DETECTION OF cagA GENE AND TYPING vacA GENE OF Helicobacter pylori IN BIOPIESIES OF PATIENTS WITH GASTRIC SYMPTOMS IN CUMANA, SUCRE STATE, VENEZUELA

INTRODUCTION

Helicobacter pylori is a Gram negative bacteria, microaerophilic which persistently colonizes the human gastric mucosa. Over 50% of the world population is infected with the bacteria and in developed countries can reach 80%. Although most are asymptomatic, the presence of H. pylori is associated with diseases such as peptic ulcers, gastric adenocarcinoma and gastric lymphoma (Cavazza et al. 2001).

The rapid changes observed in the epidemiology of gastric pathogens associated with H. pylori is likely...
due to the interaction between environmental factors and host factors or changes in the prevalence of more or less virulent strains (Jafari et al. 2008). Two phenotypic characteristics allow the identification of strains capable of virulence, the vacuolating cytotoxin encoded by vacA gene, which is present in most strains of H. pylori, and the high molecular weight protein associated with cagA gene which is also cytotoxic. The cagA gene is located on a pathogenicity island (PAI) of 40 kb that was horizontally transferred from a source not known (Censini et al. 1996). The cag PAI encodes the type IV secretion system, through which CagA is carried into the cell. The structure of the 3' cagA gene contains a motif EPIYA that phosphorylate the tyrosine of CagA once it enters the cell, variations in this region differ with strains of H. pylori and can, inclusive, differentiate their Asian or Western origin (Mane et al. 2010, Suzuki et al. 2012).

Vacuolating cytotoxin VacA, is produced by most strains of H. pylori, besides being a cytotoxin which has no similarity with other bacterial proteins and eukaryotic proteins (Cover and Blanke 2005), once it is produced may remain on the surface of the bacteria or being secreted as a toxin of approx. 88 kDa (Ilver et al. 2004, El-Bez et al. 2005), dissociates upon exposure to non-neutral environments, which when exposed to conditions of alkalinity or acidity amplifies its activity (Cover et al. 1997, Yahiro et al. 1999).

Gene structure of vacA allows variations in the vacuolating activity of the strains, possesses the region signal (s1, s2), the middle region (m) and a third intermediate region newly assigned as (i). In the region of vacA is found the p33 portion of the toxin that influence vacuolating activity and efficiency in forming anion channels, due to the hydrophobic nature of the amino acid residues that are close to the proteolytic cleavage site (McClain et al. 2001), the s1 variant contains more hydrophobic amino acids near the region of cleavage that s2 variant, which allow better integration into the membrane of the host cell (McClain et al. 2001). The middle region (m1 and m2) (Atherton et al. 1997) is found in the p55 portion of the toxin and influence the tropism of H. pylori in the host cells: the region m1 is toxic to a wide range of host cells (Pagliaccia et al. 1998, Amieva and El Omar 2008). Combinations of these regions result in strains that may be more or less virulent m1s1 genotype produces the highest level of toxin, the s1m2 strains produce low to moderate level of toxin, the m2s2 are considered nontoxic while s2m1 while not been recognizes like toxic strains.

The aim of this study was to characterize genotypes vacA and the presence of cagA gene detected in gastric biopsies of patients with gastric pathologies positive for infection with H. pylori, who attended to the Gastroenterology Service of the University Hospital Antonio Patricio de Alcalá of Cumaná, Sucre state.

MATERIALS AND METHODS

Patients

120 patients attending the Gastroenterology Service, Hospital Universitario Antonio Patricio de Alcalá of Cumaná, who expressed by prior written consent to the taking of blood samples and biopsies following the guidelines of the Bioethics Committee of the Autonomous Service, Institute of Biomedicine (Ministerio del Poder Popular de la Salud-Universidad Central de Venezuela) (MPPCTII-FONACIT 2011). Each patient provided its epidemiological data by filling out a form designed for that purpose by modified Graffar test (Méndez-Castellano y Méndez 1994). The exclusion criteria of this study were, treatment with antibiotics and drugs containing bismuth or omeprazole two weeks before the test.

Gastroscopy examination

The stomach endoscopy was performed based on guidelines protocols of the hospital work, for it biopsies of the lesser curvature of the antrum were taken. Antrum samples for molecular testing were placed in Eppendorf tubes, identified with the number of the patient and immediately frozen at -80°C.

