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ORIGINAL ARTICLE

Characterization of Gastric Cardia Tumors: Differences in *Helicobacter pylori* Strains and Genetic Polymorphisms

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Abstract
Background Gastric cancer results from a multifactorial process and is one of the most common causes of death worldwide. These tumors can arise in the distal stomach (non-cardia) and in the cardia region, presenting different characteristics and frequency of occurrence worldwide.
Aims To search for differences between tumors of different locations that could explain the presence of cardia tumors, considering *Helicobacter pylori* strains and genetic polymorphisms.
Materials and Methods DNA was extracted from gastric adenocarcinoma tissue of 127 patients. *Helicobacter pylori* genes were detected by PCR, and polymorphisms by PCR–RFLP.
Results Most of the tumors were located in non-cardia. The genotype 28152GA of *XRCC1* showed an increase in risk of cardia tumors. In analysis performed considering gender, women carrying *TNF*-308GA genotype showed a decreased risk of non-cardia tumors, while in men the decreased risk of non-cardia tumors was associated with *TNF*-308GG genotype. Genotypes combinations showed that the SNPs *RAD51* 135G>C, *XRCC3* 18067C>T, and *XRCC1* 28152G>A had some combinations more frequent in cardia tumors, with an increased risk. Patients infected by *cag*E-positive strains presented a positive correlation with non-cardia tumors.
Conclusion The results showed some susceptibility differences between tumors of different locations. There was an increased risk relationship between three repair enzyme SNPs and cardia tumors, and the G allele of the cytokine gene *TNF* negatively influenced the development of non-cardia tumors. *Helicobacter pylori* strains seemed to be different in the cardia region, where they were less virulent than those located in the distal region of the stomach.

Keywords Cardia tumors · *Helicobacter pylori* · Repair enzymes · Interleukins

Introduction

Gastric cancer is one of the most common causes of death worldwide. The prognosis of this disease is poor, particularly in Brazil, with a 5-year survival rate of 20–30 %, since most cases are diagnosed with advanced stage [1]. While *Helicobacter pylori* infection is a well-established cause of gastric cancer, different environmental and genetic factors (e.g., *H. pylori* virulence genes and single nucleotide polymorphisms (SNPs) in the host's genes) are involved in different stages of the cancer process [2]. Gastric tumors can arise in the distal stomach (non-cardia) and in the cardia region, and in the last four decades, there has been an increase in cases of cardia cancer, mainly in developed countries with remarkable geographic aggregation [3, 4]. These tumors seem to be different in some characteristics, and they are now recognized as two different clinical entities. However, what predisposes the

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gastric cancer development in a specific region of the stomach is still not clear [4]. Thus, the objective of this study was to search for differences between tumors of different locations that could explain the presence of cardia tumors, considering *H. pylori* strains and genetic polymorphisms of certain genes.

Materials and Methods

Subjects

The present study was approved by the Hospital Ethics Committee of the Federal University of Ceará, Brazil, and all subjects signed an informed consent form before inclusion. Adenocarcinoma samples included in this study were obtained from patients who had undergone gastrectomy at University Hospital Walter Cantídeo, Santa Casa de Misericórdia Hospital, and General Hospital located in Fortaleza (Ceará—Brazil). The histopathological data were obtained from histopathological records and reviewed by a pathologist of the team.

DNA Extraction

Genomic DNA was extracted from frozen tumor tissue using the cetyltrimethyl ammonium bromide (CTAB) technique, adapted from Foster and Twell [5]. DNA extraction was done only in fragments that showed more than 80 % tumor cells. DNA quality was analyzed by 1 % agarose gel electrophoresis, and quantity was determined using the NanoDrop® 3300 fluorospectrometer (Wilmington, DE, USA).

Detection of *H. pylori* Infection and *vac*A Alleles and the Presence of *cag*A, *cag*E, and *vir*B11 Genes

Helicobacter pylori infection was detected by amplification of the *ure*C gene using primers for PCR, as described by Lage et al. [6]. For the *H. pylori*-positive samples, the *vac*A alleles and *cag*A, *cag*E, and *virB*11 genes were identified by PCR according to Lima et al. [7] (Supplemental Table 1).

