, 5, 6]. 8-oxoguanine DNA glycosylase-1 (*OGGI*) is an enzyme involved in excision repair of 7,8-dihydro-8-oxoguanine (8-OHGua) that is considered to be an abundant type of DNA damage induced by reactive oxygen metabolites and is responsible for single base mutation. However, since the DNA adducts are only partially repaired via enzymatic pathways that are initiated by a DNA glycosylase, the inadequate activity of DNA glycosylase might

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cause the accumulation of DNA adducts [7]. A C to G polymorphism in exon 7 of the *OGGI* gene is associated with the substitution of cysteine for serine at codon 326. The DNA repair activity of the *OGGI*-Cys326 protein is stated to be weaker than that of wild-type *OGGI*-Ser326 [8]. The data published up to date is inconsistent, about the relation between gastric cancer and *OGGI* polymorphism, with only some of them reporting a significant association between the two [9–11]. On the other hand bulky DNA adducts are repaired by nucleotide excision repair (NER) pathway in which xeroderma pigmentosum complementation group D and C (*XPD*, *XPC*) are involved. Single nucleotide polymorphism in *XPD* in codon 751 is related with altered repair capacity [12]. Thus, variants generated by substitution of A to C can change Lys751Gln in the conserved region of *XPD* protein leading to lower DNA damage repair efficiency [13]. The fate of *XPC* in subsequent steps of NER has not been totally elucidated, yet. However, the dramatic increase in *XPC* levels after damage implied a significant role in NER in addition to the rapid initial lesion binding as an early factor and it has been reported that *XPC* is a DNA damage inducible gene [14]. Therefore, polymorphism in codon 939 might alter the damaged lesion binding.

On the other hand, several factors, including persistent *Helicobacter pylori* (*H. pylori*) infection are effective in the initiation of gastric cancer. *H. pylori* has been recognized as Group 1A carcinogen by International Agency for Research on Cancer (IARC) and may cause gastric atrophy, cell mutation leading to cell proliferation and tumour growth [15]. Considering the possible ethnical/genetically and environmental factors, diagnosis of gastric cancer at earlier ages, prediction of risk for developing gastric cancer might be a life saving issue [2, 16]. Thus, in our study, the association between *OGGI* Ser236Cys, *XPC* Lys939Gln and *XPD* Lys751Gln polymorphisms and susceptibility to gastric carcinoma were evaluated regarding extracellular oxidative stress.

## Materials and methods

### Patients and controls

This prospective randomised study involved 106 consecutive patients (Mean  $\pm$  Standard error of the mean (SEM);  $60.4 \pm 1.3$  years of age, body mass index (BMI);  $23.5 \pm 0.5$  kg/m<sup>2</sup>) with a diagnosis of gastric cancer and 116 cancer free patients ( $55.5 \pm 1.3$  years of age, BMI:  $26.6 \pm 0.4$  kg/m<sup>2</sup>) referred to Gazi University, Faculty of Medicine, Department of General Surgery for surgical intervention. None of the patients in the control group had neither malignant nor metabolic disorders. Neither of them

had cardio-pulmonary or metabolic risks that could be an obstacle for surgery. The primary disease of the cancer patients was suitable for surgical intervention.

The patients who had immune system disorders, could not receive surgical intervention or receive neoadjuvant chemotherapy due to the late stage carcinoma, who has either malnutrition, autoimmune diseases, systemic inflammatory response syndrome, intraabdominal sepsis, chronic granulomatosis, collagen tissue or neurodegenerative diseases were excluded from the study.

All participants' rights were protected and informed consents were obtained according to the Helsinki Declaration. Local Ethic Committee approved the study protocol.

Peripheral blood from each individual was collected into one EDTA containing and one anticoagulant free blood collection tube. The first tube was kept untreated, while the second tube was used for the serum separation. Both samples were kept in  $-20^{\circ}\text{C}$  until the assays.

Measurement of serum albumin levels were done by auto analyser, in biochemistry laboratory of Gazi University, Faculty of Medicine, while total antioxidant status (TAS) was detected spectrophotometrically (Randox Total Antioxidant Status, TAS, Randox, UK) according to the instructions of manual. For each individual, *H. pylori* IgG seropositivity was determined by ELISA (*H. pylori* IgG ELISA, Demeditec, Germany). Serum NO concentrations were calculated as nitrite and Griess method was used for the measurement of serum NO levels [17].