DNA extraction from biopsy

The procedure followed the guidelines of Perrone et al. (2009), briefly: To the tubes containing the biopsy were added 50-100 μL of Proteinase K (100 μg/ μL) + 50 μL of Lysis Buffer (10 mM Tris-HCl, pH 8.1 + 0.1% Sarcosine.), then stirred in Vortex and placed in water bath at 55°C for two hours. The inactivation of the proteinase K was achieved by heating at 95°C for 5 minutes. Subsequently 1 V of chloroform: phenol: isomyl was added. Vorterising and centrifuging at 14,000 rpm for 10 minutes.

PCR amplification of 349 bp region of cagA

All PCR mixtures consisted of 1 μL of DNA, 1X PCR Buffer (Gibco BRL, Gaithersburg, MD), 1.5 mM MgCl₂, 0.2 mM each deoxynucleotide (Gibco, BRL), 0.5
Detection of cagA gene and typing vacA gene of Helicobacter pylori...

mM each specific primer and 1.25 U of Taq polymerase (Gibco, BRL) in a final volume of 25 µL. A 349 bp region of cagA gene was amplified by PCR using the F1/B1 (Tummuru et al. 1993) primers. Aliquots of isolated DNA from each patient were taken and processed by performing the following amplification program in a thermocycler (Gene Amp 9700 Perkin Elmer Applied Biosystems, USA): 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72 for 2 minutes and final extension of 72°C for 6 minutes.

**Amplification of the 335 bp region of cagA**

The 335 bp region of cagA was amplified by PCR using the B7628/B7629 initiators (González-Valencia et al. 2000). Aliquots of DNA isolated from each patient were taken and were processed by performing the following amplification program in a thermocycler (Gene Amp 9700 Perkin Elmer Applied Biosystems, USA): 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes and final extension of 72 for 6 minutes.

For previous experiences in the laboratory it was decided to use two sets of primers for the detection of cagA and taken as positive anyone that achieve amplification.

**PCR amplification of vacA alleles s1/s2 and m1/m2**

For identification of allelic variants of the signal sequence S1/S2-F primers VA1/VA1-R (Atherton et al. 1995) were used to amplify the conserved regions of vacA of 259 bp and 286 bp respectively. A second set of primers R-VAG/VAG-F (Atherton et al. 1997) was used to amplify the middle regions 567 bp BEEF (m1) or 642 bp (m2). Aliquots of DNA extracted from biopsies from each patient and processed in a thermocycler (Gene Amp 9700 Perkin Elmer Applied Biosystems, USA), by 35 cycles of 94°C for 1 minute, 52 for 1 minute and 72 for 1.5 minutes was taken, with a final extension of 72°C for 6 minutes for m1/m2. As positive controls were used specific strains, 8822 (vacA s2m2) and 8823 (vacA s1m1) (ATCC 49503) and 84183 (ATCC 53726) (vacA s1m1) and as negative control sample without DNA and DNA of Escherichia coli (ATCC 2225) (Table 1).

<table>
<thead>
<tr>
<th>Gen and amplified region</th>
<th>Genotype</th>
<th>Primers designating</th>
<th>Primers sequence</th>
<th>Size of PCR products (Location)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vacA r middle</td>
<td>m1</td>
<td>VA3-F</td>
<td>5'GGTCAAAAATGCCGTCATGG-3'</td>
<td>290 bp (2741-3030)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VA 3-R</td>
<td>5'CCATTGTACCTTGAGAAA-3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>VA4-F</td>
<td>5'GGAGCCGAGAAGTATGG-3'</td>
<td>352 bp (976-1327)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VA4-R</td>
<td>5'CATACTAGGGCCTTGACCC-3'</td>
<td></td>
</tr>
<tr>
<td>vacA signal</td>
<td>s1/s2&quot;</td>
<td>VA1-F</td>
<td>5'ATGGAAATACACAAACACAA-3'</td>
<td>259/286 (797-1055/284-569)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VA1-R</td>
<td>5'CTGCTTAAATGGCAAAAC-3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m1/m2</td>
<td>VAG-F</td>
<td>5'CAATCTGTCTTCAAACACGCAG-3'</td>
<td>567/642 (2071-2640/639-1283)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VAG-R</td>
<td>5'CGGTAATAATATCAGAGG-3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>s2</td>
<td>SS2-Fb</td>
<td>5'GCTAACACGCGAAATATGAC-3'</td>
<td>199 (371-569)</td>
</tr>
<tr>
<td>cagA</td>
<td>cagA+</td>
<td>F1</td>
<td>5'GATAACAGGGAAGCTTTTAGG-3'</td>
<td>349 (1228-1576)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B1</td>
<td>5'CTGCCAAAAAAGTGTTGCGACG-3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B7628</td>
<td>5'AAGAAAGGGCAAGAAGGAAA-3'</td>
<td>335</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B7629</td>
<td>5'ACACAGAAGACGAGCGTTATT-3'</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Oligonucleotides used for typing cag A and vac A.

