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Interleukin-8 gene polymorphism and susceptibility to gastric cancer in a brazilian population

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ABSTRACT

BACKGROUND: Studies have demonstrated that some polymorphisms in different interleukin genes may increase the risk of cancer. The aim of this study was to investigate the association between the *IL-8* (rs4073) –251A/T gene polymorphism and the risk of gastric cancer (GC). PATIENTS AND METHODS: A case-control study was conducted on patients with noncardia gastric cancer. DNA was extracted from leukocytes and the *IL-8* (rs4073) –251A/T polymorphism was analyzed by PCR-RFLP. Infection with *Helicobacter pylori* was investigated in the serum by ELISA. RESULTS: The sample consisted of 104 patients with GC and 196 controls. Cigarette smoking (P=0.007) and high fat intake (P=0.01) were more frequent in patients with GC. The proportion of patients infected with *H. pylori* was similar in the two groups (P=0.101). The frequency of the genotype A/T was higher in the cancer group (P=0.008). An increased risk of GC was found in subjects carrying the genotype A/T (OR=2.50, CI: 1.27-4.90), subjects with high fat intake (OR=1.92, CI: 1.17-3.15), and smokers (OR=2.00, CI: 1.20-3.31). CONCLUSIONS: Subjects with the heterozygous A/T genotype, high fat intake and smokers or ex-smokers presented an increased risk of GC. Individuals with A/A genotype may have protective effect for GC.

Key words: IL-8, polymorphism, gastric cancer.

INTRODUCTION

Gastric cancer (GC) is the fourth most common type of cancer in the world and the second leading cause of death due to cancer. In general, the incidence of GC is two to three times greater in developing countries and the frequency of this cancer is higher among men than among women (Matysiak-Budnika and Me´graudb, 2006; Ferlay et al., 2007; Wen and Moss 2009; INCA, 2010).

IL-8 is a chemokine produced by macrophages and other cells such as epithelial and endothelial cells (Wolff et al., 1998). This cytokine is involved in the inflammatory response to infection with *Helicobacter pylori*, recruits phagocytes and causes damage to the gastric mucosa by inducing the release of reactive oxygen radicals (Torok et al., 2005; Ohyauchi et al., 2005). Furthermore, studies have shown that IL-8 induces cell proliferation and angiogenesis (Karashima et al., 2003; Taguchi et al., 2005).

In the early 1990s, Clore et al., (1990) described the three-dimensional structure of IL-8. The locus in the human genome was identified on chromosome 4q21.1. In 2002, IL-8 was renamed CXCL8 by the Chemokine Nomenclature Subcommittee of the International Union of Immunological Societies, although the symbol IL-8 has remained (Bacon et al., 2002).

Several risk factors associated with GC have been identified, including *H. pylori* infection, dietary factors such as salt-preserved foods and dietary nitrite, as well as smoking and alcohol drinking habits (Ladeiras-Lopes et al., 2008; Joossens et al., 1996).

More than 50% of the world population is infected with this bacterium (Danesh, 1999). Most case-control and cohort studies have shown that the risk of patients with *H. pylori* infection for developing GC is increased from two to six fold (Eslick et al., 1999). Moreover, some of the trials on *H. pylori* eradication revealed that cure of its infection reduces the development of GC in high risk populations (Wong et al., 2004; Fukase et al., 2008). Accumulated evidence indicates that there are three steps in gastric carcinogenesis: *H. pylori* infection, development of gastric precancerous conditions and carcinogenesis (Hamajima et al., 2006). Severe gastric atrophy and corpus-predominant gastritis, intestinal metaplasia and dysplasia are well recognized as predominant predispositions to GC (Correa, 1988; Uemura et al., 2001).

Studies have indicated that activation of the transcription factor NF-κB has a dominant role in *H. pylori*-induced IL-8 production in gastric epithelial cells. NF-κB can be activated by phosphorylation via different signaling pathways leading to subsequent proteolytic degradation of IκB. Activated NF-κB translocates to the nucleus where it up-regulates *IL-8* gene transcription (Crabtree et al., 1995; Yamaoka et al, 2000).