DNA of each patient was isolated from whole blood samples by using sodium perchlorate/chloroform extraction [18]. *OGGI* (GenBank ID: AJ131341, rs: 1052133, C $\rightarrow$ G) Ser326Cys, *XPC* (GenBank ID: AF261901, rs: 2228001, A $\rightarrow$ C) Lys939Gln and *XPD* (GenBank ID: L47234, rs: 13181, A $\rightarrow$ C) Lys751Gln genotypings were done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the modified protocols of Karahalil et al., Khan et al. and Hemminki et al., respectively [16, 18, 19]. PCR primers for *OGGI* Ser326Cys were F 5'-actgtcactagtctaccag-3' and R 5'-tgaattcggaaagtgcttgggaat-3', for *XPC* Lys939Gln F 5'-ggaggtggactctctctgatg-3' and R 5'-tagatcccagcagatgacc-3' and for *XPD* Lys751Gln F 5'-atcctgtccctactggccattc-3' and R 5'-tggacgtgacagtgagaat-3' which generate fragments of 207, 765 and 322 bp, respectively, by using about 50 ng genomic DNA. Briefly, for *OGGI* Ser326Cys genotyping, PCR was performed in 10  $\mu\text{l}$  of reaction volume, containing *Taq* buffer (75 mM Tris-HCl (25 $^{\circ}\text{C}$ , pH 8.8), 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.01% Tween 20), 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.3  $\mu\text{M}$  of each primer and 0.03 U/ $\mu\text{l}$  *Taq* polymerase. Thermal cycling conditions were 94 $^{\circ}\text{C}$  for 2 min followed by 33 cycles at 94 $^{\circ}\text{C}$  (15 s), 60 $^{\circ}\text{C}$  (30 s) and 72 $^{\circ}\text{C}$  (15 s) and a final elongation at 72 $^{\circ}\text{C}$  for 10 min. PCR products were digested overnight at 37 $^{\circ}\text{C}$  by

restriction endonuclease; Sat I (Fermentas Company, Lithuania). The digested PCR products were separated by electrophoresis on 2% agarose gel in TBE buffer (90 mM Tris–HCl, 90 mM boric acid and 2 mM EDTA), and stained in ethidium bromide. The genotype results were confirmed by using controls whose DNA sequencing was done [18]. PCR for genotyping analysis of *XPC* Lys939Gln was performed in 10 µl of reaction volume, containing *Taq* buffer (10 mM Tris–HCl (25°C, pH 8.8), 50 mM KCl, 0.08% Nonidet P40), 2 mM MgCl<sub>2</sub>, 0.06 mM of each dNTP, 0.4 µM of each primer and 0.025 U/µl *Taq* polymerase. Thermal cycling conditions were 94°C for 2 min followed by 35 cycles at 94°C (20 s), 59°C (30 s) and 72°C (2 min 30 s) and a final elongation at 72°C for 10 min. PCR products were digested for 30 min at 37°C by restriction endonuclease; Pvu II (Fermentas Company, Lithuania) The digested PCR products were separated as was done for *OGGI*. The genotype results were confirmed by using controls whose DNA sequencing was done [16]. For *XPB* Lys751Gln genotyping PCR was performed in 10 µl of reaction volume, containing *Taq* buffer (10 mM Tris–HCl (25°C, pH 8.8), 50 mM KCl, 0.08% Nonidet P40), 2 mM MgCl<sub>2</sub>, 0.04 mM of each dNTP, 0.4 µM of each primer and 0.025 U/µl *Taq* polymerase. Thermal cycling conditions were 94°C for 2 min followed by 35 cycles at 94°C (15 s), 60°C (15 s) and 72°C (15 s) and a final elongation at 72°C for 7 min. PCR products were digested for 30 min at 37°C by restriction endonuclease; Pst I (Fermentas Company, Lithuania) The digested PCR products were separated as was done for *OGGI*. The genotype results were confirmed by using controls whose DNA sequencing was done [19].

