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

DETECCIÓN DEL GEN *cagA* Y TIPIFICACIÓN DEL GEN *vacA* DE *Helicobacter pylori* EN BIOPSIAS DE PACIENTES CON SINTOMATOLOGÍA GÁSTRICA EN CUMANÁ, ESTADO SUCRE, VENEZUELA

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ABSTRACT

Helicobacter pylori has been associated with gastric diseases such as gastritis, ulcers, lymphoma gastroduodenal and is considered a risk factor for the development of gastric cancer. The aim of this study was to characterize *vacA* genotypes and to determine the presence of *cagA* gene in biopsies from patients with gastric symptoms treated at the University Hospital Antonio Patricio de Alcalá of Cumaná, Venezuela. Sixty nine patients with endoscopic indication were evaluated using the polymerase chain reaction (PCR). Only 42 (60.86%) of those patients were positive for amplification of *vacA* and/or *cagA* and of these, 54.76% amplified for both genes. The predominant genotype was *m1s1* (61.90%) followed by mixed infections (33.33%), being the most frequent associations among strains with genotypes *m1s2*, *m2s2* and *m1s1*. From the biopsies performed 72.5% were negative for the *cagA* gene. However, this was found to be associated mainly to genotype *m1s1* (17.39%). Statistically highly significant correlations were found among the allelic forms *m1* and *s1* ($\rho = 0.732$), *s2* and *m2* ($\rho = 0.643$), significant *m1* and *s2* ($\rho = 0.279$). Gene *cagA* showed highly significant correlations with *m1* ($\rho = 0.509$), *m2* ($\rho = 0.447$) and significant with *s1* ($\rho = 0.319$) and *s2* ($\rho = 0.263$). These results show the existence of co-infections with different genotypes of *H. pylori* in the studied population. They, would explain in part the low incidence of severe gastric pathologies despite being present in the region virulent genotypes of this bacterium. The high percentage of coinfections observed could be the result of the influence of geographic and ethnicity factors in the region where the study was conducted..

Key words: *H. pylori*, genotyping, gastritis.

RESUMEN

Helicobacter pylori, ha sido asociado a patologías gástricas y es considerado un factor de riesgo para el desarrollo de cáncer gástrico. El objetivo de este estudio fue caracterizar los genotipos de *vacA* y la presencia del gen *cagA* en biopsias provenientes de pacientes con sintomatología gástrica atendidos en el Hospital Universitario Antonio Patricio de Alcalá de Cumaná, Venezuela. Se evaluaron 69 pacientes con indicación endoscópica, empleando la reacción en cadena de la polimerasa. Sólo 42 de ellos (60,86%) mostraron ser positivos para la amplificación de *vacA* y/o *cagA* y de éstos el 54,76% amplificó para ambos genes. El genotipo predominante fue *m1s1* (61,90%) seguido de infecciones mixtas (33,33%), siendo más frecuentes las asociaciones entre cepas *m1s2* con los genotipos *m2s2* y *m1s1*. El 72,5% de las biopsias fueron negativas para el gen *cagA*. Sin embargo, este se encontró asociado principalmente al genotipo *m1s1* (17,39%). Estadísticamente, se encontraron correlaciones muy significativas entre las formas alélicas *m1* y *s1* ($\rho = 0,732$), *s2* y *m2* ($\rho = 0,643$), significativas *m1* y *s2* ($\rho = 0,279$). El Gen *cagA* mostró correlaciones muy significativas con *m1* ($\rho = 0,509$), *m2* ($\rho = 0,447$) y significativas con *s1* ($\rho = 0,319$) y *s2* ($\rho = 0,263$). Estos resultados muestran la existencia de coinfecciones por distintos genotipos de *H. pylori* en la población estudiada, que explicaría en parte la baja incidencia de patologías gástricas severas a pesar de estar presente en la región genotipos virulentos de esta bacteria. El elevado porcentaje de coinfecciones observado podría ser el resultado de la influencia de factores geográficos y de etnicidad de la región donde se realizó el estudio.

Palabras Clave: *H. pylori*, genotipficación, gastritis.

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 pathologies associated with *H. pylori* is likely

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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 s 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 Cumana, 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.), them 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 95ºC for 5 minutes. Subsequently 1 V of chloroform: phenol: isoamyl 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 $MgCl_2$, 0.2 mM each deoxynucleotide (Gibco, BRL), 0.5

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 for 1 minute, 55 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).