a. Localized and sequenced a strain 60190
b. located and sequenced in strain 87-203
c and d. The vacA s1 and s2 types differ based on differences in size of the PCR products
c and d. The Tx30a strain sequenced. GeneBank U2940 and Sequencing of the Gene bank strain 60190 U05676
Sequencing of TX30 strain bank strain U29401
g. Used with the reverse primer VA1-R

432
Statistical analysis

The correlation between alleles of vacA and cagA gene present were evaluated by Spearman correlation which is a measure of linear association using serial numbers of each group of alleles and compare these ranges (Spearman rho) using SPSS version 17.0.

RESULTS

In 120 symptomatic patients who underwent endoscopic study, only 69 met the requirements for entry in this study, of whom 21 (30.4%) were male and 48 (69.6%) female aged between 10 and 85 years (mean age 38.5 years). Selected patients were positive for at least two diagnostic tests for the detection of H. pylori, the data are not shown in this work. Patients came from the social strata C and D of the population according to modified Graffar (Méndez-Castellanos 1994). By provision of the Ethic Committee of the Institute of Biomedicine, endoscopic examinations were not performed in healthy people, which precluded the inclusion of control patients in the study.

Genotyping of Helicobacter pylori

Determination by PCR of the genes encoding for the vacuolating toxin VacA or cytotoxin CagA showed that 42 of the 69 patients (60.86%) were positive for one of two such genes. 23/69 (33.33%) of patients showed the vacA gene. The 19/69 (27.33%) were positive for both genes. Strains with the genotype cagA only were not observed. 27/69 (39.13%) of the biopsies did not amplify for any of the initiators used, testing of these strains by PCR technique were done in duplicate to rule out any error in its realization.

Analysis of vacA alleles genotype

In 60.86% (42/69) of patients amplification for different allelic forms of the vacA gene was observed. Percentages of allele isolation were: in 80.95% (34/42) of patients allelic form s1 and 28.57% in (12/42) allelic type s2 was detected. For the middle region of vacA analysis revealed that 95.23% (40/42) were vacA m1, while 33.33% (14/42) were vacA m2 (Table 2). The frequency of combination of the different allelic types of middle and signal vacA regions in the gastric biopsies evaluated, showed that the most frequent combination was m1s1 + m2s2 (61.90%). It is to be noted that 14 out of 42 patients (33.33%) presented infections with more than one strain of H. pylori; being the more frequent combination m1s2 + m2s2 with 16.66% (Table 3).

Table 2. Allele frequencies of the middle region m1 and m2 and signal region s1 and s2 of the vacA gene of Helicobacter pylori in symptomatic patients, Cumaná, Sucre State.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequencies</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1</td>
<td>40</td>
<td>95.23</td>
</tr>
<tr>
<td>m2</td>
<td>14</td>
<td>33.33</td>
</tr>
<tr>
<td>s1</td>
<td>34</td>
<td>80.95</td>
</tr>
<tr>
<td>s2</td>
<td>12</td>
<td>28.57</td>
</tr>
</tbody>
</table>

Table 3. Frequency vacA allelic combinations of Helicobacter pylori in gastric biopsies of patients, Cumaná, Sucre State.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1s1</td>
<td>26</td>
<td>61.90</td>
</tr>
<tr>
<td>m2s1</td>
<td>1</td>
<td>2.38</td>
</tr>
<tr>
<td>m2s2</td>
<td>1</td>
<td>2.38</td>
</tr>
<tr>
<td>m1s1 + m2s1</td>
<td>3</td>
<td>7.14</td>
</tr>
<tr>
<td>m1s2 + m2s2</td>
<td>7</td>
<td>16.66</td>
</tr>
<tr>
<td>m1s1 + m2s2</td>
<td>2</td>
<td>4.76</td>
</tr>
<tr>
<td>m1s1 + m1s2</td>
<td>2</td>
<td>4.76</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

Amplification of cagA gene in samples from biopsy-positive patients was 19/42 (45.23%). The predominance of negative cagA strains was observed only associated with vacA positive strains.

