Gastric cancer and detoxifying genes in a colombian population

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Abstract

The objective was to establish whether there are associations between gastric cancer (GC) and unfavorable polymorphisms in GSTM1, GSTP1 and GSTT1 detoxifying genes. Simultaneously interactions of smoking habits, alcohol consumption and socioeconomic levels were investigated as possible risk factors in a Colombian population with a high incidence of GC.

87 patients affected by GC and 87 controls from the same population group in the Department of Caldas participated in the research. All patients were genotyped by PCR for GSTM1-null, GSTP1-val and GSTT1-null polymorphisms. Information about tobacco smoking, alcohol consumption and socioeconomic levels was collected. Results suggest that the GSTM1-nul genotype and GSTP1-val allele are significant risk factors as are low and medium socioeconomic levels. Significant interaction between tobacco smoking, low socioeconomic levels and GC risks were also detected. To conclude, there is significant interaction between environment and genes, particularly between the GSTT1-nulle genotype and GSTP1-val allele and low socioeconomic levels, tobacco smoking and the risk of GC development within this Colombian region's population.

Keywords

Polymorphisms, *GSTP1*, *GSTT1*, *GSTM1*, tobacco smoking, socioeconomic level, gastric cancer.

INTRODUCTION

In the last 50 years, the incidence and mortality rates for gastric cancer (GC) have decreased significantly in industrialized countries (1). Nevertheless, this pathology is the second leading cause of cancer related death worldwide (2, 3). Incidence, prevalence and mortality rates of GC differ in different parts of the world, and even vary within different regions of the same country. In Colombia, high mortality rates are registered among both men and women (3), especially in the country's mountainous regions (4) where the Department of Caldas is located.

GC etiology is complex. Recent evidence suggests that environment-gene interactions confer susceptibility for developing this pathology (2). GC presents higher incidences among populations with low socioeconomic levels, and among those who live in high risk zones where several unfavorable factors converge. These factors include poor environmental quality, especially low quality water with high levels of nitrites and nitrates (5). Generally, these populations consume more carbohydrates and less protective food that modifies the gut flora to favor final nitrosamine production which affects the gastric mucosa (6). At the same time, other factors of lifestyle such as tobacco smoking (7) and alcohol consumption (8) are associated with risks of developing GC.

To counteract the injurious effects of environment and/or lifestyle, cells spontaneously conjugate different compounds with electrophile motifs which are potentially carcinogenic to the reduced glutathione (gamma-glutamylcysteine) prior to excretion from the body. But this conjugation is much more efficient when is catalyzed by the

glutathione transferases (GSTs) which are a group of enzymes coded for which several genes code (9). They are responsible for generating less toxic, and more water soluble, compounds which are excreted in urine or bile. Through this action, they decontaminate the cells and protect them from possible DNA, protein and/or lipid damage.

There are several forms of GSTs, but for detoxification the most important are the soluble or cytosolic forms (10). Various polymorphisms have been found in many soluble GST genes. Among them are unfavorable ones coding for the mu (M), pi (P) and teta (T) families of proteins which are involved in the risk of developing different kinds of cancer.

Glutathione S-transferase M1 (GSTM1) is expressed in the liver, stomach, brain and other tissues (11). Some of the substrates of this type of enzymes are oxidative stress products and activation derivatives of polycyclic aromatic hydrocarbons (PAHs) which are found in air, water, cigarette smoke, medicines and food (11). There are many polymorphisms of the gene that codes for GSTM1. One of them is *GSTM1*0* (null genotype) which does not express proteins because it is a homozygote from which both alleles have been deleted. Carriers of *GSTM1*0* genotype have detoxification deficiencies which have been associated with the risk of developing several malignant neoplasias including as GC (12-14).

Glutathione S-transferase P1 (GSTP1) is abundant in fetal tissues and is also expressed in most adult organs (15). These enzymes conjugate electrophile products with glutathione. These products result from phase I reactions or from oxidative stress (15). GSTP1 deficiency or inactivation increases DNA's oxidative vulnerability which can lead to tumoral transformation. Several polymorphisms of the GSTP1 gene can compromise exons five and six. The *GSTP1*A* (*Ile105/Ala*114) variant is considered to be a wild type. One of the other polymorphic variants, *GSTP1*B (Val105/Ala114),* has been associated with the risk of developing various different kinds of cancer, including GC (16).

