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HELICOBACTER PYLORI AND EPSTEIN–BARR CO-INFECTION IN GASTRIC DISEASE

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Abstract

The incidence of gastrointestinal diseases and in particular gastric cancer (GC) is high worldwide. Over the last few years, numerous studies have speculated that Helicobacter pylori (H. pylori) and Epstein-Barr virus (EBV) can be correlated with gastric cancerogenesis.
Virulence factors of H. pylori can contribute to the variability of clinical outcomes: among the most important virulence factors is the pathogenicity island (CagPAI), vacA and oipA gene. EBV infection usually persists in B cells and induces an inflammatory reaction in cooperation with H. pylori. In Sicily, H. pylori and EBV infections are particularly prevalent, and to our knowledge no study has addressed this yet. The aim of our study was to examine the association of H. pylori and EBV infection in patients with gastric diseases in Sicily. Gastric biopsies were collected from 24 adult patients with chronic gastritis active (CGA) and from 24 adult patients without any gastric disease (NGD) who underwent upper gastrointestinal endoscopy. H. pylori infection was diagnosed by PCR for ureaseA gene while EBV-DNA was detected by Real time PCR for region Bam HI-W. Moreoever, we investigated the presence of CagPaI and the status of vacA and oipA genes. Percentage of resistance to Clarithromycin of H. pylori was evaluated also. We established that H. pylori and EBV infection was present in 42% of patients, while dual infection with H. pylori and EBV-DNA was present in 54% of the patients with CGA. In patients with NGD we found that H. pylori and EBV infection was present in 46% and in 21% of patients respectively, while co-infection was present in 33% of patients. CagPAI was present in only 20% of patients with GCA and in 9% of patients with NGD. As regards vacA alleles, s2i2m2 were predominant, present in 80% and 82% of patients with CGA and NGD respectively. The status “ON” of oipA gene was present in the same percentage. Finally, we found that 38% of patients positive for H. pylori infection showed resistance to Clarithromycin. In our study, there was a strong association between the simultaneous presence of H. pylori and EBV infection in patients with CGA compared to patients with NGD. Furthermore, our data confirmed the high percentage of resistance among H. pylori strains circulating in Sicily, underlining the importance of establishing a therapy that is effective in eradicating them and reducing the frequency of coinfections and evolution towards gastric cancerogenesis.

Keywords: *H. pylori, EBV, coinfection, Italy, gastric diseases*.

Introduction

In recent years infections involving bacteria and viruses have been associated with the development of gastric diseases such as gastric cancer (GC), chronic gastritis and MALT Lymphoma [1].

In particular, the role of *Helicobacter pylori* and *Epstein-Barr virus* (EBV) in gastric cancerogenesis has been assessed [1-5]. In the past the authors have shown that *Helicobacter pylori* can produce a Helicobacter-like vacuolating toxin and may be responsible for cases of childhood diarrhea [3].

H. pylori was defined as a group 1 carcinogen in 1994 by the International Agency for Research on Cancer, while EBV maintains its genome inside host cells avoiding cell death and evading the immune system [6,7]. Nowadays, the role of EBV infection in inflammatory gastro intestinal disease is emerging and under debate [8-10].

The virulence factors of *H. pylori* are mainly involved in the development of gastric diseases; among these are the genes *cagA*, *vacA* and *oipA*. CagA is the most extensively studied *H. pylori* virulence factor and it is a polymorphic gene [11]. There are diverse numbers of repeat sequences located in the 3′ region of the gene. All repeat regions of the CagA protein contain Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs. VacA is the next most considered *H. pylori* virulence factor. Three different regions are known in the *vacA* gene structure, at the signal (s) region (s1 and s2), intermediate (i) region (i1 and i2) and the middle (m) region (m1 and m2) [11].

Finally, OipA was recognized as a proinflammatory-response-inducing protein, conditioning the production of IL-8 from gastric epithelial cells [12]. It is clear that the eradication of the bacterium appears to decrease the frequency of gastric cancer in high risk patients [13]. In particular, resistance to Clarithromycin, a key drug used for eradication, may have an influence on the efficiency of eradication schemes [14]. The point mutations in *23S rRNA*gene explain common clarithromycin resistance [15].

EBV infects cells from the oropharynx and then extends to the lymphoid tissue where it infects B lymphocytes [16]. Prevalence of the agent responsible for mononucleosis syndrome has been assessed in different geographic areas [17,18].

In vitro and in vivo studies have been formulated on the viral interaction between EBV and autoimmune disorders and as a pathogenic mechanism in Multiple Sclerosis (MS) [19,21].

Recently, secondary hemophagocytic lymphohistiocytosis (HLH) characterized by histiocyte proliferation and hemophagocytosis described by Cascio et al. in severe infection [22-25] was also reported in patients with mononucleosis syndrome [25].