Table 4 shows the frequency of distribution of the various combinations of vacA and cagA gene. Prevalence of cagA negative 23/42 (54.76%) strains were observed. Also shown that 14/42 (33.33%) of patients positive by PCR had mixed infections, predominantly the combination m1s2 + m2s2cagA- with 5/42 (11.90%). The predominant genotype strains m1s1 12/42 (28.57%) were associated with the cagA.
Less virulent strains \(m2s2\) and \(m2sl\), not associated with \(cagA\) gene were detected in low numbers 1/42 (2.38%). Fourteen of the 42 positive strains for either gene, presented mixed genotypes had average double \(vacA\) regions allelic combinations and of these only (7/14) mixed genotypes were associated with \(cagA\) gene, so that highlights the existence of co-infections with different genotypes in the same patient.

Table 4. Frequency of combinations of alleles of \(vacA\) and the \(cagA\) gene in gastric biopsies of patients, Cumaná, Sucre State.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mlslcagA^+)</td>
<td>12</td>
<td>28.57</td>
</tr>
<tr>
<td>(mlsl + m2s1 cagA^+)</td>
<td>3</td>
<td>7.14</td>
</tr>
<tr>
<td>(mls2 + m2s2 cagA^+)</td>
<td>2</td>
<td>4.76</td>
</tr>
<tr>
<td>(mlsl + m2s2cagA^+)</td>
<td>2</td>
<td>4.76</td>
</tr>
<tr>
<td>(mlslcagA^-)</td>
<td>14</td>
<td>33.33</td>
</tr>
<tr>
<td>(m2s2cagA^-)</td>
<td>1</td>
<td>2.38</td>
</tr>
<tr>
<td>(m2slcagA^-)</td>
<td>1</td>
<td>2.38</td>
</tr>
<tr>
<td>(mls2 + m2s2cagA^-)</td>
<td>5</td>
<td>11.90</td>
</tr>
<tr>
<td>(mlsl + m2s2cagA^-)</td>
<td>2</td>
<td>4.76</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>42</strong></td>
<td></td>
</tr>
</tbody>
</table>

Statistically Spearman Rho showed significant correlations \((p < 0.05)\) between alleles of the middle region of \(vacA\), \(ml\) with \(m2\) \((p = 0.255)\) and between \(ml\) and \(s2\) \((p = 0.279)\). Very significant correlations were observed \((p < 0.01)\) between the presence of \(cagA\) gene with the middle regions of \(vacA\) \(ml\) \((p = 0.509)\), \(m2\) \((p = 0.447)\) and significant \((p < 0.05)\) with \(s1\) \((p = 0.319)\) and \(s2\) \((0.263)\), explaining that the presence of \(cagA\) gene may be associated with any of the combinations of these alleles. The \(cagA\) gene showed a direct and significant relationship \((p < 0.05, \ p = 0.253)\) with genotype \(mlsl + m2s2\), as the \(cagA\) gene tends to be associated with the genotype \(mlsl\), it is possible that this combination be favored by this feature.

