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World J Gastroenterol 2006 September 14; 12(34): 5465-5472
World Journal of Gastroenterology ISSN 1007-9327

H pylori status and angiogenesis factors in human gastric carcinoma

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Received: 2004-12-21 Accepted: 2005-02-21

Abstract

AIM: To investigate *H pylori* expression in gastric cancer patients in relation to primary tumor angiogenic markers, such as microvessel density (MVD), thymidine phosphorylase (TP), vascular endothelial growth factor receptor-1 (VEGF-R1), p53 and circulating VEGF levels.

METHODS: Angiogenic markers were analyzed immunohistochemically in 56 primary gastric cancers. *H pylori* cytotoxin (*vac*A) and the cytotoxin-associated gene (*cag*A) amplification were evaluated using PCR assay. Serum *H pylori* IgG antibodies and serum/plasma circulating VEGF levels were detected in 39 and 38 patients by ELISA, respectively.

RESULTS: A total of 69% of patients were positive for circulating IgG antibodies against *H pylori*. *cag*A-positive *H pylori* strains were found in 41% of gastric patients. *vac*A was found in 50% of patients; s1 strains were more highly expressed among *vac*A-positive patients. The presence of the s1 strain was significantly associated with *cag*A (P = 0.0001). MVD was significantly correlated with both tumor VEGF expression (r = 0.361, P = 0.009) and serum VEGF levels (r = -0.347, P = 0.041). Conversely, neither VEGF-R1 expression nor MVD was related to p53 expression. However, *H pylori* was not related to any angiogenic markers except for the plasma VEGF level (P = 0.026).

CONCLUSION: *H pylori* antigen is related to higher plasma VEGF levels, but not to angiogenic characteristics. It can be hypothesized that the toxic effects of *H pylori* on angiogenesis occurs in early preclinical disease phase or in long-lasting aggressive infections, but only when high *H pylori* IgG levels are persistent.

Key words: *H pylori*; Gastric carcinoma; Angiogenesis

Mangia A, Chiriatti A, Ranieri G, Abbate I, Coviello M, Simone G, Zito FA, Montemurro S, Rucci A, Di Leo A, Tommasi S, Berloco P, Xu JM, Paradiso A. *H pylori* status and angiogenesis factors in human gastric carcinoma. *World J Gastroenterol* 2006; 12(34): 5465-5472

http://www.wjgnet.com/1007-9327/12/5465.asp

INTRODUCTION

H pylori infection is a well-known risk factor for the development of pre-neoplastic and neoplastic gastric mucosal alterations[1,2]. An increase in proliferative activity of gastric epithelial cells without a corresponding increase in apoptosis has been implicated in *H pylori* gastric carcinogenesis[3,4]. In addition, specific virulence determinants of *H pylori* strains can influence the outcome of the infection. Urease, vacuolating cytotoxin *vac*A, and the pathogenicity island (*cag* PAI) gene products are the main virulence factors of this organism involved in the development of gastric carcinoma. Thus, individuals infected with strains that express these virulence factors are prone to develop severe local inflammation which may induce the development of peptic ulcers and gastric cancers. Also, *H pylori* activity may be associated with virulence; in fact, urease activity may be an important colonization factor and exert a direct toxic effect upon intercellular junctions, resulting in alteration of gastric mucosal permeability[5]. The subsequent passage toward cancer is probably prompted by other factors, such as the onset of infection or other agents independent of *H pylori*.

Several studies have suggested that angiogenesis might also contribute to gastric tumorigenesis[6-8]. Angiogenesis is a complex multistep cascade modulated by positive soluble factors, such as the vascular endothelial growth factor (VEGF). The tumor neo-angiogenesis has been demonstrated in almost all solid tumors using various morphological techniques. The current method of angiogenesis quantification is the evaluation of CD34 antigen expression, a cell surface glycoprotein also present in the vascular endothelium permitting the study of intratumor endothelial cells[9]. The cellular receptor for VEGF, VEGF-R1 or Flt-1, is highly expressed in gastric carcinoma cells, suggesting that this pathway could influence tumor growth and metastasis through paracrine and autocrine mechanisms[10]. An additional tissue factor is thymidine phosphorylase (TP), an enzyme involved in pyrimidine nucleoside metabolism, which is identical to the platelet-derived endothelial cell growth factor and is endowed with angiogenic activities in various solid tumors[11] Furthermore, the *p53* oncosuppressor gene has been reported to be involved in inhibition of tumor vascularization by fostering unopposed angiopoietin-2 activity[12].