### Statistical analysis

All results were expressed as mean ± SEM. The significance of the difference between controls and patients was analysed by Mann–Whitney *U* test. The deviation from Hardy–Weinberg equilibrium were checked among cases and controls by a  $\chi^2$ -test, with one degree of freedom. To estimate the association between gastric cancer risk and *OGGI* Ser326Cys, *XPC* Lys939Gln and *XPB* Lys751Gln polymorphisms, odds ratios (ORs) with 95% confidence intervals (CIs) were determined by using logistic regression models and the calculations were performed by using statistical software SPSS.

### Results

Both the gastric cancer and control patients were evaluated for the *OGGI* Ser326Cys, *XPC* Lys939Gln and *XPB* Lys751Gln polymorphisms and the allele frequencies were

**Table 1** Allele frequencies of control and gastric cancer patient groups for *OGGI* Ser326Cys, *XPC* Lys939Gln and *XPB* Lys751Gln polymorphism

Alleles	Control group		Cancer group	
	Alleles (n)	Allele frequencies	Alleles (n)	Allele frequencies
<i>OGGI</i> (codon 326 C/G)				
C	149	C = 0.64	148	C = 0.70
G	83	G = 0.36	64	G = 0.30
<i>XPC</i> (codon 939 A/C)				
A	105	A = 0.45	107	A = 0.51
C	27	C = 0.55	105	C = 0.49
<i>XPB</i> (codon 751 A/C)				
A	123	A = 0.53	116	A = 0.55
C	109	C = 0.47	96	C = 0.45

calculated (Table 1). None of the genotype distributions deviated from Hardy–Weinberg equilibrium. The polymorphisms were found to be not associated with increased risk of gastric cancer (Table 2).

The oxidative stress status of the cancer patients was assessed via the measurement and comparison of their TAS, NO and albumin values to the controls. Serum albumin and NO levels were significantly decreased in the cancer patients ( $P < 0.05$ , Table 3). Also, serum NO, albumin and TAS values of heterozygous or homozygous mutant *OGGI*, *XPC* or *XPB* genotyped cancer patients and *OGGI*, *XPC* or *XPB* wild type controls were compared (Table 3). While the serum albumin concentrations of heterozygous *OGGI* carrier gastric cancer patients had significantly decreased, their TAS values were increased compared to the wild type controls (both,  $P < 0.05$ ). The homozygous mutant *OGGI*, *XPC* or *XPB* carriers and heterozygous *XPC* or *XPB* genotype carrier cancer patients had significantly lower albumin than control group (for all,  $P < 0.05$ ). We detected a significant decrease in serum NO levels of *OGGI* Ser allele carrier and *XPB* Lys/Lys genotyped cancer patients compared to the *OGGI* Ser/Ser and *XPB* Lys/Lys controls, respectively ( $P < 0.05$ ). Serum NO levels of cancer patients with either of *XPC* genotypes were significantly decreased compared to the *XPC* Lys/Lys controls ( $P < 0.05$ ) (Table 3).

The frequency of the IgG seronegative controls and cancer patients were 31.9 and 41.5%, respectively, while the seropositive percentage of the same groups were 61.2 and 50.9%, respectively. However, 6.9% of the control group and 7.6% of the gastric cancer patients were in grey zone. Thus, the *H. pylori* IgG seropositivity seems not to be associated with increased gastric cancer risk (OR = 0.640, 95% confidence interval (CI); 0.364–1.122;  $P > 0.05$ , Table 4).

**Table 2** Odds ratios for gastric cancer according to the genotypes of *OGGI* Ser326Cys, *XPC* Lys939Gln and *XPB* Lys751Gln polymorphism (OR odds ratio, CI confidence interval)

Genotype	Control group n (%)	Cancer group n (%)	ORs (95% CI)	P
<i>OGGI</i>				
Ser/Ser	51 (44.00)	53 (50.00)	1	
Ser/Cys	47 (40.50)	42 (39.60)	0.860 (0.488–1.515)	0.601
Cys/Cys	18 (15.50)	11 (10.40)	0.588 (0.253–1.366)	0.214
<i>XPC</i>				
Lys/Lys	25 (21.60)	22 (20.80)	1	
Lys/Gln	55 (47.40)	63 (59.40)	1.302 (0.661–2.563)	0.445
Gln/Gln	36 (31.00)	21 (19.80)	0.663 (0.302–1.455)	0.304
<i>XPB</i>				
Lys/Lys	40 (34.50)	30 (28.30)	1	
Lys/Gln	43 (37.10)	56 (52.80)	1.736 (0.936–3.222)	0.079
Gln/Gln	33 (28.40)	20 (18.90)	0.808 (0.390–1.676)	0.567