**DISCUSSION**

The \(vacA\) gene structure allows variations in the vacuolating activity of strains, possesses the region signal \((s1, s2)\), the middle region \((m)\) and a third intermediate region newly assigned as \((i)\). The region \(s\) of \(vacA\) is associated to the domain p33 toxin and influence vacuolating activity and efficiency in forming anion channels, due to the hydrophobic nature of the amino acid residues that are close to the proteolytic cleavage site (McClain et al. 2001), the \(s1\) variant contains more hydrophobic amino acids near the region of cleavage that \(s2\) variant, which allow better integration into the membrane of the host cell (McClain et al. 2001). The middle region \((ml\) and \(m2)\) (Atherton et al. 1997) is in the domain p55 of the toxin and influence tropism of \(H.\ pylori\) in the host cells: the region \(ml\) is toxic to a wide range of host cells (Amieva and El-Omar 2008). Combinations of these regions result in strains that may be more or less virulent. Among the genotypes \(s1\), the strains \(slml\) are more toxic that strains \(slm2\) (Letley et al. 2003). The \(s2\) form has a short N-terminal peptide in the mature protein, which blocks the biological activity of \(VacA\) (Letley et al. 2003). Strains \(vacA\) \(m2s2\) encoding proteins of low toxicity and are not often associated with peptic ulcer or gastric cancer. It has been described that vacuolating activity \(slml\), depend on the type \(il\), being associated with peptic ulcer or adenocarcinoma (Basso et al. 2008). The \(s2ml\) combination is rare (Salama et al. 2007) but has been reported in Chile (Martinez et al. 2001), Colombia (Citelly et al. 2002) and Cuba (Torres et al. 2009). In this work was not detected the presence of unique \(vacA\) allelic genotypes, strains without a single allele were found, but combined, unlike those reported by Ghose et al. (2005), in strains of \(H. pylori\) isolated from different Venezuelan populations, especially Amerindians Piaroas and Guahibos. Recently Chiurillo et al. (2013) found genotypic diversity of \(H. pylori\) in patients from western Venezuela with more than one allele of \(vacA\).

The results of this study established that the allelic combination of \(vacA\) most frequent was \(slml\) (69.90%; 26/42), lower than that reported by Ortiz-Prinçez et al. (2010), but higher than that observed by Perrone et al. (2009), both studies carried out in other regions of Venezuela. The genotype \(mlsl\) has established itself as the most frequent in other Latin American countries (Martinez et al. 2001, Vega et al. 2010). In other Latin American countries such as Mexico, Argentina, Colombia, were found patients colonized with strains possessing multiple \(vacA\) genotypes. This may be a result of the acquisition of the bacterium from childhood, domestic infection and transmission route oral and fecal of different strains that can coevolve on the individual
as demonstrated in murine models (Mane et al. 2010),
establishing synergies between them favoring the
permanence of the strains in the niche and a relationship
with the host that allows colonization indefinitely. Ghose
et al. (2005) and Salama et al. (2007) State that these
variations can be innergeographics, i.e. as a result of
isolation and/or progressive contact between different
geographical locations.

In this study, 37.68% of biopsies not amplified for any
gene despite the positivity of biopsies for the presence
of H. pylori. This not genotyping has been observed in
works by other authors (Gatti et al. 2005, Jafari et al.
2008, Torres et al. 2009) and has been suggested that non-
typeable strains, is due to the existence of subfamilies
allelic forms s and m that are not recognized by the
primers available today. Atherton et al. (1999), failed to
establish middle regions of 22 strains from patients from
South America and Asia, by changes in the alignment in
the middle region divider, so they suggested the design of
a new genotyping for these populations.

In our study, the frequency of strains m2s2 was
low compared with those reported by Ortiz-Princz et
al. (2010) in another study in Venezuela. Furthermore,
not only the presence of strains mls2 was detected,
but a percentage of mixed infections in 33.33% of
biopsies and genotyping within these coinfections were
observed mls2 strains mainly associated to mls1 and
m2s2 genotypes. Detecting strains with genotype vacA
mls2, was consistent with other studies which detected
this genotype (Kidd et al. 1999. Morales-Espinosa et
al. 1999, Panagua et al. 2009, Sugimoto and Yamaoka
2009, Torres et al. 2009). The pathogenicity of this
genotype is not well defined, because it has a selective
disadvantage to develop disease (Francesco et al. 2009),
but it is known that the m1 allele is associated with high
virulence strains (Atherton et al. 1995).