Gen and amplified region	Genotype	Primers designating	Primers sequence	Size of PCR products (Location)
***vacA* r meddle**	*m 1*	VA3- F	5`GGTCAAAATGCGTCATGG-3`	290 bp (2741- 3030)[a]
		VA 3-R	5`CCATTGGTACCTGTAGAAA-3	
	m 2	VA4-F	5´GGAGCCCCAGGAAACATTG-3´	352bp (976-1327)[b]
		VA4-R	5´CATAACTAGCGCCTTGCACC-3´	
***vacA* signal**	*s1/s2*[a]	VA1-F	5´ÁTGGAAATACAACAAACACA-3´	259/286 (797-1055[c]/284-569)[d]
		VAI-R	5´CTGCTTGAATGCGCCAAA-C3´	
	m1/m2	VAG-F	5´CAATCTGTCCAATCAAGCGAG3´	567/642 (2071-2640[e]/639-1283[f]
		VAG-R	5´GCGTAAAATAATTCCAAGG-3´	
	s2	SS2-F[b]	5´GCTAACACgCCAAATgATC-3´	199 (371-569)[g]
cagA	*cag A+*	F1	5´GATAACAGGCAAGCTTTTGAGG-3´	349 (1228-1576)[h]
		B1	5´CTGCAAAAGATTGTTTGGCAGA-3´	
		B7628	5´AAGAAAGGCAAGAAGCAGAAA-3´	335
		B7629	5ÁCACAGAAGACAGAGCGTTATT-3´	

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 f TX3O genebank strain U29401

g. Used with the reverse primer VA1-R

h. Sequenced in strain ATCC 53726 (Gene Bank L117714)

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 alelles 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* 26/42 (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.

Allele	Frequencies	Percentage
m1	40	95.23
m2	14	33.33
s1	34	80.95
s2	12	28.57

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

Genotype	Frecuency	Percentage
m1s1	26	61.90
m2s1	1	2.38
m2s2	1	2.38
m1s1+m2s1	3	7.14
m1s2+m2s2	7	16.66
m1s1 + m2s2	2	4.76
m1s1+m1s2	2	4.76
Total	42	

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 *m2s1*, 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.

Genotype	Frecuency	Percentage
m1s1cagA+	12	28.57
m1s1 + m2s1 cagA+	3	7.14
m1s2 + m2s2 cagA+	2	4.76
m1s1 + m2s2cagA+	2	4.76
m1s1cagA-	14	33.33
m2s2cagA-	1	2.38
m2s1cagA-	1	2.38
*m1s2 + m2s2*cag A-	5	11.90
m1s1 +m2s2cagA-	2	4.76
Total	42	

Statistically Spearman Rho showed significant correlations ($p < 0.05$) between alleles of the middle region of *vacA*, *m1* with *m2* ($\rho = 0.255$) and between *m1* and *s2* ($\rho = 0.279$). Very significant correlations were observed ($p < 0.01$) between the presence of *cagA* gene with the middle regions of *vacA m1* ($\rho = 0.509$), *m2* ($\rho = 0.447$) and significant ($p < 0.05$) with *s1* ($\rho = 0.319$) 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 ($p < 0.05$, $\rho = 0.253$) with genotype *m1s1+m2s2*, as the *cagA* gene tends to be associated with the genotype *m1s1*, 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 (*m1* 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 *m1* 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 *s1m1* are more toxic that strains *s1m2* (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 *s1m1*, depend on the type il, being associated with peptic ulcer or adenocarcinoma (Basso *et al.* 2008). The *s2m1* combination is rare (Salama *et al.* 2007) but has been reported in Chile (Martínez *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* solated 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 *s1m1* (69.90%; 26/42), lower than that reported by Ortiz-Princz *et al.* (2010), but higher than that observed by Perrone *et al.* (2009), both studies carried out in other regions of Venezuela. The genotype *m1s1* has established itself as the most frequent in other Latin American countries (Martínez *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 *m1s2* was detected, but a percentage of mixed infections in 33.33% of biopsies and genotyping within these coinfections were observed *m1s2* strains mainly associated to *m1s1* and *m2s2* genotypes. Detecting strains with genotype *vacA m1s2*, was consistent with other studies which detected this genotype (Kidd *et al.* 1999, Morales-Espinosa *et al.* 1999, Paniagua *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 highest 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 *m1s1cagA* +. 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 *m1s1cagA* + 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 *m1s1* + *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

(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* and 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 despite 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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