**Table 3** Comparison of the oxidative stress parameters of control group and gastric cancer patients regarding to the *OGGI* Ser326Cys, *XPC* Lys939Gln or *XPB* Lys751Gln genotypes

Group	Albumin (g/dl) mean ± SEM	NO (μM) mean ± SEM	TAS (mM) mean ± SEM
Control (n = 116)	4.65 ± 0.10	24.44 ± 1.62	1.28 ± 0.08
Cancer (n = 106)	3.74 ± 0.08*	17.92 ± 1.09*	1.31 ± 0.11
<i>OGGI</i>			
Control (Ser/Ser) (n = 51)	4.57 ± 0.07	23.51 ± 1.90	1.10 ± 0.05
Cancer (Ser/Ser) (n = 53)	3.76 ± 0.12**	16.54 ± 1.24**	1.42 ± 0.21
Cancer (Ser/Cys) (n = 42)	3.76 ± 0.11**	19.81 ± 2.13**	1.23 ± 0.08
Cancer (Cys/Cys) (n = 11)	3.58 ± 0.26**	17.36 ± 2.85	1.14 ± 0.15
<i>XPC</i>			
Control (Lys/Lys) (n = 25)	4.49 ± 0.11	32.02 ± 6.02	1.29 ± 0.14
Cancer (Lys/Lys) (n = 22)	3.62 ± 0.16***	16.24 ± 1.76***	1.45 ± 0.26
Cancer (Lys/Gln) (n = 63)	3.81 ± 0.11***	18.91 ± 1.56***	1.26 ± 0.16
Cancer (Gln/Gln) (n = 21)	3.64 ± 0.14***	16.69 ± 2.26***	1.33 ± 0.10
<i>XPB</i>			
Control (Lys/Lys) (n = 40)	4.70 ± 0.19	21.72 ± 1.90	1.22 ± 0.08
Cancer (Lys/Lys) (n = 30)	3.89 ± 0.17****	16.21 ± 1.43****	1.60 ± 0.35
Cancer (Lys/Gln) (n = 56)	3.67 ± 0.10****	18.94 ± 1.84	1.22 ± 0.07
Cancer (Gln/Gln) (n = 20)	3.71 ± 0.16****	17.62 ± 1.52	1.16 ± 0.12

\*  $P < 0.05$ ; statistically significant, control versus cancer patients

\*\*  $P < 0.05$ ; statistically significant. Comparison between wild type *OGGI* Ser326Cys controls and either heterozygous or homozygous Cys allele carrier gastric cancer patients

\*\*\*  $P < 0.05$ ; statistically significant. Comparison between wild type *XPC* Lys939Gln controls and either heterozygous or homozygous Gln allele carrier gastric cancer patients

\*\*\*\*  $P < 0.05$ ; statistically significant. Comparison between wild type *XPB* Lys751Gln controls and either heterozygous or homozygous Gln allele carrier gastric cancer patients

*H. pylori* IgG seropositive gastric cancer patients with either of *OGGI* Ser/Ser, *XPC* Lys/Lys or *XPB* Lys/Lys carriers had significantly lower NO levels compared to the wild type *H. pylori* IgG seronegative controls ( $P < 0.05$ , Table 5). On the other hand, *H. pylori* IgG seronegative *OGGI* Cys, *XPC* Gln or *XPB* Gln allele carrier controls did not have significantly different albumin, NO or TAS values

compared to the wild type *OGGI*, *XPC* or *XPB* *H. pylori* IgG seronegative controls ( $P > 0.05$ ). Also, *H. pylori* IgG seropositive *OGGI* Cys, *XPC* Gln or *XPB* Gln allele carrier cancer patients did not have significantly different albumin, NO or TAS values compared to the wild type *OGGI*, *XPC* or *XPB* *H. pylori* IgG seropositive gastric cancer patients.