Recent publications have suggested that *H pylori* infection may regulate the angiogenesis and invasion of gastric carcinoma. In fact, *H pylori* influences *in vitro* angiogenesis-related gene expression; in particular, it has been demonstrated to up-regulate VEGF expression in gastric epithelial cells, an effect which appears to be related to *vac*A-expression[13,14]. Moreover, *H pylori* has been shown to up-regulate the expression of epidermal growth factor (EGF)-related growth factors and COX-2 in *in vitro* human gastric epithelial cells as well as in human gastric mucosa *in vivo*[15,16]. Lastly, its relationship with p53, which has been described as an angiogenesis-related factor, has been documented[17-20]. In spite of these evidences originating from *in vitro* studies, suggesting a relationship between pathophysiological roles for *H pylori* in the induction of tumor neo-angiogenesis**,** to our best of knowledge, no data are available in literature in patient series. Our hypothesis was that *H pylori*-related gastric cancer could involve different neo-angiogenic characteristics with respect to tumors without bacterial infection.

To verify the association between *H pylori* infection and different angiogenesis-related characteristics, 56 gastric cancer patients were studied for microvessel density (MVD), thymidine phosphorylase (TP), vascular endothelial growth factor-receptor (VEGF-R1) and p53 expressions in addition to circulating serum and plasma VEGF levels. *H pylori* was investigated at the molecular and at circulating blood levels.

MATERIALS AND METHODS

Patients

Fifty-six patients (37 men and 19 women; median age 64 years, range 42-83 years) with T_{1-4} N_{0-1} M_{0-1} gastric carcinoma were enrolled in this study. All patients had primary surgery for gastric cancer at National Cancer Institute of Bari. Primary tumor tissues were utilized for the immunohistochemical analysis of MVD, p53, VEGF-R1 and TP expressions.

Table 1 Clinicopathological features and distribution of *cagA*, *vacA* and IgG anti-*H pylori* in a series of 56 gastric cancer patients

Clinicopathological features	*n*
Sex	
Male	37
Female	19
Tumour category	
pT_{1-2-3}	28
pT_4	28
Location	
Antrum	23
Other	33
IgG anti *H pylori* (ELISA)	39
IgG - (≤ 7 KU/L)	12
IgG + (> 7 KU/L)	27
cagA (PCR)	56
cagA -	32
cagA +	23
NE	1
vacA (PCR)	56
NEG	28
s1m1	10
s2m2	5
s1m2	9
s1m1/s1m2[a]	1
NE	3

NEG: Negative; NE: Not evaluable; [a] Multiple genome.

Formalin-fixed and paraffin-embedded specimen of the primary tumor was selected by the pathologist for each patient on the basis of the quality of morphological preservation and neoplastic cellularity. In accordance with standardized sampling protocols, the sample was comprehensive both at the deeper portions of tumor, as well as the edges of the lesions. Five-micrometer thick sections were cut for immunohistochemical assay and for determination of *H pylori* status by means of polymerase chain reaction (PCR). A section contiguous to those selected for immunohistochemistry and DNA extraction was always stained with haematoxylin and eosin and confirmed by the pathologist as rich in neoplastic cellularity. Enzyme-linked immuno-sorbent assay (ELISA) for IgG antibodies against *H pylori* was performed on blood samples from 39 patients. Circulating VEGF levels were detected by ELISA in serum and plasma of 38 patients. The patients characteristics are shown in Table 1.

DNA extraction and PCR analysis

DNA extraction from paraffin-embedded specimens was performed using the method described by Lin *et al*[21]. Briefly, samples were incubated with a lysis buffer and proteinase K for 3 h at 55℃. Total DNA was extracted with phenol/chloroform, precipitated with acidic ethanol, and dissolved in sterile water.

Amplification of cagA and vacA

The extracted DNA was subjected to PCR for detection of *H pylori* genes, *cagA* and *vac*A. The *cag*A gene was amplified using the primers described elsewhere[22,23]. The *vac*A gene

was amplified using primers described by Atherton *et al*[24] which evaluate the mid region (*m*) and the region encoding for the signal peptide (s) of the gene. Four different PCR products were obtained: *s1* or *s2* from the *s* region, and *m1* and *m2* from the *m* region. PCR products were analyzed by electrophoresis on 20 g/L agarose gel. Positive and negative controls were examined with each batch of PCR.