Many investigators have reported associations between single nucleotide polymorphisms (SNPs) in genes that regulate the host's inflammatory response and GC, with sometimes conflicting results. The inflammatory response-related genes that have been most frequently studied in relation to GC are interleukin (IL) genes *IL1B, IL1RN, IL-8,* and *IL10*, coding for the proteins IL-1β, IL-1ra, IL-8, and IL-10, respectively. These cytokines are important mediators in gastric physiology and pathophysiology and could play important roles in the etiology of GC (Sharma et al., 1998). There is growing interest in identifying the association between IL-8 (rs4073) genotypes and the risk of GC (Ohyauchi et al., 2005; Taguchi et al., 2005; Sarvestani et al., 2006; Shirai et al., 2006 Persson et al., 2011).

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The aim of this study was to determine the frequency of the IL-8 (rs4073) promoter polymorphism (–251A/T) genotypes A/A, A/T and T/T in patients with GC and to correlate this polymorphism with *H. pylori* infection, consumption of red meat, fat and alcohol and smoking.

PATIENTS AND METHODS

A case-control study was conducted on patients of both genders with adenocarcinoma of the stomach aged > 18 years and treated at the Gastroenterology Division of Universidade Federal de Sao Paulo and Santa Casa de Misericordia de Sao Paulo, Brazil. Patients with cancer of the cardia or diffuse-type adenocarcinoma were excluded. The control group consisted of healthy subjects who attended the Central Laboratory of the hospital for blood collection. Patients with cancer were excluded from the control group. The study was approved by the Ethics Committee of the two hospitals and all patients agreed to participate in the study by signing an informed consent form.

All patients filled out a questionnaire on the frequency of food intake, including meat, fat, vegetables, and fruits. Habits such as smoking and alcohol consumption were also recorded and classified as 'never used' and 'current user'.

Serum anti-*H. pylori* IgG levels were measured by a commercially available kit (R-Biopharm GmbH, Germany) based on the enzyme-linked immunosorbent assay (ELISA) method.

Genotyping procedure: DNA was extracted from peripheral venous blood leukocytes collected with EDTA as anticoagulant using the Set Invisorb™ (Invisorb, Blood Spin Mini kit, Germany). The samples were submitted to the polymerase chain reaction (PCR) and subsequently genotyped by restriction fragment length polymorphism (RFLP) analysis. The following primers were used: forward primer: 5' – TTCTAACACCTGCCACTCTAG – 3' and reverse: 5' – CTGAAGCTCCACAATTTGGTG – 3'. PCR was performed in a final volume of 10 μL containing 40 ng DNA, 1x buffer reagent, 0.125 mmol/L of each deoxynucleotide triphosphate (dNTP), 1.5 mmol/L $MgCl_2$, 0.75 mmol/L of each primer, and 0.5 units Platinum Taq DNA polymerase. The PCR conditions were denaturation at 94 ºC for 4 min, followed by 35 cycles at 94 ºC for 30 s, 60 ºC for 30 s and 72 ºC for 30 s, with a final extension at 72 ºC for 7 min. After amplification, the PCR products were digested with 5 units of*Mfe*I for 12h at 37 ºC. The digestion products were separated by electrophoresis on 5% agarose gels stained with ethidium bromide (Taguchi et al., 2005). Genome sequencing was used to confirm the PCR and RFLP techniques using random samples of the two groups. The amplicons were purified bywith the Big Dye XTerminator Kit (Applied Biosystems) and sequenced in an ABI Prism 3100 sequencer (Applied Biosystems). The reverse primer was used for sequencing. The electropherogram was analyzed with the Sequence Scanner v1.0 program.

Statistical analysis:

The data were analyzed using Minitab (Version 15) (Minitab, State College, PA, USA). The Student t-test and χ^2 test were used for comparison and odds ratios (OR) and confidence intervals (CI) (95%) were calculated using the Cox regression model to examine the impact of the polymorphism on GC risk. Multivariate logistic regression was applied to identify risk factors. A P value < 0.05 was considered statistically significant.