Genotyping of Host's Polymorphisms

The genetic polymorphisms of *RAD51* 135G>C (rs1801320), *XRCC3* 18067C>T (rs861539), *MLH1*-93G>A (rs1800734), *MGMT* 533A>G (rs2308327), *XRCC1* 28152G>A (rs25487), *XPD* 35931A>C (rs13181), *IL1B*-511C>T (rs16944), *IL1RN* (VNTR), *TNF*-308G>A (rs1800629), *IL6*-174G>C (rs1800795), and *IL8*-251A>T (rs4073) were detected by PCR–RFLP, as described in Supplemental Table 2.

Statistical Analyses

The statistical analyses were carried out using the programs SPSS® version 15.0 (Chicago, IL, USA), SNPStats, and UNPHASED® version 3.1.7 (London, UK). Statistically significant differences were evaluated by the Chi-square test (χ^2) or Fisher exact test. The results were considered statistically significant when p was less than 0.05.

Results

Study Population

Of a total of 127 collected gastric tumor samples, 30 (20.62 %) were located in the cardia region, and 97 (76.38 %) in non-cardia. The patients' median age was 65 years, independent of tumor location. Among the tumors located in the cardia region, there were a significantly higher proportion of males (Table 1).

SNP Analysis

A total of 11 SNPs were analyzed, individually and in groups. The genotype and allele distributions and risk analysis are shown in Table 2. Considering all cases, only the heterozygote genotype of *XRCC1* 28152G>A (Arg399Gln) showed an increase in risk of tumor located in the cardia region. This result was confirmed by SNPStats analysis, where this genotype was associated with a

Table 1 Characteristics of gastric cancer patients according to tumor location

	Cardia *N* (%)	Non-cardia *N* (%)	χ^2	*p*
Tumor location	30 (29.62)	97 (76.38)	–	–
Gender				
Male (65 %)	26 (20.5)	57 (44.9)		
Female (35 %)	4 (3.1)	40 (31.5)	7.88	0.005*
Age				
<60	6 (4.7)	32 (25.2)		
≥60	24 (18.9)	65 (51.2)	1.84	0.174
Tumor stage ($n = 124$)				
I	3 (2.4)	13 (10.5)	–	–
II	5 (4.1)	24 (19.3)	0.02	1.00
III	14 (11.3)	22 (17.7)	2.04	0.153
IV	7 (5.6)	36 (29.1)	0.05	1.00

Table 2 Genotype and allelic distribution of gastric cardia and non-cardia tumors