The relevance of inflammatory response is hypothesized by recent studies that showed how *H. pylori* and EBV co-infection can cause injury to the tissue through inflammatory reactions or through more contact between CagA protein of *H. pylori* and EBV supporting augmented activation of B cells transiting through the gastric mucosa [26].

This study aimed to evaluate the frequency of *H. pylori* and EBV infection, as well as the presence of *cagA*, *vacA* and *oipA* genes and the percentage of resistance to Clarithromycin in gastric biopsies from adult patients with and without gastric disease in Sicily.

Materials and Methods

Patients

Twenty-four gastric biopsies were collected from patients with chronic gastritis active (CGA) and from 24 adult patients without any gastric diseases (NGD) undergoing upper gastrointestinal endoscopy. Data and clinical information, such as age, sex, symptoms, clinical diagnosis and histology evaluation were collected; data are shown in Table 1. Patients under antibiotic or antacid treatment were omitted from the study.

Two biopsies were collected from each patient: one biopsy from the antrum was used for molecular detection of *H. pylori*, *cagA* status, *vacA* genotyping, status of *oipA* and EBV through polymerase chain reaction (PCR). The other biopsy was for histological analysis.

DNA extraction

The genomic DNA was extracted by means of QIAamp DNA Mini Kits (QIAGEN), in agreement with the producer's information [27].

Polymerase chain reaction (PCR)

DNA of *H. pylori* was detected in biopsies by PCR using *ureaseA* primers according to Fasciana et al. [14].

Instead, the BAMHI-W fragment region of the EBV genome was used as the target to evaluate the presence of the virus according to P. Di Carlo et al. [28].

Positive samples for *H. pylori* were analysed with PCR in order to define the *cagA*status, the EPIYA motif, *vacA s, i* and *m* subtypes, *oipA* status and presence of point mutation in the 23S rRNA gene using specific primers, as previously reported [13,14].

Results

We found that *H. pylori* and EBV presence occurred in 42% of patients while dual prevalence of *H. pylori* infection and EBV-DNA was detected in 54% of the patients with CGA. In patients with NGD we found that *H. pylori* and EBV infection was present in 46% and in 21% of patients respectively, while co-infection was present in 33% of patients. In Table 2 we show the percentage of infections related to sex of the patients.

The assessment of *vacA* gene mosaicism in patients with GCA has revealed: alleles s2i2m2 in 80% of the cases, alleles s1i1m2 in 10% of cases, and alleles s1i1m1 in 10% of cases. In patients with NGD alleles s2i2m2 were found in 82% of the cases, alleles *s1i1m2* in 9% of cases, and alleles s1i1m1 in 9% of cases. The cagA gene was present, with EPIYA motif ABC, in only 20% of patients with GCA, and in 9% of patients with NGD with a EPIYA motif ABCC.

The distribution of *vacA* mosaicism, presence of *cagA* gene motif EPIYA and status of *oipA* gene of strains related to diagnosis is shown in Table 3.

Finally, the status "OFF" of gene *oipA* was present in 80% of patients with GCA and in 90% of patients with NGD. We also evaluated the presence of CTAA nucleotides that may influence the status of genes. CT patterns and their distribution are shown in Table 4.

The analysis of the *23S rRNA* gene revealed that 38% of patients were infected with *H. pylori* resistant to Clarithromycin and the predominant point mutation observed was A2142G in 7 cases (88 %) and A2143G in 1 case (12%).

Conclusion

H. pylori and EBV infections are acquired during childhood, and both microorganisms can cooperate to induce the alteration of gastric mucosa.

After preliminary infection by *H. pylori,* patients can develop acute gastritis, and clinical outcome is determined by the diverse interactions between host factors and bacteria.

In our study, *H. pylori* infection was present in 43% of all patients enrolled, with a percentage of 41% in patients with GCA and 45% in patients with NGD respectively.

The three central *H. pylori* genes associated with pathogenicity are: *cagA*, *vacA* and *oipA*.

Here, the presence of *cagA* genotype was lower compared to a previous study conducted in Sicily, and it is associated with motif EPIYA ABC in 66% of the cases and with motif EPIYA ABCC in 34% of the cases. Moreover, *cagA* gene is only associated with *s1i1m1* or *s1i1m2* mosaicism of the *vacA* gene [13,14].

Lastly, the status "OFF" of gene *oipA*, established through the number of repeat CT and based on the presence or not of CTAA, was present in 80% of the patients with GCA and in 90% of patients with NGD.

A close correlation was found between the status of the *oipA, vacA* and *cagA* genes and, indeed, the 'on' status occurred in *cagA*-positive strains, being associated with *s1m1* mosaicism.