Detection of anti-H pylori IgG

An enzyme-linked immuno-sorbent assay was used to detect *H pylori*-specific IgG serum antibodies (Anti-*H pylori* EIA Quant- COBAS2- Roche Diagnostics). The anti-*H pylori* IgG EIA is a second-generation two-step EIA for the detection of IgG antibodies to *H pylori* in human serum, based on a set of fast protein liquid chromatography-purified cell surface antigens, including the native urease enzyme[25]. According to the manufacturer, patients were considered positive for IgG against *H pylori* when IgG value was higher than 7 U/mL.

Immunohistochemistry

Serial sections of paraffin-embedded gastric tissue were deparaffinized and rehydrated. For antigen retrieval, the sections were microwaved at 500 W for 10 min in citrate buffer (pH 6) and endogenous peroxidase activity was blocked with 30 mL/L hydrogen peroxide solution. Adjacent slides were incubated with different monoclonal antibodies. The bound antibody was visualized using a biotinylated secondary antibody, avidin-biotin peroxidase complex, and 3-amino-9-ethylcarbazole (Ultra Vision Detection System anti-Polyvalent, HRP/DAB, Lab Vision Corporation). For negative control sections, primary antibody was replaced with phosphate-buffered saline and processed in the same manner. Gastric carcinomas known to express high levels of CD34, p53, TP and VEGF-R1 proteins were used as positive controls.

Anti-CD34 (QB–END/10, Novocastra Laboratories Ltd) was diluted at 1:50 for 1 h at room temperature as a pan-endothelial marker for MVD analysis. The modified Weidner's method was utilized for the evaluation of MVD according to CD34 endothelial cell immunostaining[26]. For the microvessel counting, positive stainings for MVD, in five most highly vascularized areas ('hot spots') in each slide, were counted in 400 × fields with an image analysis system (Quantimet 500 Leica; 0.19 mm^2/field) and MVD was expressed as the average of the microvessel count in these areas[27]. Any EC or endothelial cluster positive for CD34 (brown yellow staining) was considered to be a single countable microvessel. Sclerotic areas, both hypocellular and necrotic, within the tumor were not considered for vessel evaluation.

Anti-p53 monoclonal antibodies (PAb 1801, Neo-Markers), grown against human p53 and recognizing wild-type and mutant forms of the p53 protein, were diluted at 1:150 for 1 h at room temperature. Tumor cells expressing p53 immunoreactivity were quantified by evaluating a total of 1000 neoplastic cells in random fields from representative areas. Exclusive nuclear staining was scored as positive. The immunoreactive cells were expressed as percentages[28]. Anti-TP monoclonal antibodies (P-GF.44C Neo-Markers), recognizing full length human TP protein, were diluted at 1:100 for 1 h at room temperature. TP positivity was determined at 400 × fields with the image analysis system and was evaluated on the basis of percentage of stained epithelial tumor cells. Tumor cells with moderate or strong staining intensity were counted. TP expression in macrophages was considered an internal positive control. The polyclonal antibody anti-VEGF-R1 (Flt-1 polyclonal rabbit antibody, Santa Cruz Biotechnology Inc.), recognizing the carboxyl terminus of the receptor for VEGF, VEGF-R1 or the Flt-1 protein of human origin, was used at a dilution of 1:100 for 1 h at room temperature. VEGF-R1 positivity was scored as cytoplasmic immunostaining using an image analysis system (Quantimet 500 Leica). Immunoreactivity was expressed as the percentage ratio between the area of immuno-positive tumor cells and the entire area of invasive neoplastic tissue.

The laboratory, where the immunohistochemical analyses were performed, participated to Quality Control programs managed by INQAT[29].

Circulating VEGF detection

Blood samples were collected before surgery. Venous blood was dispensed into a serum separator tube (Becton Dickinson Vacutainer Systems) for serum obtainment, and into sodium citrate, theophylline, adenosine, dipyridamole (CTAD) tubes for plasma (Becton Dickinson Hemogard Vacutainer Systems).