	Cardia		Non-cardia		χ^2	OR	*p*
RAD51 (G>C)							
GG	23	76.7 %	77	79.4 %	–	–	–
GC	4	13.3 %	13	13.4 %	0.00	1.03 (0.25–3.87)	1.00
CC	3	10.0 %	7	7.2 %	0.25	1.43 (0.27–6.92)	0.697
G allele	50	83.3 %	167	86.1 %			
C allele	10	16.7 %	27	13.9 %	0.28	1.24 (0.52–2.90)	0.597
XRCC3 (C>T)							
CC	19	63.3 %	51	52.6 %	–	–	–
CT	8	26.7 %	41	42.3 %	1.92	0.52 (0.19–1.43)	0.165
TT	3	10.0 %	5	5.2 %	0.38	1.61 (0.27–8.90)	0.680
C allele	46	76.7 %	143	73.7 %	–	–	–
T allele	14	23.3 %	51	26.3 %	0.21	0.85 (0.41–1.76)	0.646
MLH1 (G>C)							
GG	15	50.0 %	61	62.9 %	–	–	–
GC	14	46.7 %	27	27.8 %	2.97	2.11 (0.82–5.42)	0.085
CC	1	3.3 %	9	9.3 %	0.55	0.45 (0.02–4.03)	0.680
G allele	44	73.3 %	149	76.8 %	–	–	–
C allele	16	26.7 %	45	23.2 %	0.30	1.20 (0.59–2.45)	0.582
MGMT (A>G)							
AA	25	83.3 %	74	76.3 %	–	–	–
AG	4	13.3 %	15	15.5 %	0.15	0.79 (0.20–2.88)	1.00
GG	1	3.3 %	8	8.2 %	0.90	0.37 (0.02–3.19)	0.684
A allele	54	90.0 %	163	84.0 %	–	–	–
G allele	6	10.0 %	31	16.0 %	1.32	0.58 (0.21–1.57)	0.251
XRCC1 (G>A)							
GG	12	40.0 %	53	55.2 %	–	–	–
GA	16	53.3 %	28	29.2 %	4.41	2.52 (0.96–6.66)	0.035*
AA	2	6.7 %	15	15.6 %	0.43	0.59 (0.08–3.29)	0.723
G allele	40	66.7 %	134	69.8 %	–	–	–
A allele	20	33.3 %	58	30.2 %	0.21	1.16 (0.59–2.24)	0.647
XPD (A>C)							
AA	13	43.3 %	53	54.6 %	–	–	–
AC	10	33.3 %	34	35.1 %	0.15	1.20 (0.43–3.34)	0.701
CC	7	23.3 %	10	10.3 %	3.41	2.85 (0.79–10.32)	0.107
A allele	36	60.0 %	140	72.2 %	–	–	–
C allele	24	40.0 %	54	27.8 %	3.19	1.73 (0.90–3.30)	0.104
IL1B (-511C>T)							
CC	10	34.5 %	26	27.1 %	–	–	–
CT	12	41.4 %	54	56.3 %	1.27	0.58 (0.20–1.68)	0.260
TT	7	24.1 %	16	16.7 %	0.05	1.14 (0.31–4.15)	0.826
C allele	32	55.2 %	106	55.2 %	–	–	–
T allele	26	44.8 %	86	44.8 %	0.00	1.00 (0.53–1.88)	0.996
IL1RN							
L/L	9	31.0 %	35	36.5 %	–	–	–
L/2	17	58.6 %	56	58.3 %	0.13	1.18 (0.44–3.24)	0.721
2/2	3	10.3 %	5	5.2 %	1.11	2.33 (0.35–14.80)	0.366
L allele	35	60.3 %	126	65.6 %	–	–	–
2 allele	23	39.7 %	66	34.4 %	0.54	1.25 (0.66–2.39)	0.461

Table 2 continued

	Cardia		Non-cardia		χ^2	OR	*p*
TNF (-308G>A)							
GG	24	80.0 %	79	81.4 %			
GA	6	20.0 %	17	17.5 %	0.08	1.16 (0.36–3.61)	0.776
AA	0	0.0 %	1	1.0 %	0.30	0.00 (0.00–59.84)	1.00
G allele	54	90.0 %	175	90.2 %	–	–	–
A allele	6	10.0 %	19	9.8 %	0.00	1.02 (0.35–2.90)	0.962
IL-6 (-174G>C)							
GG	9	30.0 %	16	16.7 %	–	–	–
GC	11	36.7 %	41	42.7 %	1.94	0.48 (0.15–1.55)	0.164
CC	10	33.3 %	39	40.6 %	2.11	0.46 (0.14–1.51)	0.146
G allele	29	48.3 %	73	38.0 %	–	–	–
C allele	31	51.7 %	119	62.0 %	2.02	0.66 (0.35–1.22)	0.155
IL-8 (-251A>T)							
AA	6	20.0 %	14	14.4 %	–	–	–
AT	17	56.7 %	59	60.8 %	0.51	0.67 (0.20–2.32)	0.557
TT	7	23.3 %	24	24.7 %	0.35	0.68 (0.16–2.90)	0.552
A allele	29	48.3 %	87	44.8 %	–	–	–
T allele	31	51.7 %	107	55.2 %	0.22	0.87 (0.47–1.62)	0.635

decreased risk of tumors in the non-cardia region—in codominant [OR 0.40 (0.16–0.95), $p = 0.046$] and overdominant [OR 0.36 (0.16–0.84), $p = 0.017$] models. However, when taking gender into account, women carrying the *TNF-308*GA genotype showed a decreased risk of non-cardia tumors [OR 0.07 (0.01–0.78), $p = 0.016$], while in men, a decreased risk of non-cardia tumors was associated with the *TNF-308*GG genotype [OR 0.06 (0.01–0.47), $p = 0.016$].

