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Original Article

HISTOPATHOLOGICAL DETERMINATION OF *Helicobacter pylori* IN GASTRIC CANCER

Determinação histopatológica da presença do Helicobacter pylori em câncer gástrico

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ABSTRACT - *Background* - Etiology of gastric cancer (GC) remains controversial and several factors have implicated in its carcinogenesis process, including *Helicobacter pylori* (Hp) infection. Hp infection's role on GC remains uncertain, with several conflicting studies. ***Aim* -** To look for any correlation between *H. Pylori* and gastric cancer fom gastric specimen after gastrectomy. ***Method*** - Ninety-one patients with diagnosis of adenocarcinoma of the stomach treated by surgical resection were reviewed. Pathological examination was repeated in all patients to determine the presence of Hp infection, intestinal metaplasia (IM) and confirmation of the hystologic type by conventional haematoxylin-eosin staining. Statistical analysis was performed using Chi-quadrate and log-rank tests. ***Results* -** IM was observed in 81 tumours (89%). Overall, the presence of Hp infection was observed in 46 tumours (50.5%). There was no association between age and Hp status. In the group of patients with early and advanced GC, Hp infection was present in 47.7% and 54% of tumours. Hp infection was present in 40 tumours (49%) in the group of patients with IM. In patients with tumours without IM Hp was present in five (50%) tumours. Proximal tumours had more Hp infection when compared to distal tumours. ***Conclusions* -** The infection rate had no significant association with histologic type, IM, gender or stage. These results may indicate that participation of Hp infection during GC development cannot be ruled out; however, it is probably not essential during all stages of GC development and the mechanism may be distinct of the chronic gastritis and IM progression. Finally, it is possible that the proposed association is merely coincidental and that there is no actual influence of the bacteria in the carcinogenesis process.

. **HEADINGS –** Stomach Neoplasms. *Helicobacter pylori*.

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RESUMO – ***Racional*** - A causa do câncer gástrico (CG) é controversa e tem vários fatores envolvidos no seu processo de carcinogênese, incluindo o *Helicobacter pylori* (Hp) O papel da infecção pelo Hp no CG permanece incerto, com vários estudos controversos. ***Objetivo*** - Correlacionar a presença da infecção pelo Hp com câncer gástrico, através de exame anatomopatológico convencional do estômago ressecado. ***Método*** - Noventa e um pacientes tratados por ressecção cirúrgica foram revistos. O exame anatomopatológico foi feito em todos os pacientes para determinar a presença de infecção por Hp, metaplasia intestinal (MI) e confirmação do tipo histológico por hematoxilina-eosina. A análise estatística foi realizada através do qui-quadrado e testes de log-rank. ***Resultados*** - MI foi observada em 81 tumores (89%). Em geral, a presença de infecção pelo Hp foi observada em 46 casos (50,5%). Não houve associação entre idade e Hp. Nos grupos de pacientes com CG avançado e precoce, a infecção pelo Hp estava presente em 47,7% e 54% dos tumores. A infecção pelo Hp ocorreu em 40 tumores (49%) no grupo de pacientes com MI. Nos com tumores sem MI, Hp estava presente em cinco (50%). Tumores proximais tiveram mais infecção por Hp, quando comparados aos tumores distais. ***Conclusões*** - A taxa de infecção não teve associação significativa com o tipo histológico, sexo, MI ou estágio de desenvolvimento tumoral. Esses resultados podem indicar que a participação da infecção pelo Hp durante o desenvolvimento do CG não pode ser descartada; no entanto, provavelmente não é essencial em todas as fases e o mecanismo do CG pode ser distinto da gastrite crônica e MI.Finalmente, é possível que a associação proposta é mera coincidência e que não há nenhuma influência real das bactérias no processo de carcinogênese.