**Table 4** The relation between *Helicobacter pylori* IgG seropositivity and gastric cancer risk

<i>H. pylori</i> IgG	Control group <i>n</i> (%)	Cancer group <i>n</i> (%)	ORs (95% CI)	<i>P</i>
Seronegative	37 (31.90)	44 (41.50)	1	
Seropositive	71 (61.20)	54 (50.90)	0.640 (0.364–1.122)	0.118

**Table 5** Comparison of the oxidative stress parameters of *H. pylori* IgG seronegative control group and *H. pylori* IgG seropositive gastric cancer patients regarding to the *OGG1* Ser326Cys, *XPC* Lys939Gln or *XPD* Lys751Gln genotypes

Group	Albumin (g/dl) mean $\pm$ SEM	TAS (mM) mean $\pm$ SEM	NO ( $\mu$ M) mean $\pm$ SEM
<i>H. pylori</i> IgG seronegative Control ( <i>n</i> = 37)	4.83 $\pm$ 0.21	1.50 $\pm$ 0.22	21.86 $\pm$ 1.81
<i>H. pylori</i> IgG seropositive Cancer ( <i>n</i> = 54)	3.74 $\pm$ 0.13*	1.13 $\pm$ 0.06	17.27 $\pm$ 1.36*
<i>OGG1</i>			
<i>H. pylori</i> IgG seronegative Control (Ser/Ser) ( <i>n</i> = 16)	4.61 $\pm$ 0.11	1.16 $\pm$ 0.09	22.45 $\pm$ 2.18
<i>H. pylori</i> IgG seropositive Cancer (Ser/Ser) ( <i>n</i> = 27)	3.68 $\pm$ 0.21**	1.13 $\pm$ 0.07	16.43 $\pm$ 2.00**
<i>H. pylori</i> IgG seropositive Cancer (Ser/Cys) ( <i>n</i> = 21)	3.86 $\pm$ 0.17**	1.12 $\pm$ 0.09	18.26 $\pm$ 2.01
<i>H. pylori</i> IgG seropositive Cancer (Cys/Cys) ( <i>n</i> = 6)	3.57 $\pm$ 0.42**	1.16 $\pm$ 0.27	17.59 $\pm$ 4.99
<i>XPC</i>			
<i>H. pylori</i> IgG seronegative Control (Lys/Lys) ( <i>n</i> = 7)	4.63 $\pm$ 0.30	1.39 $\pm$ 0.32	28.07 $\pm$ 6.83
<i>H. pylori</i> IgG seropositive Cancer (Lys/Lys) ( <i>n</i> = 11)	3.59 $\pm$ 0.28***	0.96 $\pm$ 0.09	15.09 $\pm$ 2.79***
<i>H. pylori</i> IgG seropositive Cancer (Lys/Gln) ( <i>n</i> = 32)	3.83 $\pm$ 0.18***	1.09 $\pm$ 0.07	17.76 $\pm$ 1.70
<i>H. pylori</i> IgG seropositive Cancer (Gln/Gln) ( <i>n</i> = 11)	3.63 $\pm$ 0.22***	1.41 $\pm$ 0.14	18.02 $\pm$ 3.70
<i>XPD</i>			
<i>H. pylori</i> IgG seronegative Control (Lys/Lys) ( <i>n</i> = 9)	4.54 $\pm$ 0.21	1.40 $\pm$ 0.23	23.37 $\pm$ 4.79
<i>H. pylori</i> IgG seropositive Cancer (Lys/Lys) ( <i>n</i> = 19)	3.85 $\pm$ 0.25****	1.22 $\pm$ 0.11	15.95 $\pm$ 1.93****
<i>H. pylori</i> IgG seropositive Cancer (Lys/Gln) ( <i>n</i> = 26)	3.67 $\pm$ 0.17****	1.11 $\pm$ 0.08	18.58 $\pm$ 2.36
<i>H. pylori</i> IgG seropositive Cancer (Gln/Gln) ( <i>n</i> = 9)	3.70 $\pm$ 0.30****	0.99 $\pm$ 0.12	16.28 $\pm$ 2.07

\* *P* < 0.05; statistically significant, control versus cancer patients

\*\* *P* < 0.05; statistically significant. Comparison between wild type *OGG1* Ser326Cys controls and either heterozygous or homozygous Cys allele carrier gastric cancer patients

\*\*\* *P* < 0.05; statistically significant. Comparison between wild type *XPC* Lys939Gln controls and either heterozygous or homozygous Gln allele carrier gastric cancer patients