Circulating VEGF levels were examined in plasma and serum using the Quantikine Human VEGF-enzyme-linked immuno-sorbent assay (ELISA, R&D System Inc.) which recognizes VEGF165. According to the manufacturer, the minimum detectable dose of VEGF is typically less than 9.0 ng/L. Values below 9.0 ng/L were equal to zero. VEGF levels in plasma and serum were analyzed, as we previously demonstrated that the two determinations provide alternative and additional information on circulating VEGF, also in relation to the role played by the activation and quantity of platelets in VEGF release[30].

Statistical analysis

The associations between MVD, TP, VEGF-R1 and p53 expression, markers of *H pylori* status and histological diagnosis were evaluated using the Chi-square test. A correlation analysis among the aforementioned biomarkers, considered as continuous variables, was performed by Pearson's correlation coefficient (*r*). In the statistical analysis, *vac*A genotypes were classified into two subgroups: "cytotoxic strains" which included s1m1, s1m2 and s1m1/s1m2 strains and "others" which included negative and s2m2. Patients with s1m1/s1m2 strains were infected by multiple genotypes. Backwards stepwise logistic regression analysis was used to estimate the independent association of any biological markers with *H pylori* characteristics. Statistical analysis was carried out using the software SPSS for windows, release 9.0.

RESULTS

H pylori status

Cytotoxin-associated gene (*cag*A)-positive *H pylori* strains

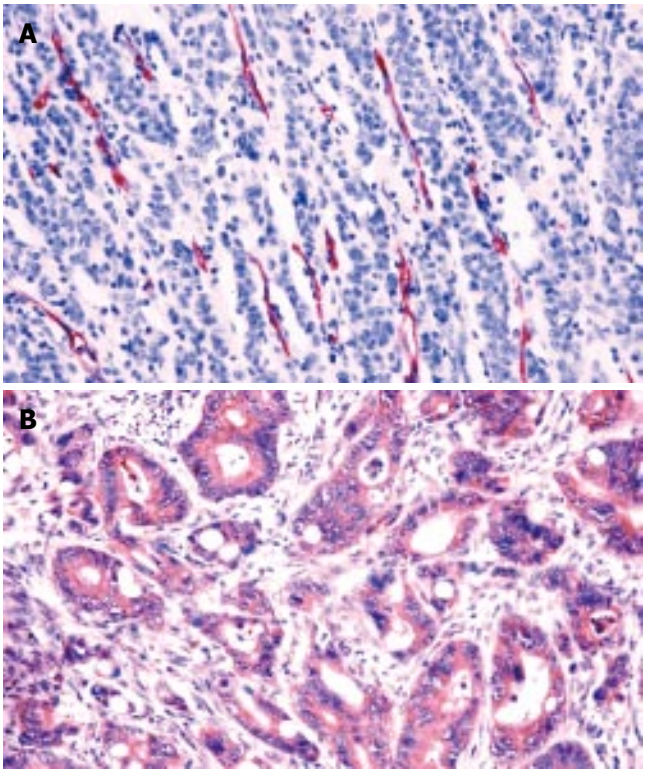

Figure 1 Immunohistochemical assay for detection of microvessel density (**A**) and VEGF-R1 protein expression (**B**) in gastric cancer. VEGF-R1 is stained in the cytoplasm of cancer cells.

were found in 41% of gastric patients. *vac*A was found in 50% of patients; s1 strains were more highly expressed among *vac*A-positive patients. Moreover, a single patient was found to be infected with different *H pylori* strains (multiple genomes). The results are summarized in Table 1. The presence of the s1 strain was significantly associated with c*agA* (P = 0.0001). Only one patient infected with the s2 strain showed *cag*A-positivity, while 26 (81%) patients were negative for both *cag*A and *vac*A. A total of 69% of patients were positive for circulating IgG antibodies against *H pylori*, with a mean and median value of 77 U/mL (range 1-476 U/mL) and 15 U/mL, respectively.

Immunohistochemical analysis

TP immunoreactivity was observed in normal epithelial cells, malignant epithelial cells, macrophages and endothelial cells. The usual pattern of positive staining was both cytoplasmic and nuclear. A mean of 5% (range 0%-80%) of cells showed TP positivity.

p53 expression was generally confined to neoplastic tissues, while the normal mucosa was rarely stained. A mean of 3% (range 0%-50%) of cells showed p53 positivity.