DESCRITORES - Neoplasias gástricas. *Helicobacter pylori*

INTRODUCTION

Gastric cancer (GC) remains as one of the most important malignancies worldwide with significant incidence and mortality rates. [27,33,35,39] Etiology of gastric cancer remains controversial and several factors have been implicated in its carcinogenesis process. Among these factors, genetic events, environmental, and dietary characteristics appear to be relevant to the development of the disease. [3,19,33,35]

Helicobacter pylori (Hp) infection has also been implicated in the carcinogenesis of gastric cancer. This association has been suggested by the observation of higher rates of Hp infection in patients with gastric cancer when compared to normal controls in western countries.[6,7,25] Furthermore, in countries with high incidence of gastric cancer, prevalence of Hp infection appears to be even higher, occurring at early ages of the general population. [13, 25]

The role of Hp infection in the development of gastric cancer may be associated with the production of specific cytokines and enzymes leading to chronic atrophic gastritis and intestinal metaplasia. [3,19,35] However, this association is still controversial as some studies report the association of Hp infection to chronic gastritis but not to gastric cancer.[13] Also, some countries with low incidences of gastric cancer may show high prevalence of Hp infection. [8]

For these reasons, in a country with high prevalence of Hp infection among the general population as in Brazil, one would expect to find high rates of Hp infection in patients with GC. Accordingly, one would expect association between Hp infection and IM or intestinal type GC.

Therefore, the aim of this study was to verify the presence of Hp infection in 91 Brazilian patients with gastric cancer by conventional pathological examination of the resected stomach.

METHODS

Ninety-one patients with diagnosis of adenocarcinoma of the stomach treated by surgical resection at our Institution between 2000 and 2002 were reviewed. Collected data included age at diagnosis, gender, tumor location, histological type (according to Laurén's classification), complete pathological report and disease stage. Stage classification was performed according to UICC-1998 recommendations.

Forty female (44%) and fifty-one (56%) male patients were eligible for the study. Mean age was 61 ranging from 19 to 87 years-old. Three patients (3.3%) had their tumor located in the proximal third (U), 33 (36.3%) in the middle third (M) and 42 (46.1%) in the distal third (L) of the stomach. Thirteen patients had tumors located in more than one region, being eight (8.8%) in the distal and middle thirds (LM), two (2.2%) in the proximal and middle thirds (UM) and three tumors (3.3%) in the entire organ (LMU). In order to perform statistical analyses concerning tumor location, proximal and middle tumors were aggregated and named "proximal tumors".

Pathological examination was revised by an experienced pathologist in all patients in order to determine the presence of Hp infection, intestinal metaplasia (IM) and confirmation of the histological type (Laurén's classification) by conventional haematoxylin-eosin staining. The presence of Hp infection was determined by direct visualization of the bacteria at the adjacent normal mucosa of gastric cancer.

Correlation between HP infection and epidemiological, clinical and pathological features were performed. Statistical analysis was performed using Chi-quadrate and log-rank tests.

RESULTS

Overall, 61 patients (68%) had intestinal type gastric adenocarcinoma according to Laurén's classification, while 30 (32%) had diffuse type. Sixty-seven patients (74%) had early gastric cancer, while 24 (26%) had advanced gastric cancer. In the group of patients with early gastric cancer, 21 (31%) had diffuse type and 46 (69%) had intestinal type gastric adenocarcinoma. In the group of patients with advanced gastric cancer, nine (30%) had diffuse type and 15 (70%) had intestinal type GC (Table 1).

TABELA 1 - Patient's demographics

Patient's demographics (n = 91)	
Gender	
Female	40 (44%)
Male	51 (56%)
Tumor location	
Upper	3 (3,3%)
Medium	33 (36,3%)
Lower	42 (46,1%)
Lower/medium	8 (8,8%)
Upper/medium	2 (2,2%)
Intire stomach	3 (3,3%)
Histological type	
Intestinal	61 (68%)
Diffuse	30 (32%)
Stage	
Early GC	67 (74%)
Advanced GC	24 (26%)

Associated intestinal metaplasia (IM) was observed in 81 tumors (89%). In the group of patients with intestinal type GC, IM was present in 54 tumors (88%). In the group of patients with diffuse GC, IM was observed in 27 tumors (90%). In the group of patients with early

GC, 63 (94%) tumors had intestinal metaplasia while in the group of patients with advanced gastric cancer, 21 (87%) tumors had IM. There was no significant correlation between the presence of IM and early/advanced GC or histological type (p>0.05) (Table 2).