RESULTS

All samples were submitted to genotyping by digestion of the amplicons by RFLP. Subjects carrying the homozygous T/T genotype showed a single band of 108 bp, A/A homozygotes had two bands of 76 and 32 bp, and subjects carrying the heterozygous A/T genotype presented three bands of 108, 76, and 32 bp as shown in Figure 1.

The case group consisted of 104 patients with GC, including 47 (45.2%) women. The control group consisted of 196 subjects, including 107 (54.6%) women. The patients of the case and control groups were admitted during the same period. There was no difference in gender (P=0.121) or age (P=0.117) between the groups. Lifestyle analysis showed no significant differences between the control and case groups in terms of the consumption of alcohol (P=0.391), fruits (P=0.809), vegetables (P=0.340), cereals (P=0.167), or red meat (P=0.499). There was a relationship between smoking and GC; nearly 61% of GC patients were current smokers plus former smokers (those who had smoked in some period during the past year), statistically different (P=0.007) than the control group (57.6%) who never smoked. The GC patients consumed significantly (P=0.005) more fat foods (48.1%) compared to the control group (31.6%). There was no statistically significant difference between GC and persons with *H. pylori* positivity IgG antibody, which was detected in 45.2% of the GC patients *vs*. 54.6% of the controls (P=0.101). The frequencies of the A/A, A/T and T/T genotypes were 26.5%, 43.4% and 30.1%, respectively, in the control group, and 14.4%, 55.8% and 29.8% in the case group. The genotypic distribution is in Hardy-Weinberg equilibrium in the control group (p>0.05). The frequency of the genotypes differed between the two groups (χ^2=6.654, P=0.036), and on the other hand, there was no statistical difference in allele frequency

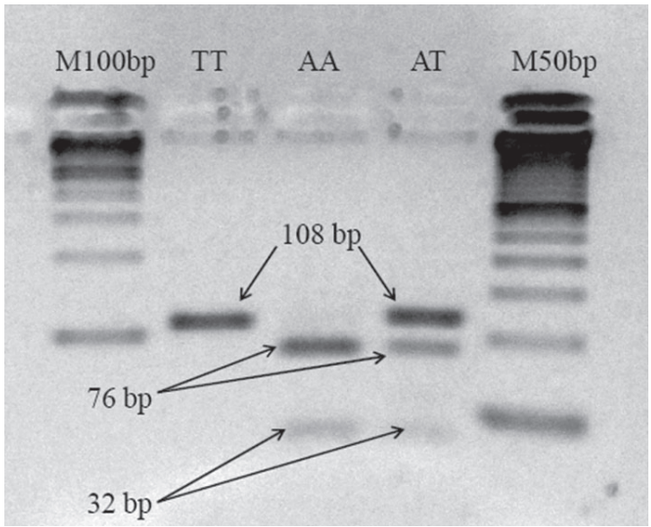

Figure 1. Subjects carrying the homozygous TT genotype showed a single band of 108 base pairs (bp), AA homozygotes had two bands of 76 and 32 bp, and subjects carrying the heterozygous AT genotype presented three bands of 108, 76, and 32 bp. DNA Molecular Weight Marker (100 bp ladder).

between groups (χ^2=1.677, *P*=0.631). Comparing GC patients with T/T genotype to the subgroup with A/T+A/A genotypes did not show a significant difference from the control group (*P*=0.866). However, comparing the sub group with A/A genotypes *vs*. A/T+T/T genotypes, there was a significant difference compared to the controls (*P*=0.003). The proportion of GC patients was significantly greater in the T carriers (A/T+T/T) than in the non–T carriers (A/A) (*P*=0.015). Although a positive association was observed among individuals for the A/T genotype and GC (OR = 2.50, CI 1.27-4.90, *P*=0.008), and among T/T genotype *vs*. A/A genotype and GC, the results were not statistically significant (*P*=0.106). Individuals with A/A genotype had a significantly lower risk of GC (OR = 0.45, CI 0.24-0.86, *P*=0.003) compared to (A/T+T/T) genotypes. The T allele was not statistically significantly associated with CG risk (*P*=0.631); however, T carriers was associated with an increased risk of GC (OR = 2.21, CI 1.17-4.20, *P*=0.015). These results remained consistent for IL-8 (rs4073) genotypes associated with GC in unadjusted and age-sex adjusted models, which are presented in Table 1.