The described high prevalence of *H. pylori resistant to antibiotics suggests* that empirical therapy with clarithromycin should be

abandoned in Sicily, confirmed by the result of our study [14].

In conclusion, the authors suggest the recruitment of a multidisciplinary team to assist with diagnostic microbiological goals in the different steps of EBV and *H. pylori* infection, such as morphological and imaging methodologies in the acute phase and during follow up, especially for the frail population, such as patients with gastrointestinal disorders and/or neoplasms [35-38].

Around 31% of our patients presented EBV DNA in gastric biopsies, with a percentage of 46% in patients with GCA and 21% in patients with NGD separately.

The dual prevalence of *H. pylori* infection and EBV-DNA was present in 54% of the patients with CGA and in 33% of patients with NGD.

Our results suggest that co-infection is more frequent in patients with gastric lesions, although additional investigations are still required to hypothesize a critical role of the two microorganisms in the development of gastric disease in the Italian population.

Recently studies have suggested that microbiology analysis should be complemented with direct visualization, such as per cyto- and/or histo-pathology analysis, especially when samples are obtained from unsterile sites, for example gastrointestinal and respiratory apparatus [35-38].

In conclusion, the authors encourage researchers to apply existing and newly acquired knowledge within a multi-disciplinary team investigation using radiological and morphological investigations [37,38] for a more clinical amplification of microbiological features of the gastrointestinal tract, [39-41].

The promise of prevention through vaccination is the goal of virologist researchers. The authors currently encourage health promotion through standardized vaccine schedules [42,43].

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Table 1. Clinical and histology diagnosis, sex and age of patients enrolled.

Diagnosis	Male (%)	Female (%)	Medium age	Range of age
CGA	37.5% (9/24)	62.5% (15/24)	47	22-87
NGD	33.3% (8/24)	66.7% (16/24)	45,7	25-71

Table 2. The percentage of infections related to sex of patients

Diagnosis/Sex	Patients *H.p*+ (%)	Patients EBV+ (%)	Patients *H.p*+/EBV+ (%)
GCA			
Male	33.3% (3/9)	33.3% (3/9)	2.2% (2/9)
Female	46.7% (7/15)	46.7% (7/15)	33.3% (5/15)
NGD			
Male	37.5% (3/8)	12.5% (1/8)	12.5% (1/8)
Female	50.0% (8/16)	25.0% (4/16)	18.75% (3/16)

Table 3. Distribution of *vacA* mosaicism, presence of *cagA* gene motif EPIYA and status of *oipA* gene.

Diagnosis	vacA mosaicism Nr. (%)	cagA Positive/ motif EPIYA Nr. (%)	CagA Negative Nr. (%)	Status oipA Nr. (%)
GCA	*s2i2m2* 8 (80%)	-	8 (82%)	OFF 8 (80%)
	s1i1m2 1 (10%)	1 (10%) ABC	-	ON 1 (10%)
	s1i1m1 1 (10%)	1 (10%) ABC	-	ON 1 (10%)
NGD	*s2i2m2* 9 (82%)	-	9 (82%)	OFF 9 (82%)
	s1i1m2 1 (9%)	-	1 (9%)	OFF 1 (9%)
	s1i1m1 1 (9%)	1 (9 %) ABCC	-	ON 1 (9 %)

GCA=chronic gastritis active; NGD=without gastric disease.

Table 4. *oipA* CT repeat patterns

Sequence of signal peptide-encoding region of *oipA*	Nr. CT	Nr. of strains
OFF Status		
ATGAAAAAAGCTCTCTTA……...CTCTCTCTCTCTCTCTCTCGTT	9	4
ATGAAAAAAGCCCTCTTACTAACTCTCTCTCTCTCTCTCGTTT	8	5
ATGAAAAAAGCTCTCTTGCTAACTCTCTCTCTCTCTCTCTCGTT	10	4
ATGAAAAAAGCTCTTTTA……CTCTCTCTCTCTCGTT	6	1
ATGAAAAAAGCTCTCTTACTAACTCTCTCTCTCTCTCGTT	7	2
ATGAAAAAGCTCTCTTA……CTCTCTCTCTCTCTCGTTCTGG	7	1
ATGAAAAAAGCCCTCTTA……..CTCTCTCTTTCTCTCGTTTT	4+2	1
ON Status		
ATGAAAAAAGCTCTCTTACTAATTCTCTCTCTCTCTCGTT	5	1
ATGAAAAAAGCTCTCTTACTAACTCTCTCTCTCTCTCGTT	6	1
ATGAAAAAAGCCCTCTTA……..CTCTCTCTCTCTCTCTCTCTCTCGT	11	1