TABELA 2 - IM according to tumor characteristics

Intestinal metaplasia	MI -	MI +	p
N	10 (11%)	81 (89%)	
Histologic type			
Intestinal	7 (12%)	54 (88%)	
Diffuse	3 (10%)	27 (90%)	ns
Stage			
Early GC	4 (6%)	63 (94%)	
Advanced GC	3 (13%)	21 (87%)	ns

TABELA 3 - *Helicobacter pylori* infection determination by HE pathological examination

Hp Infection	Hp -	Hp +	p
N	45 (49,5%)	46 (50,5%)	
Gender			
Female	18 (45%)	22 (55%)	
Male	28 (55%)	23 (45%)	ns
Tumor location			
Proximal (upper, upper/medium and medium)	22 (44%)	28 (56%)	
Distal (lower and lower/medium	23 (63%)	14 (37%)	
Intire stomach	-	3 (100%)	p=0,03
Histologic type			
Intestinal	31 (51%)	30 (49%)	
Diffuse	14 (47%)	16 (53%)	ns
Stage			
Early GC	32 (47,7%)	35 (52,3%)	
Advanced GC	13 (54%)	11 (46%)	ns
Intestinal metaplasia			
Present	41 (52%)	40 (49%)	
Absent	5 (50%)	5 (50%)	ns

Overall, the presence of Hp infection was observed in 46 tumors (50.5%). Similar frequencies of Hp infection were observed in male (45%) and female (55%) patients. There was no association between age and Hp status. In patients with diffuse type gastric cancer, Hp was present in 14 tumors (47%), while in patients with intestinal type GC, Hp was present in 31 tumors (51%). Also, in the group of patients with early gastric cancer, Hp was present in 32 tumors (47.7%), while in patients with advanced GC, Hp was present in 13 tumors (54%). These differences showed no statistical significance (p>0.05).

Hp infection was present in 40 tumors (49%) in the group of patients with IM. In patients with tumors without IM, Hp was present in five (50%). In the subgroup of patients with intestinal type gastric cancer and with IM, the presence of Hp was detected in 27 tumors (50%). These differences showed no statistical significance as well (p>0.05). Curiously, in patients with gastric cancer located in the distal third of the stomach (L and LM), the presence of Hp infection was observed in 14 tumors (37%). In patients with proximally located GC (U and M), Hp infection was present in 28 tumors (56%). Proximal tumours had more frequently Hp infection when compared to distal tumours (p=0.03) (Table 3).

DISCUSSION

In early 1900's, the presence of *H. pylori* on the surface of the gastric mucosa was first described, coincidentally in a gastric cancer specimen.[16] However, it was not until early 1980's that a relation between Hp infection and diseases of the stomach was proposed[7,11,18].

During early Hp infection, a polymorphic neutrophylic inflammatory infiltrate is observed in the gastric surface in the acute phase. Later, there is a decrease in mucus production resulting in a decrease in the mucous thickness. Chronic infection is characterized by an increase in lymphocyte and plasmocyte count in the lamina propriae.[19,29,33,35]

A significant proportion of patients with Hp infection remain asymptomatic presenting chronic active gastritis.[21,28,33,35] Some may develop gastric or duodenal ulcers. A minority of the patients with Hp infection develop chronic atrophic gastritis [17,32] and intestinal metaplasia.[2,5,34] Different disease progression may be influenced by dietary factors such as vitamin C [3,23,24], salt [9] and nitrate ingestion [23,24]. The proposed mechanism for the progression to chronic atrophic gastritis and intestinal metaplasia may be associated with the production of cytokines and enzymes with direct or indirect action on the gastric mucosae. [33] Furthermore, the inflammatory process in the gastric wall may lead to more cytokine production such as IL-6, IL-8, TNF-alpha and peroxides contributing to mucosal damage.[10,15] Hp urease activity, for instance, may result in ammonia production, which in turn, decreases mucosal integrity and elevates pH of the gastric secretions favouring bacterial proliferation.[19,33,35]

The proposed mechanism of action of Hp infection in the genesis of gastric cancer is through the development of chronic gastritis and intestinal metaplasia.[6,31,36] These situations may be considered pre-malignant conditions, specifically for intestinal type gastric cancer and thus Hp infection may be considered by some also pre-malignant.[6,12,19,36]