In multivariate logistic regression analysis adjusted for age and sex, the following factors were associated with high risk of GC in Current + Ex-smokers (*P*=0.030; OR:1.79, 95% CI = 1.06-3.01), high-fat diet (*P*=0.047; OR:1.66, 95% CI = 1.02-2.95), and

TABLE 1

Characteristics of the study population. Odds ratios (OR) for gastric cancer according to Cigarette smoking, Fat intake, *H. pylori* infection, Genotype and Alleles frequencies, T Carriers using case-control data

Variable	Cases N = 104	Controls N = 196	P	OR* (95% CI)	P	OR** (95% CI)
Gender	N (%)	N (%)				
Male	57 (54.8)	89 (45.4)	0.121	-		-
Female	47 (45.2)	107 (54.6)				
Age						
Male	59.9 ±14.4	64.0 ±10.3	0.117#	-		-
Female	60.4 ±11.6	62.0 ±14.0				
Total	60.7 ±11.8	63.0 ±12.4				
Smoker						
Never	40 (38.5)	113 (57.6)		1.00 Reference		1.00 Reference
Current +Ex-Smokers	64 (61.5)	83 (42.4)	0.007	2.00 (1.20-3.31)	0.002	2.18 (1.34-3.54)
Fat Intake						
Low[1]	54 (51.9)	134 (68.4)		1.00 Reference		1.00 Reference
High[2]	50 (48.1)	62 (31.6)	0.010	1.92 (1.17-3.15)	0.005	2.00 (1.23-3.26)
H pylori infection						
Negative	57 (54.8)	89 (45.4)		1.00 Reference		1.00 Reference
Positive	47 (45.2)	107 (54.6)	0. 101	0.71 (0.44-1.15)	0.121	0.69 (0.43-1.11)
Genotypes						
A/A	15 (14.4)	52 (26.5)	0.036+	1.00 Reference		1.00 Reference
A/T	58 (55.8)	85 (43.4)	0.008	2.50 (1.27-4.90)	0.005	2.37 (1.22-4.60)
T/T	31 (29.8)	59 (30.1)	0.106	1.83 (0.88-3.79)	0.143	1.82 (0.89-3.74)
A Allele	88 (43.3)	189 (48.2)		1.00 Reference		1.00 Reference
T Allele	120 (57.7)	203 (51.8)	0.631	1.09 (0.76-1.56)	0.195	1.27 (0.90-1.78)
A/T + A/A	73 (70.2)	137 (69.9)		1.00 Reference		1.00 Reference
T/T	31 (29.8)	59 (30.1)	0.866	0.96 (0.56-1.62)	0.958	0.99 (0.59-1.66)
A/T + T/T	89 (85.6)	144 (73.5)		1.00 Reference		1.00 Reference
A/A	15 (14.4)	52 (26.5)	0.003	0.45 (0.24-0.86)	0.014	0.47 (0.25-0.88)
No T Carriers	15 (14.4)	52 (26.5)				
T Carriers	89 (85.6)	144 (73.5)	0.015	2.21 (1.17-4.20)	0.017	2.14 (1.14-4.03)

Values are means ± standard deviation for continuous variables and n (%) for categorical variables. Low[1]: almost never, 3-4 times/mo; [2]High: 3-4 times/wk, more than once/d. ***P*** for trend Pearson Chi-Square (c^2) test; #Two-Sample T-Test. *OR (Odds Ratios) and CI (Confidence interval) adjusted by age and sex. **Unadjusted; ***+P*** for Genotypes trend.

A/T genotype individuals (P=0.014; OR: 2.36, 95% CI = 1.19-4.67) as shown in Table 2.

Figure 2 shows the survival estimated by Kaplan-Meier analysis and comparison of curves by log-rank test; our study showed no difference between the probability of survival of GC patients and IL-8 (rs4073) polymorphism (P=0.038). CG patients with advanced stage were significantly associated (P=0.003) with decreased survival.