Glutathione S-transferases T1 (GSTT1) are expressed constitutively and differentially in a wide variety of tissues. High levels are detected in the gastrointestinal tract, lungs, kidney, brain, skeletal muscle, heart, spleen and erythrocytes (17). These enzymes conjugate epoxides with glutathione, including propane and ethylene which are derived from components of cigarette smoke. They also conjugate other reactive metabolites including halogenated methane and ethane derivatives (18). Several polymorphisms of the GSTT1 gene have been identified. Individuals that present at least one functional allele are called GSTT1 positive and those that have a complete lack of any copy of the gene are *GSTT1*0* (null homozygote) genotype. They have no detectable enzymatic activity, and they present detoxifying deficiencies. As a result, this genotype has been associated with the risk of developing diverse neoplastic pathologies such as GC (20-24).

Given this evidence, polymorphic combinations of these detoxifying genes combined with unfavorable environmental factors are important modifiers of cancer risk.

This indicates the importance of evaluating this Colombian population group with high GC incidence. The study which follows evaluates, analyzes, and compares and contrasts cases and controls. It shows significant differences among the frequencies of genetic GSTM1, GSTP1 and GSTT1 polymorphisms. Simultaneously, it tests whether there are differences and/or interrelations associated with smoking habits, alcohol consumption and socioeconomic level.

MATERIALS AND METHODS

Study population

New cases of GC were studied for two consecutive years (January 2001-December 2002) at the Hospital de Caldas, the principal Hospital to which oncology patients are referred in this region. Out of 116 patients, 86 fulfilled the following inclusion criteria:

a. Most importantly, patients had to be affected by a gastric neoplasia, diagnosed for the first time, histopathologically confirmed, and without concomitant neoplasias.
b. The affected patient had to be part of the "paisa" community (parents and grandparents had to be part of the same population group).
c. Patients did not have clinical backgrounds of systemic diseases or chronic inflammations, such as asthma, arthritis or gastric ulcers.

The cases were paired with 87 unrelated controls randomly and progressively selected from visitors to the same hospital center. Controls and cancer patients were the same gender and age, from the same community and from the same ethnic group. Controls had no family medical history of cancer, systemic diseases or chronic inflammations.

Participants who fulfilled the inclusion criteria signed a written consent form approved by the Bioethics Committee of the University of Antioquia. Everyone was registered through a standardized questionnaire which included personal data, demographic variables and information about clinical history, habits and lifestyle related to smoking habits, alcohol consumption and socioeconomic level. Classification of socioeconomic levels was based upon the six Colombian *estratos* (Geographic categories used by the Departamento Nacional de 'Planeación (National Department of Planning) for determining public utility subsidies and surcharges according to economic

resources). Subsequently, three levels were defined: *estrato* 1 (the lowest socioeconomic level), medium *estratos* 2 and (medium socioeconomic levels), and *estratos* 4, 5 and 6 (highest socioeconomic levels) (Table 1).

Genotyping

The blood samples were collected with EDTA. The DNA was extracted using the "salting out" method (25). The methodology of Salama *et al* (26) was used to genotype *GSTM1*, while that of Dusinská *et al* was used to type *GSTP1* (27), and that of el-Zein *et al* (28) was used for *GSTT1*.

Statistical analysis

To evaluate the goodness of fit between the observed and expected frequencies the Chi-square test for deviation from the Hardy-Weinberg equilibrium was performed. We also used these tests to explore the associations between different factors and GC. The odds ratios were calculated step by step using logistic regression models. Then the significant variables were adjusted and included into the model. The association was estimated with 95% confidence intervals. The maximum level of significance for statistical analysis was set at 0.05. SPSS version 13 (Statistical Package for Social Sciences) was used for analysis.

RESULTS

A higher GC frequency was detected in the group of patients who were older than 60 years. In this group the intestinal subtype predominated (73.6%). The average age of these patients was 59.4 years old while the male to female ratio was 1.8 to 1.