CD34 immunostaining was detected in the endothelial cells, especially in the area surrounding the tumor (Figure 1A). In the 'hot spot' tumor area, a mean of 39 vessels (range 0-100 vessels) was found with only 2% of cases not demonstrating any microvessels. VEGF-R1 immunostaining was mainly localized at the membrane and cytoplasm of epithelial and endothelial cells (Figure 1B). By counting only the epithelial component, a mean of 24% (range 0%-100%) of VEGF-R1-immunostained cells was found.

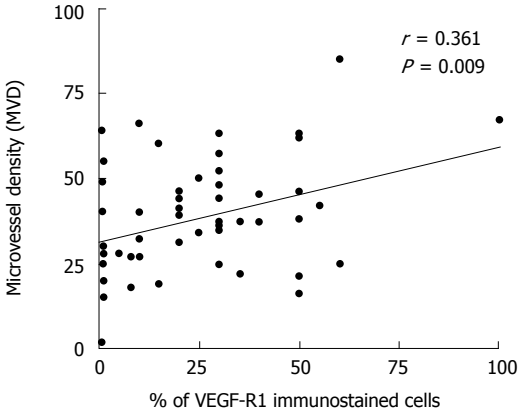

Figure 2 Correlation between percentage of VEGF-R1-immunoreactive cells and microvessel density (MVD) within each tumor.

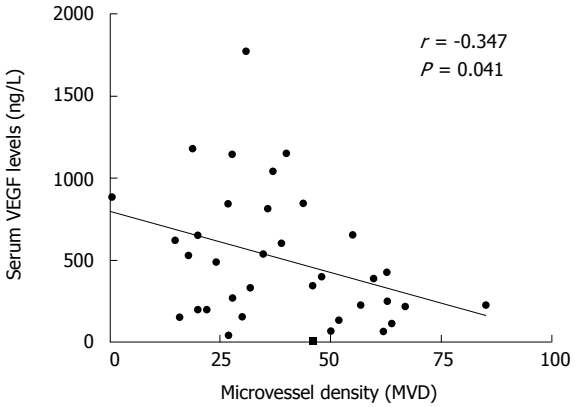

Figure 3 Correlation between serum VEGF levels and microvessel density (MVD) within each tumor.

About 80% of tumors demonstrated VEGF-R1 expression.

Regarding the relationship among the different angiogenic characteristics, only MVD was significantly correlated with both tumor VEGF expression (r = 0.361, P = 0.009; Figure 2) and serum VEGF levels (r = -0.347, P = 0.041; Figure 3). Conversely, neither VEGF-R1 expression nor MVD was related to p53 expression.

Table 2 shows the association between the markers of *H pylori* status and angiogenic factors. There was no significant association between markers of *H pylori* status, *cag*A, *vac*A and angiogenic biomarker expression. When the correlation between angiogenesis related-markers and IgG status was analysed a significant correlation between IgG status and plasma VEGF levels was observed (P = 0.026). The lack of association between *H pylori* characteristics and biomarkers was also confirmed with multivariate logistic regression analysis.

Angiogenic marker expression and markers of *H pylori* status were analyzed with respect to clinico-pathological features. Plasma VEGF levels and tumor TP expression were both significantly associated with tumor size (P = 0.030 and P = 0.035, respectively; Table 3). Regarding plasma VEGF levels in particular, T_4 tumors showed a significantly smaller percentage of low IgG cases as compared with T_{1-3} tumors (31% *vs* 74%; $P < 0.03$).

Table 2 ***cag*** **A,** ***vac*** **A, IgG anti-*H pylori* and angiogenic factors in gastric cancer patients**

	IgG anti *H pylori*		*cag* A		*vac* A	
	n (%) With IgG- (≤ 7 U/mL)	*n* (%) With IgG+ (> 7 U/mL)	*n* (%) Negative for *cag*A	*n* (%) Positive for *cag*A	*n* (%) Cytotoxic strains (s1; m1/2)	*n* (%) Others (absent; s2m2)
TP expression[1]						
0[2]	9 (31)	20 (69)	24 (57)	18 (43)	24 (60)	16 (40)
> 0	2 (25)	6 (75)	8 (80)	2 (10)	8 (80)	2 (20)
p53 expression[1]						
0[2]	8 (25)	24 (75)	27 (60)	18 (40)	28 (65)	15 (35)
> 0	3 (60)	2 (40)	5 (63)	3 (37)	4 (50)	4 (50)
MVD (CD34)[1]						
≤ 37[2]	7 (32)	15 (68)	16 (59)	11 (41)	15 (58)	11 (42)
> 37	3 (21)	11 (79)	16 (64)	9 (36)	17 (71)	7 (29)
VEGF-R1 expression[1]						
≤ 20[2]	6 (29)	15 (71)	16 (59)	11 (41)	14 (54)	12 (46)
> 20	5 (31)	11 (69)	15 (58)	11 (42)	17 (68)	8 (32)
pVEGF levels						
≤ 26[2]	9 (50)[a]	9 (50)[a]	10 (53)	9 (47)	11 (61)	7 (39)
> 26	2 (13)[a]	13 (87)[a]	9 (60)	6 (40)	9 (60)	6 (40)
sVEGF levels						
≤ 432[2]	5 (29)	12 (71)	11 (61)	7 (49)	12 (71)	5 (29)
> 432	6 (32)	13 (68)	9 (47)	10 (53)	9 (47)	10 (53)