\*\*\*\* *P* < 0.05; statistically significant. Comparison between wild type *XPD* Lys751Gln controls and either heterozygous or homozygous Gln allele carrier gastric cancer patients

## Discussion

In studies investigating the association between increased gastric cancer risk and *OGG1* Ser326Cys, *XPD* Lys751Gln or *XPC* Lys939Gln polymorphisms in different ethnical groups, inconsistent results have been found [11, 20–25]. However, there is no study in the literature for gastric cancer and *OGG1* Ser326Cys, *XPD* Lys751Gln or *XPC* Lys939Gln polymorphisms, in Turkish population. In our study we did not find any association between *OGG1* Ser326Cys, *XPC* Lys939Gln or *XPD* Lys751Gln polymorphisms and susceptibility to gastric carcinoma. Our results supported by the studies done in different populations from Asia, Eastern Europe and South America. Takezaki et al. found that the proportional distribution of the Cys/Cys alleles in Chinese did not differ between

stomach cancer cases and controls [10]. Hanaoka et al. examined the distribution of the *OGG1* Ser326Cys polymorphism and its presumed correlation with gastric cancer risk in two case–control studies of different ethnic groups in São Paulo, Brazil. They concluded that the ethnic difference in *OGG1* Ser326Cys polymorphism was much greater than the case–control difference, and this polymorphism is unlikely to be associated with gastric cancer [11]. Long et al. showed an increased risk of gastric antrum adenocarcinoma in individuals who carry *XPD* Gln allele at codon 751. However, *XPC* Gln allele at codon 939 was not found to be associated with gastric cancer risk in the same Chinese study [26]. On the other hand, in another study, *XPD* Lys751Gln polymorphism did not show any relation with increased gastric carcinoma risk [27]. Finally Huang et al. studied selected DNA repair enzyme polymorphisms

in a population-based, case–control study, in Warsaw, Poland. Evaluating 281 incident gastric cancer cases and 390 controls, they did not find independent effects of *XPD*-Lys751Gln on gastric cancer risk, as well [24].

The oxidative stress represents one of the mutagenicity mechanisms in cancer. The serum TAS is a dynamic balance, which represents the total of variety of antioxidant molecules. However, we did not find any alteration in the TAS levels of the cancer patients compared to the controls. Also, we did not find any variation in the TAS of the cancer patients carrying neither homozygous Cys allele of *OGG1* nor homozygous or heterozygous Gln allele of *XPC* or Gln allele of *XPD*. The resultant balance in TAS might be attributed to the compensation of the oxidative stress via different antioxidant molecules in serum. In this respect, human serum albumin is claimed to represent the unique and major source of reactive thiol group in serum, particularly efficient in extracellular scavenging and antioxidant protection under increased ROS and RNS formation circumstances [28, 29]. The redox state of the Cys-34 on albumin plays an important role in ligand binding of this plasma protein. Reversible modification of the Cys-34 by NO and oxidative stress might play regulatory roles in the binding and transport of organic anions and heavy metals in the circulation [30]. In order to further evaluate the oxidative status of the patients, we measured the serum levels of NO and albumin.

The formation of nitrite and nitrate are important indicators of the biology of NO, which is a highly reactive free radical, and quickly turn into nitrite, nitrate, S-nitrosothiol or peroxynitrites. Nitrate may be considered as NO end product, while nitrite can still be considered as an active substrate. Kinetically, one of the primary sources of nitrite formation is the production of NO in the presence of ROS. Under oxidative conditions nitrite may be immediately oxidized further to form nitrate. This has been proposed as a mechanism to consume and control NO [31]. Nitrate is biologically inactive and does not participate in S-nitrosylation reactions of albumin [32]. Therefore, in our study we did not measure the amount of nitrate. The mechanism of formation and biological action of circulating S-nitrosoalbumin in humans are still poorly understood. NO reacts directly with thiols by a slow oxidative mechanism to form disulfides [33]. Reaction of NO with sulfhydryl groups of reduced thiol of peptides and proteins has been reported to lead in part to S-nitrosated compounds by nitrosation of sulfhydryl groups and in part to sulfenic acids by oxidation of sulfhydryl groups [34]. Sulfur-containing amino acid residues, namely cysteine and methionine residues are particularly sensitive to oxidation. Of the 35 cysteines of the human serum albumin molecule, 17 disulfide bonded cysteines are engaged, leaving only Cysteine-34 available for reactions. So the cysteine residue is

chiefly involved in free radical scavenging activity of human serum albumin [35]. Ultimately in this study, albumin levels of cancer patients were significantly lower than the controls and independent of the *OGG1*, *XPC* or *XPD* genotypes.