The genotypes were also analyzed in combination. Among all possible genotype combinations, only the SNPs *RAD51* 135G>C, *XRCC3* 18067C>T, and *XRCC1* 28152G>A showed combinations with statistically significant association with an increased risk of cardia tumors ($p = 0.029$) (Table 3). Interestingly, among the significant combinations, the presence of the *RAD51* GG genotype was associated with at least three polymorphic alleles of the other two genes, while for the *RAD51* CC genotype, only one polymorphic allele of the other SNPs appeared to be required to establish the relationship of risk.

Helicobacter pylori Genotype

Almost all samples were *H. pylori* positive (117/127; 92.1 %). Of these, 64.9 % (76/117) were *cag*A+, 51.3 % (60/117) *cag*E+, 59 % (69/117) *vir*B11+, 85.5 % (100/117) *vac*A s1, 70.1 % (82/117) *vac*A m1, 66.6 % (78/117) *vac*A s1m1, and 11.1 % (13/117) *vac*A s2m2. The distribution of these genes according to tumor location showed that the *cag*E and *vir*B11 genes were significantly more frequent in tumors of the non-cardia region ($p = 0.020$ and 0.046, respectively), while other genes had the same distribution in tumors from both regions. Corroborating these data, a positive correlation was found between patients infected by *cag*E-positive *H. pylori* and non-cardia tumors ($r = 0.192$; $p = 0.030$).

Discussion

An increasing incidence of adenocarcinomas of the cardia has been observed in recent years, accompanying the increased incidence of adenocarcinomas at the esophagogastric junction mainly in the USA and Western Europe [4, 8]. Two pathways have been described for gastric cardia carcinogenesis, one associated with *H. pylori* atrophic gastritis, resembling non-cardia cancer, and the other associated with non-atrophic gastric mucosa, resembling esophageal adenocarcinoma. Also, cardia tumors are especially more frequent in males, as found in the present study [4, 23].

The relationship of genetic factors with tumors located in the cardia has not been widely explored, besides the finding that the risk of gastric tumors is generally associated with certain polymorphisms. In this study, considering the polymorphisms in DNA repair genes, a statistical difference between cardia and non-cardia tumors was related to the SNP *XRCC1* 28152G>A, in which the heterozygous genotype (GA) was associated with a risk of the cardia region and a risk reduction for non-cardia. *XRCC1* gene

Table 3 Genotype combination of SNPs *RAD51*, *XRCC3*, and *XRCC1* in patients with cardia and non-cardia tumors ($p = 0.029$)

RAD51 (G>C)	XRCC3 (C>T)	XRCC1 (G>A)	Cardia/non-cardia frequencies	OR
GG	CC	GG	0.2577/0.2333	1
GG	CC	GA	0.1237/0.1667	0.672 (0.1763–2.562)
GG	CC	AA	0.06186/0.03333	1.68 (0.1724–16.37)
GG	CT	GG	0.1443/0.06667	1.96 (0.3573–10.75)
GG	CT	GA	0.08247/0.1667	0.448 (0.1108–1.811)
GG	**CT**	**AA**	**0.07216/0**	**1.894e + 010 (1.245e + 010–2.88e + 010)**
GG	TT	GG	0.02062/0.06667	0.28 (0.03322–2.36)
GG	TT	GA	0.02062/0.03333	0.56 (0.04405–7.119)
GG	**TT**	**AA**	**0.01031/0**	**5.846e + 007 (2.152e + 007–1.588e + 008)**
GC	**CC**	**GG**	**0.03093/0**	**3.156e + 009 (1.734e + 009–5.744e + 009)**
GC	CC	GA	0.01031/0.1	0.09333 (0.008354–1.043)
GC	**CC**	**AA**	**0.01031/0**	**1.173e + 008 (4.317e + 007–3.186e + 008)**
GC	CT	GG	0.03535/0.03333	0.96 (0.08716–10.57)
GC	**CT**	**GA**	**0.04713/0**	**3.779e + 010 (2.267e + 010–6.302e + 010)**
CC	**CC**	**GG**	**0.03093/0**	**2.663e + 009 (1.463e + 009–4.847e + 009)**
CC	CC	GA	0/0.06667	0
CC	CC	AA	0/0.03333	0
CC	**CT**	**GG**	**0.03093/0**	**3.156e + 009 (1.734e + 009–5.744e + 009)**
CC	**CT**	**GA**	**0.01031/0**	**1.777e + 008 (6.54e + 007–4.827e + 008)**