A significant association was detected between smoking and the risk of developing GC (OR=1.5, 95% CI, 1.15 to

Table 1. Distribution of demographic variables for GC cases and controls.

Variables	Controls n (%)	Intestinal tumors n (%)	Diffuse tumors n (%)	Total GC n(%)	OR CI(95%)	p
Age groups						
< 41 years	9 (10,3)	5 (7,8)	3 (13,0)	8 (9,2)	1,0	
41-50 years	20 (23,0)	16 (25,0)	3 (13,0)	19 (21,8)	1,0(0,3-3,9)	0,90
51-60 years	17 (19,6)	12 (18,8)	4 (17,5)	16 (18,4)	1,0(0,3-4,0)	0,92
61 or more years	41 (47,1)	31 (48,4)	13 (56,5)	44 (50,6)	1,0(0,3-,4)	0,92
TOTAL	87	64 (73,6)	23 (26,4)	87		0,97
Average Age	57,7±13,8	58,7± 14,11	61,7±14,61	59,4±14,2		
Gender						
Female	32 (36,8)	24 (37,5)	7 (30,4)	31 (35,6)		
Male	55 (63,2)	40 (62,5)	16 (69,6)	56 (64,4)		
TOTAL	87	64	23	87		0,87
Tobacco Smoking						
Non-smokers	47 (54,0)	22 (34,4)	7 (30,4)	29 (33,3)	1,0	
Low (1-20 packs/year)	8 (9,2)	9 (14,0)	1 (4,4)	10 (11,5)	2,0(0,6-6,4)	0,17
Moderate (21-40 packs/yr)	21 (24,1)	13 (20,3)	8 (34,8)	21 (24,1)	1,6(0,7-3,7)	0,21
Excessive (>40 packs/yr)	11 (12,7)	20 (31,3)	7 (30,4)	27 (31,1)	4,0(1,7-9,2)	0,001
Total smokers	40(46,0)	42(65,6)	16(69,6)	58(66,7)	1,5(1,15-1,9)	0,002
Alcohol Consumption						
0 g/day	43 (49,4)	28 (43,8)	7 (30,4)	35 (40,2)	1,0	
Low (1-19 g/day)	30 (34,5)	27 (42,2)	11 (47,8)	38 (43,7)	0,61(0,1-2,6)	0,51
Moderate (20-39 g/day)	8 (9,2)	8 (12,5)	3 (13,0)	11 (12,6)	0,39(0,9-1,7)	0,21
Excessive (40+ g/ day)	6 (6,9)	1 (1,5)	2 (8,8)	3 (3,5)	0,36(0,6-1,9)	0,23
Total Alcohol consumers	44(50,6)	36(56,2)	16(69,6)	52(59,8)		0,35
Socio-economic Levels						
High (*estratos* 4,5,6)	28(32,2)	6(9,3)	0	6(6,9)	1,0	
Medium(*estratos* 2,3)	55(63,2)	44(68,8)	16(69,6)	60(69,0)	5,1(1,9-13,3)	0,002
Low (*estrato* 1)	4(4,6)	14(21,9)	7(30,4)	21(24,1)	24,5(6,1-97,9)	0,000
TOTAL	87	64	23	87		0,001

1.9, *p=0.002*). This risk increased as the quantity of cigarettes smoked increased (OR=4.0, 95% CI, 1.7-9.2, *p=0.001*) (Table 1).

We also found a significant association between low and middle socioeconomic levels and GC risks (Table1). However, no association with alcohol consumption was detected.

There was no association between the *GSTM1*0* genotype and GC risk *p=0.34)* (Table 2). Given the low frequency of homozygotes for the mutated allele (*val/val*) of *GSTP1*, all *GSTP1* cases were considered as one group (Including individuals with the homozygote allele (*val/val*) and the heterozygote allele (*ile/val*)). An association with GC risks was found when the adjusted OR was calculated for age, sex and tobacco smoking (OR=1.9) (Table 2). The risk increased slightly when the socioeconomic level was considered (OR=2.3). The *GSTT1*0* polymorphism was highly associated with GC risks (OR=2.5), and this risk increased when it was compared with the socioeconomic level (OR=2.8) (Table 2).