DISCUSSION

Gastric cancer is the fourth most common cancer and the second cause of death from cancer worldwide (Crew et al., 2006; Jemal et al., 2008). Data from the Brazilian National Cancer Institute (INCA) show that GC is more frequent among men (64.3%), with a higher incidence in patients over the age of 50 in both genders (INCA, 2010). A higher prevalence among men (54.8%) was also observed in the present study, with the mean age being 60.7 years in the two genders.

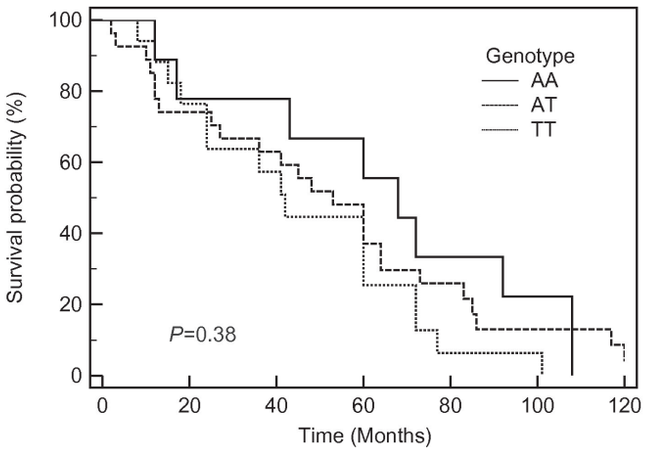

Figure 2. Kaplan-Meier survival plot for gastric cancer patients by interleukin-8 genotype. Survival was estimated Kaplan-Meier analysis and comparison of curves by long-rank test, our study showed no difference between the probability of survival of GC patients and IL-8 polymorphism.

Studying patients from Nagoya, Japan, Shirai et al. (2006) demonstrated an association between GC and infection with *H. pylori*, with a higher rate of infection being observed among patients with GC (91.1%) compared to those without cancer (58.8%). In the present study, no significant difference in the proportion of patients infected *H. pylori* was diagnosed (P=0.101). Similarly, Lu et al. found no increased frequency of patients infected with *H. pylori* in the cancer group (P=0.18) (Lu et al., 2005).

The occurrence of GC has also been associated with exposure to intrinsic (genetic) and extrinsic factors, such as the consumption of diets containing high concentrations of sodium chloride, nitrates and nitrites found in smoked foods, sausages and potato chips (Teixeira and Nogueira, 2003). In the present study, no significant differences in dietary habits (consumption of fruits, cereals, red meat, and vegetables) were observed for patients with GC. However, the consumption of fried foods and fats was significantly greater among cancer patients compared to subjects without cancer.

Epidemiological studies have demonstrated a relationship between nutrition and GC, with the observation of a protective effect of diets containing fresh vegetables and fruits, probably due to the presence of vitamin C and carotene which reduce the risk of cancer (Kono and Hirohata, 1996; Latorre, 1997). However, this relationship was not observed in the present study, possibly because of the difficulty in measuring the intake of these foods throughout life. A food frequency questionnaire on the consumption of certain foods and beverages was applied to the subjects to evaluate the relationship between diet and disease (Salvo and Gimeno, 2002). In a multicenter study involving 10 European countries, Buckland et al. (2010) found a significant reduction in the risk of GC in countries where adherence to the Mediterranean diet was greater.

The –251A/T polymorphism in the promoter region of the IL-8 (rs4073) gene has been the target of studies investigating which genetic profile is associated with a higher risk of or

TABLE 2

Multivariate logistic regression analysis stratified by the selected variables

Variable	Cases N = 104	Controls N = 196	*P*	OR* (95% CI)
Smoker				
Never	40 (38.5)	113 (57.6)		1.00 Reference
Smokers**	64 (61.5)	83 (42.4)	**0.030**	1.79 (1.06-3.01)
Fat Intake				
Low[1]	54 (51.9)	134 (68.4)		1.00 Reference
High[2]	50 (48.1)	62 (31.6)	**0.047**	1.66 (1.02-2.95)
Genotypes				
A/A	15 (14.4)	52 (26.5)		1.00 Reference
A/T	58 (55.8)	85 (43.4)	**0.014**	2.36 (1.19-4.67)
T/T	31 (29.8)	59 (30.1)	**0.130**	1.78 (0.84-3.74)