There is conflicting evidence about the role of NO in tumour development. In some studies NO has been shown to accelerate the tumour progression via increasing the angiogenesis and vasodilatation in tumour tissue [36]. Conversely, some researchers indicated an inverse association between NO synthesis and gastric cancer [37]. Recent studies showed enhanced NO expression in gastric cancer [38, 39]. Increased synthesis of NO in gastric cancer supports the immune system by extending the necrosis of tumour tissues. Whereas decrease of NO in tumour tissue might conduce the tumour cells escape from apoptosis and preserve their reproducing ability [38]. Most of our cancer patients were histopathologically in the higher grade and had significantly lower serum NO levels than controls. This decrease might be attributed to the declined tumoricidal activity or the downstream production of superoxide upon reaction with NO, thus the tumour cells might escape from apoptosis and these cells might continue to proliferate leading to bad prognosis. This assumption is supported by the fact that most of the gastric cancer cases are at late stage when they are diagnosed. However, we detected a non-significant decrease in serum NO levels of *OGG1* homozygous Cys allele carrier and *XPD* Gln allele carrier cancer patients. The decrease of NO in these genotypes may depend on the limited number of the patients.

Higher levels of oxidative DNA damage in the gastric mucosa during the early phase of *H. pylori* infection supports the hypothesis that the oxygen free radicals persistently produced in the gastric mucosa due to *H. pylori* infection are the driving force that transforms the chronic gastritis ultimately into gastric carcinoma. Mutation on the DNA in the stem cells induced by free radical damage could lead to intestinal metaplasia, dysplasia, and gastric carcinoma in the long term [40]. It was proposed that a modified nucleoside 8-Oxo-deoxyguanosine which is resulting from oxidative DNA damage, might affect the initiation step of *H. pylori*-related gastric carcinogenesis [41]. However, evidence showed that the gastric cancer risk did not happen to decrease despite of the *H. pylori* eradication in individuals with *H. pylori* seropositivity [42]. In our study, it was not clear whether the patients had a precancerous disorder like atrophic gastritis or intestinal metaplasia, thus the association between *H. pylori* and gastric cancer could not be demonstrated by histopathological examination. On the other hand, there was a significant decrease in both serum albumin and NO levels of *H. pylori* seropositive cancer patients compared to the seronegative control group. That result might be attributed

to the enhanced oxidative stress in *H. pylori* infected situation. However, a recent study from Turkey indicated that gastric cancer patients were characterized by increased protein oxidation, whereas there was no significant difference in oxidative stress parameters and antioxidant enzyme activity between the *H. pylori* seropositive and negative gastric cancer patients [43]. Likewise, we did not find any significant alteration in TAS levels of the *H. pylori* seropositive cancer patients compared to the seronegative controls. The NO concentrations of the *H. pylori* IgG seropositive gastric cancer patients who are either carrier of wild type *OGG1* Ser/Ser or *XPC* Lys/Lys or *XPB* Lys/Lys were significantly decreased compared to the wild type *OGG1* Ser/Ser or *XPC* Lys/Lys or *XPB* Lys/Lys *H. pylori* IgG seronegative controls, respectively. However, no statistical difference was found between the NO levels of heterozygous or homozygous variant allele carrier *H. pylori* IgG seropositive cancer patients compared to the wild type *H. pylori* IgG seropositive cancer patients.

Our study has some limitations. As a hospital-based case–control study, this study may be subject to potential selection bias. However, as our study was testing a genotype-driven hypothesis rather than an environment-driven hypothesis, selection bias is of less concern. These results are based on a limited number of SNPs and further larger scaled studies are needed to determine the interaction between genetical and environmental factors and the risk of gastric carcinoma.

Consequently, in our study, *OGG1* Ser326Cys, *XPC* Lys939Gln or *XPB* Lys751Gln polymorphisms have found not to be associated with gastric carcinoma. It might be stated that development of gastric cancer is a multistep disorder affected by oxidative stress and sophisticated interactions between genetical and environmental factors.

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