In this setting, it would be of great interest to determine the frequency of Hp infection in gastric cancer. One would expect to find higher rates of Hp infection in patients with gastric cancer when compared to normal controls in the general population. Furthermore, if the mechanism of action of Hp infection in GC is

considered, it would be expected to find even higher rates of Hp infection in intestinal type gastric cancer with associated intestinal metaplasia. Confirmation of such propositions would favour Hp participation in gastric carcinogenesis and strength the importance of Hp eradication in asymptomatic patients including the possibility of populational prevention measures such as mass vaccination of high-risk areas to reduce the GC incidence. [6,28,31,33]

Determination of Hp infection in some countries, such as Netherlands and UK, showed higher rates in gastric cancer patients (61-69%) when compared to normal subjects (31-47%).[6] However, in other regions such as in Italy, these differences were not observed. [26,37] Also, regions with high incidences of gastric cancer appear to have high prevalences of Hp infection, such as Japan, where approximately 75% of the general population has serological evidence of such infection and where the GC incidence is extremely high, compared to other countries and the western world. [1,6] In this country, it is considered that there is a 3-fold increased risk for the development of GC in normal subjects with Hp infection when compared to Hp free subjects, since the great majority of the infections appear to occur early during lifetime.[4,6] On the other hand, in Africa where there is a high prevalence of Hp infection in the general population, there is a very low incidence of GC.[8,31] Also, the high frequency of distal and intestinal type GC in the elderly may facilitate bacterial proliferation, being Hp infection merely circumstantial.[22] Finally, the low economic status of developing countries may be associated to high rates of infectious diseases (including Hp infection). Specifically in Brazil, where the prevalence of infectious diseases and GC are high, the association between them could be merely coincidental.

There is some more evidence that may not favour Hp infection participation during GC carcinogenesis. First, the association between Hp infection and chronic gastritis is well established, but the association between Hp infection and GC is not widely accepted. [31,38] Some authors believe that GC arises *de novo* and not through chronic gastritis and intestinal metaplasia. [13,38] Also, there is a significant association between GC and gender (male subjects being more frequently affected). Therefore we would expect to find the same association between gender and Hp infection. However, the prevalence of Hp infection shows equal distribution between male and female controls, including the results observed in the present study in patients with CG. [38]

In this study, determination of Hp infection in 91 gastric cancer specimens was made by conventional pathological examination, using haematoxylin-eosin staining. This method is considerably expensive, meticulous and the presence of an experienced pathologist is crucial. On the other hand it gives unequivocal information about the presence of Hp infection. Also, it may be useful in cases of gastric cancer where surgical resection eventually will be performed, but questionable in normal controls where endoscopic biopsies are some times scant and insufficient for accurate determination of Hp infection.

The prevalence of Hp infection in this study was surprisingly low, 50.5%. A previous study performed in our country with 18 patients with gastric cancer, showed an infection prevalence of over 80%, considerably higher than our results. [25] Moreover, the infection rate in the present study is considerably lower than Hp infection prevalence in normal controls in Brazil, determined by serological studies. [20,30] In these latter, this country is considered to present high prevalence of Hp infection in the general population reaching rates of infection as high as 80%. [20,30] The Hp infection in GC patients in this study occurred at similar rates between both genders, according to previous observations noted among normal controls.[1,30,38]

Interestingly, there was a significant correlation between Hp infection and proximal tumour location of GC. However, the low incidence of proximal tumours (U) may be responsible for this difference, since for statistical reasons, tumours located in the proximal and middle third (M and U) had to be aggregated. Surprisingly, the infection rate had no significant association with histological type, gender or stage (early vs. advanced GC) even though one would expect higher infection rates in intestinal type GC. Surprisingly, there was no association between Hp infection and intestinal metaplasia, one of the proposed evidences of Hp infection for GC development.

CONCLUSION

The results of Hp infection in GC patients indicate that this event can be determinated approximately 50% of patients by histopathological study of the resected specimen. Participation of Hp infection during GC development cannot be ruled out, however it is probably not essential during all stages of the development of the disease. Also, if there is any participation of Hp infection in gastric cancer development, the mechanism may be distinct of the chronic gastritis and intestinal metaplasia progression. Finally, it is possible that the association between GC and Hp infection is merely coincidental and that there is no actual influence of the bacteria in the carcinogenesis process.

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