There was also an interaction between the unfavorable polymorphisms, tobacco smoking and socioeconomic level (table 2). The three unfavorable genotypes all interact with tobacco smoking. Their ORs varied from 3.0 for *GSTM1-null* to 4.9 for *GSTT1-null* (Table 3).

DISCUSSION

As in other Colombian population (29), intestinal GC predominated in this population group. This was particularly true in high risk zones where there is a noticeable environmental effect (5, 30). The average age of the cases studied, 59.4 years old, does not differ significantly from the world-wide population affected by GC which has averages in the range of 61 to 70 years old (31). It is also similar to the average age in other studies of Colombian populations (29, 32, 33). Cancer is most commonly diagnosed at this time of life. With aging, the physiological processes are not so efficient, the protective and repair mechanisms of gastric mucosa decline (34), and the injurious effects of environmental risk factors and unfavorable lifestyles become noticeable. All of these factors induce the development and progression of cancer (35).

In people affected by GC, the male population predominated over the female population with a 1.8 to1.0 proportion. This tendency was also found in the study by Adrada *et al* (29). It might be explained in part to the fact that men are more frequently exposed to injurious environmental factors than are women. These factors could be occupational factors or unfavorable lifestyles (tobacco smoking, alcohol consumption, diet, among others). In addition, men have more hepatic mass than women (36).

There is an association between low and medium socioeconomic levels with the risk of developing GC *p*=0.000 (Table 1). Comparing medium and high levels, a high socioeconomic level has a protective effect against developing GC (*p*=0.006,OR=0.14, 95% CI:0.03 to 0.06). It is possible that at this level there are protective environmental factors and healthier lifestyles. Low and medium

Table 2. Genotype distribution and GC risk.

Genotype	Controls n(%)	GC cases n(%)	Raw OR (CI-95%)/*P*	Adjusted OR* (CI-95%)/*P*	Adjusted OR ** (CI-95%)/*P*
GSTM1					
Present (normal)	59 (67,8)	53 (60,9)	1,0	1,0	1,0
Null (-/-)	28 (32,2)	34 (39,1)	1,4 (0,7-2,5)/0,34	1,3 (0,7-2,5) /0,42	1,5(0,8-3,0)/0,20
GSTP1					
ile/ile (normal)	55(63,2)	42(48,3)	1,0	1,0	1,0
ile/val	29(33,3)	36(41,4)	1,6(0,9-3,0)/0,132	1,8(0,9-4,0)/0,12	2,0(0,9-4,6)/0,1
val/val	3(3,4)	9(10,3)	3,9(1,001-15,4)/0,05	3,7(0,9-13,5)/0,24	2,2(0,4-12,6)/0,37
ile/val+ val/val	32(36,7)	45(51,7)	1,7(0,9-3,1)/0,07	1,9(1,03-3,7)/0,04	2,3((1,2-4,2)/0,016
GSTT1					
Present (normal)	68 (78,2)	53 (60,9)	1,0	1,0	1,0
Null (-/-)	19 (21,8)	34 (39,1)	2,1(1,04-4,1)/0,02	2,5(1,2-4,9)/0,011	2,8(1,3-5,7)/0,06
GSTM1+GSTT1+GSTP1					
Normal	30(34,5)	18(20,7)	1,0	1,0	1,0
Null+ ile/val+ val/val	57(65,5)	69(79,3)	2,0(1,02-3,98)/0,04	2,5(1,2-5,1)/0,014	3,0(1,4-6,5)/0,006

*Adjusted by age, gender and tobacco smoking.
** Adjusted by age, gender and tobacco smoking and socio-economic level.

Table 3. Genotypes and Tobacco smoking vs. CG.