Low[1]: almost never, 3-4 times/mo; [2]High: 3-4 times/wk, more than once/d; ***P*** for trend; *OR (Odds Ratios) and CI (Confidence interval) adjusted by age and sex; **Current Smokers + Ex-Smokers.

protection against gastrointestinal disease. In a recent study, Song et al. (2010) found a significant association between the IL-8 (rs4073) polymorphism and cachexia in GC patients (OR=1.765, 95% CI: 1.192-2.615; *P*=0.004). However, the number of cases and controls was relatively small in that study. A Polish study reported no association between GC and the –251A/T polymorphism (Savage et al., 2006). However in another case-control study, Japanese investigators found no significant difference in genotype (*P*=0.95) or allele (*P*=0.93) frequencies of the IL-8 (rs4073) polymorphism (Shirai et al., 2006). Similar results have been also reported by Kamangar et al. (2006) in Finland (*P*=0.82) who also studied the *IL-8* (rs4073) polymorphism (OR=0.92, 95% CI: 0.42-2.00). Sarvestani et al. (2006) studied Iranian patients with GC, and observed a higher frequency of genotype T/T (*P*=0.04) and a marginal difference (*P*=0.07) in the frequency of allele T between patients (62%) and controls (56%). Similarly, Lee et al. (2005) found a higher frequency of genotype T/T in Chinese patients with GC (*P*=0.002), with 90% of these patients showing an increased risk of this malignancy (OR=1.93, 95% CI: 1.26-2.95). These authors also reported a significant difference (*P*=0.004) in T allele proportion between the cancer group (64.8%) and the control group (57.5%). No association between allele frequency and the risk of GC was observed in our study.

In contrast, a study conducted in Mexico showed that the *IL-8* (rs4073) –251A allele is a risk factor for the development of noncardia GC (Garza-Gonzalez et al., 2007). In China, Lu et al. (2005) observed a greater risk of GC in subjects with genotype A/A and *H. pylori* infection (OR=2.54, 95% CI: 1.38-4.72; *P*=0.012). In the study of Taguchi et al. (2005), the A/A genotype was found to be strongly associated with gastric carcinogenesis compared to genotype T/T in Japanese patients (OR=2.22, 95% CI: 1.08-4.56; *P*=0.03).

In the present study, the individuals with homozygous A/A genotype may have a protective effect on CG (OR=0.45, 95% CI: 0.24-0.86; *P*=0.003). The heterozygous A/T genotype increased more than twice the risk of GC (OR=2.50, 95% CI: 1.27-4.90; *P*=0.008). Similar results have been reported by Ohyauchi et al., (2005) who showed that this genotype was more frequent in patients with GC (OR=2.02, 95% CI: 1.37-2.97; *P*=0.0005).

Sarvestani et al. (2006) also compared the frequency of *IL-8* (rs4073) genotypes between patients with gastritis, peptic ulcer and GC and found a higher prevalence of the A/A genotype in patients with GC compared to those with benign diseases (*P*=0.013). These differences in genotypes may be due to differences in ethnic groups and disease etiology and/or sample size limitations.

We compared the *IL-8* (rs4073) polymorphism with survival time in 104 patients with GC. The median survival time was 68 months for patients with the *IL-8* (rs4073) –251A/A genotype, 53 months for patients with the A/T genotype, and 42 months for patients with the T/T genotype. The *IL-8* (rs4073) –251 genotypes were not associated with the prognosis of GC (*P*=0.38, log-rank test). By contrast, Lurje et al. (2010) found a significant result (P<0.001) between the probability of survival of GC patients and *IL-8* (rs4073) polymorphism, and they found the A/A genotype associated with increased survival. The limitation of the study was the relatively small sample size, which limits the generality of the results.

In conclusion, the presence of the A/T genotype was associated with an increased risk of GC in the population studied. Individuals with A/A genotype may have protective effect on GC. No association was observed between allele frequency and the risk of GC. Subjects consuming large amounts of fried foods and ex-smokers/smokers are at higher risk of GC.

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FOOTNOTES

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