Bold informations are statistically significant ($p = 0.029$)

encodes the key protein of the base excision repair pathway, and the SNP 28152G>A causes an amino acid change that alters the efficiency of the repair process [9, 10]. One possible explanation for this association is that this genotype (GA) leads to a less efficient repair favoring the development of adenocarcinoma in the cardia even at a low rate of *H. pylori* infection.

The literature is contradictory about the association of *XRCC1* 28152G>A polymorphism with gastric cardia tumor risk. Shen et al. [11] found a borderline association with the risk of the genotypes *XRCC1* 28152 GA/AA, but the association was significant when combined with the CC genotype of the *XRCC1* (*26304C>T*) SNP. Also, Miao et al. [12] found risk associated with *XRCC1* allele A. Besides these two studies with a Chinese population showing a relative risk associated with the A allele, other studies with a Chinese population or other ethnicities found no association [13–16].

In our study, only the genotype combinations of *RAD51*, *XRCC3*, and *XRCC1 SNPs* were associated with increased risk of cardia tumors. The SNP that more strongly established associations with such risk in combination with others was *RAD51* 135G>C. Although previous studies have not established a relationship between this SNP and cardiac gastric cancer, this polymorphism seems to be associated with gastric cancer in general and esophageal squamous cell carcinoma (ESCC) [17, 18]. Gastric cardia adenocarcinoma and ESCC have many similarities (etiologic determinants and anatomical proximity), which argues for similar etiology for the two tumors [19]. RAD51 has been demonstrated to be involved in homologous recombination repair of double-stranded DNA breaks, which may cause genomic instability and cancer [20]. Furthermore, it has been demonstrated that the XRCC3–RAD51 interaction is biologically relevant, since XRCC3 protein promotes the assembly or stabilizes a multimeric form of RAD51 required for DNA repair [21].

With regard to cytokine polymorphisms, we found that the GG and GA genotypes of *TNF*-308G>A were less frequent in patients with tumors located in the non-cardia region. These genotypes are associated with a normal and intermediate inflammatory response, respectively [22]. Since tumors in the non-cardia region are associated with infection by *H. pylori* [23], the inflammation caused by this bacterium does not get worse and appears to decrease the risk of cancer development in this region. Polymorphisms of interleukins have been widely studied in gastric cancer, but few studies classify their data according to tumor location.

Data from the literature show that non-cardia gastric tumors have a strong positive association with *H. pylori* infection, whereas the cancer of the cardia does not have an established relationship with the presence of this microorganism [2]. In our study, the *cag*E and *vir*B11 genes were

more frequent in non-cardia tumors and *cag*E was positively related to them. Similar results were obtained in a previous study by our group, where *cag*E-/*vir*B11-negative *H. pylori* strains showed a slight increase in the incidence of gastric cardia tumors [24]. Therefore, the relationship between *H. pylori* infection and tumor development in non-cardia region seems to be more influenced by the virulence status of the strain than by the presence of bacteria. Thus, another carcinogenic pathway appears to act in the development of tumors of the cardia region.

In conclusion, this study indicates that there are some susceptibility differences between tumors of different locations. Patients with *XRCC1* 28152G>A (Arg399Gln) have an increased risk of cardia tumors, also associated with some genotypes of the repair enzyme SNPs *RAD51* 135G>C and *XRCC3* 18067C>T. The G allele of the cytokine *TNF*-308G>A may negatively influence the development of non-cardia tumors. Furthermore, *H. pylori* strains seem to be different in the cardia region, where they are less virulent than those located in the distal region of the stomach.

Conflict of interest None.

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