Genotype	Smoke	Controls n (%)	Total GC		
			n (%)	OR (IC 95%)	P
GSTM1					
Present	NO	37(42,5)	25 (28,7)	1,0	
	SI	22(25,3)	28 (32,2)	1,9(0,9- 4,0)	0,099
Null	NO	18(20,7)	14 (16,1)	1,2(0,5-2,7)	0,749
	SI	10(11,5)	20 (23,0)	3,0(1,2-7,4)	0,018
GSTP1					
Ile/ile	NO	33(37,9)	21 (24,1)	1,0	
	SI	22(25,3)	21 (24,1)	1,5(0,7-3,7)	0,326
ile/val+val/val	NO	22(25,3)	18 (20,7)	1,3(0,6-3,0)	0,552
	SI	10(11,5)	27 (31,0)	4,2(1,7-10,5)	0,001
GSTT1					
Present	NO	43(49,5)	24(27,6)	1,0	
	SI	25(28,7)	29(33,3)	2,1(1,0-4,3)	0,049
Null	NO	12(13,8)	15(17,2)	2,2(0,9-5,6)	0,079
	SI	7 (8,0)	19 (21,8)	4,9 (1,8-13,2)	0,001

socioeconomic levels have a higher incidence of GC, especially intestinal gastric cancer (IGC). This population group has a less favorable lifestyle. It is common for them to cook with charcoal or firewood, to smoke tobacco and to consume alcohol. They live in areas with poor environmental quality and are constantly exposed to garbage, toxins, polluting agents, air pollution, bad water quality, noise, overcrowding, accommodation problems, limited educational facilities, inadequate work atmospheres and unhealthy conditions in their neighborhoods. In addition, low incomes among do not allow them to have healthier nutrition or opportune access to health care. These factors generate difficult health conditions. It is possible that accumulation of multiple and suboptimal physical conditions, rather than one single environmental event could explain how socioeconomic levels affect the health gradient (37).

Although other studies have shown no association between the smoking habit and GC risk (33), in the population we studied there was a significant association (OR=1.5) and in excessive smokers the risk was higher (OR=4.0) (Table 1). This is probably due to greater exposure genotoxic agents (38). The global risk to GC for smokers is on the order of 1.5 to 1.6 (39-40) times higher than it is for nonsmokers. It is estimated that the annual number of GC cases associated with cigarette smoking throughout the world is 80,000 (11%). This is a higher number than those estimated for other cancers associated with tobacco smoke such as pancreatic and renal cancer (39). It is known that cigarette smoke contains more than 4,700 chemical components, among which at least 60 are carcinogenic. There is evidence that some of them are involved in human gastric carcinogenesis. These include [α]pyrene, aromatic amines, nitrosamines, like 4-(methylnitrosamino)-1-(3 pyridyl)-1 butanone (NNK) and free radical generators (42). These could act by direct contact with the gastric mucosa or indirectly through the blood flow (42). In addition, clinical evidence indicates that cigarette smoke promotes the transition of precancerous gastric lesions to become cancerous lesions (41, 43). Risk increases with the intensity and the duration of the smoking habit (42). Other molecular evidence of the action of tobacco addiction are given by the increase of P450 enzyme activity (44) and by high DNA adduct levels of smokers affected by GC (45).

Alcohol consumption has been associated with different kinds of cancer including GC (8). However, in our study there was no association as in the study done by Zuleta *et al.* in 2009 (33). This is probably due to the infrequency of alcohol consumption among these patients.

Several studies have found association between the GSTM1-null genotype and GC risk (14, 38, 46), including in another Colombian population (32). Nevertheless, in this study similar to one of a Chinese population (20), no significant association was found between *GSTM1 null* and GC risk, p= 0.34 (Table 2). The contradictory results in different Colombian populations may be a product of different genotype frequencies and/or of exposure to diffe-

rent environmental factors which correspond to substrates of the GSTM1 enzyme. Patients with this genotype and a tobacco addiction were significantly associated with high GC risk (OR=3.0). In this case it was caused by a the greater genotoxic load contributed by the cigarette smoke.

GSPT1 gene polymorphisms have been associated with the risk of developing GC (16), but in this study we also found that the *GSTP1-val* gene polymorphism was associated with GC risk (OR=1.9). In addition, an interaction between the *GSTP1* genotype and the *val* allele and tobacco consumption increased the risk of developing GC by 4.2 times. Given that this enzyme has a wide specificity of endogenous and environmental substrates (47), it is possible that it participates in chemical detoxification and/or modulation of exposure to suboptimal concentrations of multiple unfavorable factors within the gastric mucosa which were not considered in this study but which are present in populations with low socioeconomic levels. Accumulation of these toxic factors could increase GC risk. Concomitantly, allele frequencies for *GSTP1-val*, did not show significant differences between the control group (0.20) and the group affected by GC (0.31) (Table 4). This might be due to exposure to different environmental factors.