sVEGF: Serum VEGF; pVEGF: Plasma VEGF; [1] % of immunostained cells; [2] Cut-off median value of the series; [a]$P = 0.026$.

Table 3 Association between angiogenic characteristics, markers of *H pylori* status and clinicopathological features

Biomarkers	Tumor stage			M status		
	T_{1-2-3} (*n* = 28)	T_4 (*n* = 28)	*P*	M_0 (*n* = 38)	M_1 (*n* = 18)	*P*
cagA negative (*n* = 32)	54	63	NS	58	59	NS
vacA no cytotoxic strains (*n* = 33)	62	63	NS	58	71	NS
TP negative (*n* = 42)	93	65	0.035	83	71	NS
p53 negative (*n* = 46)	78	93	NS	78	100	0.038
low MVD values (*n* = 27)	56	46	NS	54	44	NS
Low VEGF-R1 expression (*n* = 27)	56	44	NS	51	47	NS
High IgG levels (*n* = 27)	65	74	NS	77	54	NS
Low pVEGF levels (*n* = 19)	74	31	0.03	52	58	NS
Low sVEGF levels (*n* = 19)	35	67	NS	50	50	NS

NS: Non-significant.

Lastly, p53 expression was significantly associated with metastatic status ($P = 0.038$), as 100% of patients with metastatic disease did not express p53. No association was found between cytohistological tumor grading or *H pylori* infection site and angiogenesis-related markers and *H pylori* characteristics.

DISCUSSION

The role of *H pylori* in gastric cancerogenesis has been extensively investigated; conversely, information is lacking regarding the biological impact of *H pylori* on the progression of gastric cancer. Several factors emphasize the importance that various *H pylori* components can have their roles on the development of pre-neoplastic and neoplastic alterations of the gastric mucosa. Specific virulence factors produced by the bacterium, such as the vacuolating cytotoxin (vacA) or the cytotoxin-associated protein (cagA), contribute to gastroduodenal mucosal injury and impair the healing process of the damaged mucosa[31,32]. In addition, the host response to the infection and the presence of environmental factors are thought to be involved in the pathogenesis of *H pylori*-related gastroduodenal disease[33,34]. The vacuolating toxin (vacA) is believed to be a major determinant of *H pylori*-associated gastric disease[35,36]. The vacuolating cytotoxin gene A (*vacA*), which encodes the v*acA* protein, is present in all *H pylori* strains, but its encoded products are associated both with and without *in vitro* vacuolating activity[37]. It has been suggested that the *vac*A s1a genotype is closely associated with

high cytotoxin production, while the *vac*A s2 allele can demonstrate a negative *in vitro* association with cytotoxin activity. The presence of these virulence factors can be used to identify patients at risk to develop gastric cancer; in fact, patients with neoplastic transformation of the gastric mucosa are more likely to be infected by the *cag*A+ strain[19,38,39]. However, conflicting results regarding the association between these virulence factors and clinical outcome of gastric cancer are found in the literature[36,40-43].

p53 mutations and the genotypic characterization of *H pylori* have also been thoroughly studied to identify possible links between *H pylori* infection and p53 alterations without reaching definitive conclusions. Alterations of the *p53* gene and/or its abnormal protein accumulation have both been described during the later stage of gastric carcinogenesis[44,45] and in precancerous gastric lesions[46]. As *cag*A+ *H pylori* strains induce particularly severe inflammation in the gastric mucosa[41,47]. it has been hypothesized that gastric tumors from subjects infected with *cag*A+ *H pylori* might have a higher prevalence of p53 mutation than tumors from non-infected subjects.