Co-infection with different strains, especially
combinations of s1m1, with s1m2 strains in one patient,
have been reported by several authors in different
countries (Morales-Espinosa et al. 1999, González-
Valencia et al. 2000). The results of this study establish
that 33.33% of the genotyping showed that patients had
multiple colonization, which is consistent with previous
observations (Taylor et al. 1995, Chen et al. 2003),
who state that the rates of co-infection are higher in
countries with a high prevalence of H. pylori, as has been
established for Venezuela (Cavazza et al. 2001, De Sousa
et al. 2006). Another factor to consider for variations
of strains and co-infection is ethnicity in Venezuela,
in addition to being variable according to its historical
process as a nation, geographical position places it at the
gateway of people from other continents especially in
the early twentieth century, they settled in the country
or continued to other areas of the continent which would
allow the exchange of strains of Helicobacter among
different human groups (Atherton and Blaser 2009,
Mahomed et al. 2009, Sugimoto and Yamaoka 2009,
Yamaoka 2009).

Most biopsy specimens were negative for the
presence of cagA gen (72.5%), similar results were
observed in countries of Asia, Europe and Africa (Letley
et al. 1999, Maeda et al. 1999, Yamaoka et al. 1999,
2008) rather than in Western countries. It has been noted
that the populations of Western countries are exposed to
antibiotic consumption from an early age which would
have a cumulative effect and selection in strains of H.
pylori colonizing the stomach (Perez-Perez et al. 1990,
Marais et al. 1998) favorable for cagA negative strains that
are less susceptible to treatment by antibiotics than cagA
positive strains (Perez-Perez et al. 2001). The decline of
the strains cag+ according to Perez-Perez et al. (2002) in
a study conducted in Finland, is due to socio-economic
development which results in low transmissibility of
strains especially in childhood and adulthood decline.
Another hypothesis is that the loss of cagA gene is an
adaptive way to the bacteria remain in the host, because if
the damage persists severely as to reach a metaplasia,
the bacteria disappear for not having receptors for adhesion
(Yahiro et al. 1997). cagA association was specially
found with the middle regions of vacA m1 and m2 and
signal sequences s1 and s2, being the most frequent
combination of strains mls1cagA+. These results
coincide with those observed by Citelly et al. (2002) in
Colombia, Martins et al. (2005) in northern Brazil, Torres
et al. (2009) in Cuba. While this genotype mls1cagA+
is highly associated with severe gastrointestinal disease,
its association with other genotypes allows is linked to
inflammatory gastric diseases increasing virulence of
these genotypes (Van Doorn et al. 1999, Kidd et al. 2001,
Zambon et al. 2003), the association of cagA mainly
observed with the combined genotype mls1 + m2s2,
explain in patients with chronic gastritis the virulence of
the combined genotype.

The association among the most virulent vacA
genotypes and cagA gene may be a coincidence or a
preference of cagA for vacA genotypes. However, the
idea that the cag pathogenicity island containing the
cagA gene is a genomic island unstable and should prefer
virulent vacA alleles during insertion into the genome
Detection of cagA gene and typing vacA gene of Helicobacter pylori...

(Nagiyev et al. 2009) is further assumed. In addition to the synergy of these virulence factors to produce pathogenic effect (Argent et al. 2008, Oldani et al. 2009), consider that there is no single polymorphism in vacA, but also in the cag PAI, as evidence of polymorphism among others, the IL-1B gene are associated factors for the initiation and amplification of the inflammatory response in chronic infection with H. pylori (Yamaoka et al. 1996).

Statistically it was showed significant positive correlations between alleles of the middle region vacA m1 and m2, between signal region s2, this result showed polymorphism having vacA in the population studied, where the observed genotypes showed allelic combinations middle regions and signal, being the most frequent genotype m1s1, followed by m2s2, these allelic combinations have been observed preferably in the Americas (Torres et al. 2009). Although it has been widely established that the region m2 of vacA has little toxic activity in vitro that affect the tropism of the bacteria. Pagliaccia et al. (1998) demonstrated in RK-12 cells that this form of H. pylori induced vacuolation, thus the genotypes m1s2 and m2s2 may have activity induce infectious disease processes.

Correlations between vacA allelic regions with cagA and the combination of these genes can decrease the effects of each of the toxins by themselves, so as to lengthen the survival time in infected host cells (Argent et al. 2008). CagA and VacA are the most studied virulence factors of H. pylori, both toxins have a high degree of polymorphism and the evidence shows that this polymorphism, alone or together, is responsible for strains of H. pylori may affect patients with varying severity. This would explain why the presence of genotypes known by its virulence have not observed in this study patients with severe gastric diseases such as cancer or metaplasia.

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