Different studies (38, 48, 49), one of them of a Colombian population (32), have not shown associations between *GSTT1-null* genotype and GC risk. However, in this population, as in others (24, 50, 51), there is a significant association between this genotype and GC risk (OR= 2.8). If tobacco addiction is also considered the risk is even higher (OR= 4.9) (Table 3).

Frequencies for *GSTT1-null* allele differ significantly between cases (0.47) and controls (0.63) (Table 4). This suggests that this population may be exposed to a series of factors in which the GSTT1 enzyme plays an important role in detoxification. Consequently a deficiency of this enzyme could confer a risk for development of GC.

Homozygous null individuals do not have detectable enzyme activity. There seems to be a link between this genotype, smokers and oxidative DNA damage to pyrimidine bases. For this reason it is considered to be a risk factor for developing certain kinds of maladies (27). Possibly, *GSTT1-null* patients do not eliminate reactive metabolites generated efficiently by gastric cells. This could induce adduct formation that causes DNA degeneration and could trigger mutations in genes that control tumorigenesis. Discordant results between Colombian populations could be due to different frequencies of *GSTT1-null* and to different environmental factors.

An interaction was found between the unfavorable genotypes (*GSTM1-null, GSTP1-val and GSTT1-null*) and tobacco smoking with OR=2.5 and *p=0.014. T*his risk increases when the socioeconomic level is also studied (OR=3.0, *p=0.006*) (Table 2). These results indicate that besides tobacco smoking, there are other suboptimal exposures, exogenous or endogenous, which were not taken into account, but which are present.

Table 4. Allele and genotype frequencies for *GSTM1, GSTP1* and *GSTT1* polymorphisms.

Genotypes	Control frequency n (%)	Frequency of GC cases n (%)	Total frequency n (%)
GSTM1 gene			
Genotype			
Normal (+)	59 (0,678)	53 (0,609)	112 (0,64)
Null (-/-)	28 (0,322)	34 (0,391)	62 (0,36)
Alleles			
+	0,43	0,37	0,40 (IC 95%:0,33-0,47)
-	0,57	0,63	0,60 (IC 95%:0,53-0,67)
GSTP1 gene			
Genotype			
ile/ile	55 (0,632)	42 (0,483)	97 (0,557)
ile/val	29 (0,333)	36 (0,414)	65 (0,374)
val/val	3 (0,034)	9 (0,103)	12 (0,069)
Alleles			
ile	0,80	0,69	0,74(IC 95%:0,67-0,81)
val	0,20	0,31	0,26(IC 95%:0,19-0,33)
Gen GSTT1			
Genotype			
Normal (+)	68 (0,782)	53 (0,609)	121 (0,70)
Null (- / -)	19 (0,218)	34 (0,391)	53 (0,30)
Alleles			
+	0,53	0,37	0,45 (IC 95%:0,38-0,52)
-	0,47	0,63	0,55 (IC 95%:0,48-0,62)

CONCLUSIONS

It is possible that the population we studied may be vulnerable due to exposure to risk factors from early ages, because of genetic susceptibility due to unfavorable polymorphisms for detoxifying enzymes, or due to a combination of both factors. One or the other, or a combination of both of these possibilities, could trigger the gastric physiological alterations which lead to malignant neoplasias. Perhaps these are the factors that explain the presence of a such high percentages of cases between the ages of 41 and 60 years (40.2%) and which have made the Department of Caldas an epidemic zone for gastric cancer.

Environmental factors have been shown to have a very large impact, for which reason it would be very advisable to promote effective campaigns to efficiently improve the atmosphere and to foment healthy life styles which would decrease tobacco consumption. One important consequence would be decreased morbidity and mortality from gastric cancer. An exhaustive study with greater population samples must be made to confirm the results obtained in this study. Other genetic and environmental factors that might be GC risk factors in this population should be included in such a study. All this information could be very useful for establishing the impact of environmental GC risk factors and for identifying genetic modifiers of risk.

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Declaration of conflicts of interest

We do not have any conflicts of interest. We acted independently of the financial institutions involved in this project.

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