H pylori infection may also regulate the angiogenesis and invasion of gastric carcinomas[13,14], but whether *H pylori* exerts its effects to induce neovascularization early in the development of gastric pre-neoplastic lesions or late in clinical phases of the disease is still unknown. However, it is clear that *H pylori* infection can increase the expression of the platelet-derived endothelial cell growth factor by infiltrating interstitial cells in pre-malignant lesions, such as intestinal metaplasia, thereby assisting in creating a favourable environment for tumor development[48]. Furthermore, it has been demonstrated that *H pylori* is able to up-regulate VEGF expression in gastric epithelial cells determining effects related to vacA-expression.

Recently, for the first time, Caputo *et al*[13] showed that *H pylori* up-regulated VEGF expression in gastric mucosa cells *in vitro* and that this effect was strictly *vac*A-dependent; and, interestingly, this result was not observed when using an isogenic mutant specifically lacking *vac*A. Moreover, *in vitro* and *in vivo* up-regulation of a number of EGF-related growth factors have also been reported[15,16,49].

In our sufficiently large series of gastric cancer patients, a percentage of *H pylori* infection was demonstrated, either as IgG-circulating level or as *cag*A/*vac*A DNA, which is in agreement with the previous studies[50,51]. In addition, the neo-angiogenesis characteristics reported did not significantly differ from those of other series of gastric cancers[7,52,53]. It is also possible to verify a clear relationship between p53, TP, VEGF and the clinicopathological characteristics considered in our series, further stressing the impact that angiogenesis has on tumor aggressiveness[52,53]. In fact, TP expression and plasma VEGF levels were both associated with tumor size, while p53 expression was associated with metastatic status.

However, when addressing the main objective of our study, it is possible to demonstrate an association only between higher levels of plasma VEGF and high levels of IgG (Table 3). Conversely, *H pylori*-infected tumors did not show p53, MVD, TP, VEGF-R1 characteristics which obviously differed from those without presence of *H pylori* infection. These results only partially agree with previous data[17,54] which, however, were all obtained from experimental *in vitro* models, in some cases referring to mRNA analyses utilizing only quantitative molecular or immuno-enzymatic approaches[14], therefore not exploiting morphological antigen tissue distribution. Furthermore, the increase of gene expression induced by the co-culture of gastric tumor cells with *H pylori* has been reported to be generally modest with more evident positive modulation for angiogenic factors not investigated in the present study, such as interleukin-8[14]. Caputo *et al*[13] also recently suggested that *vac*A-induced up-regulation of VEGF expression could depend on the functionality of epidermal growth factor receptor (EGFR)-, mitogen-activated protein kinase (MAPK)- and COX-2-mediated pathways, the biological targets which are largely heterogeneous in human gastric cancer. In conclusion, our study seems to suggest that the relationship between the *H pylori* toxic effect and angiogenic factors demonstrated *in vitro* could be influenced in human gastric tumor tissues by other key biological factors not considered in the present study.

A last comment regarding IgG and VEGF association concerns the blood of gastric cancer patients. The level of IgG antibodies has been suggested to be useful, not for diagnosis of infection, but for monitoring the outcome of *H pylori* infection over time, and specifically the efficacy of therapies aiming to eradicate *H pylori* infection. Thus, an elevated serum level before primary surgery for gastric cancer could be a signal of long-lasting and probably *H pylori* infection resistant to antimicrobial therapy[55].

The results of our study indicate that, from the angiogenic point of view, *H pylori*-related gastric cancers do not differ from those in which exposure to the bacterium cannot be demonstrated. Different explanations for these findings can be proposed: an angiogenetic relationship, if any, could be only induced by a long-lasting *H pylori* infection demonstrated by high IgG levels in the plasma; an alternative hypothesis might regard the ability of *H pylori* to modulate angiogenesis only during early phase of disease genesis and progression to be then lost during the clinically evident disease phases. This hypothesis would concord with the presumed role that angiogenesis plays, especially during the extremely early phases of cancer[9,17,56].

ACKNOWLEDGMENTS

We thank Mr. Baldassarre Stea for statistical analysis, Mr. Cesare Salvatore for technical support and Mrs. Paulene Maselli-Campagna for her help in the elaboration of the manuscript.

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