Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

Effect of Interleukin (IL-1B) Gene Polymorphism with the Development of *H. Pylori* Infection and Treatment Efficiency in Iraq

Nada Saad Omer
University of Çankırı Karatekin Department of Biology, Molecular biology ,
nadasaad7997@gmail.com

Alaq Kareem Jawad Kinanah
University of Çankırı Karatekin Department of Microbiology
Alaq93kareem@gmail.com

Yaqot Raad Wahabe
Yanka kupala state university, Department of Microbiology, Alnakkash

Abstract

There are numerous transcription factors and cytoplasmic signals that influence the expression of the IL-1B gene and its translation into protein. As a result, there are three key processes in the development and release of IL-1B, which include: Bioactive pro-IL-1B is produced in (I). caspase-1 cleaves pro-IL-1B, resulting in the physiologically active mature version of IL-1B. (III) The maturation of IL-1B and its release. Therefore, caspase-1 is critical to the processing of pro-IL-1B and the quick release of mature IL-1B from the cell. However, the process of secretion is still a bit of an open question. There are various ways in which IL-1B can be secreted and these cannot be mutually exclusive, but each of them can contribute to IL-1B-dependent inflammation in unique and distinct ways.

Among the virulence factors of *H. pylori*, two proteins differ in their expression among isolates of this microorganism. The vacuolizing cytotoxin VacA has been associated with more severe disease. The toxin is encoded by the vacA gene that is present in all isolates, whether or not they produce the toxin. The CagA protein is related to differences in the disease process that occurs, when it is a cagA + or cagA-28 strain. The CagA cytotoxin is encoded by the cagA gene, which seems to be part of a region with other genes called Isla of pathogenicity (PAI)

Introduction

Marshall and Warren were able to isolate from the gastric mucosa of patients with chronic gastritis and peptic ulcer a curved bacterium with an appearance similar to that of campylobacteria

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

(Boyanova, 2011). Initially, this agent was named Campylobacter pyloridis according to its morphological characteristics and its location: bacteria similar to that of genus Campylobacter present in the mucosa of the pyloric antrum. In 1989, Campylobacter pylori and a similar organism, Campylobacter mustelae, isolated from the gastric mucosa of ferrets, were included by Goodwin et al. in a new genus called Helicobacter. PAMPs or DAMPs must be present to trigger the rapid processing and release of IL-1B, which is controlled by the interleukin-1 converting enzyme (ECI) active caspase-1. caspase-1. A cysteine protease, this enzyme has two subunits of 10 KDa and two 20 KDa and its activation requires the assembly and activation of inflammosomes, multiprotein complexes containing an intracellular adapter protein associated with ASC and NLR apoptosis whose stimulus induces rapid and efficient transformation of IL-1B and its subsequent release. Lacking a second stimulation, cells progressively secrete mature IL-1B, which is then processed by cells outside the cell's membrane. The processing and quick release of IL-1B is regulated by active caspase-1, also known as interleukin-1 converting enzyme (ECI), once the creation of pro-IL-1B is obtained from PAMPs or DAMPs. A cysteine protease, this enzyme has two subunits of 10 KDa and two 20 KDa and its activation requires the assembly and activation of inflammosomes, multiprotein complexes containing an intracellular adapter protein associated with ASC and NLR apoptosis whose stimulus induces rapid and efficient transformation of IL-1B and its release.

Different species of the genus Helicobacter can be found in humans and other mammals. *Helicobacter pylori* is described as an S-shaped or curved rod-shaped gram-negative rod (0.5-0.9 μm wide and 2-4 μm long) and with one to three coils. Other forms of *H. pylori* have been observed, in culture or in vivo, such as coccoid, V-shaped or U-shaped, and also straight; in solid media cultures the rod shape predominates. Under the light microscope, *H. pylori* shows between 5 and 7 polar flagella. These bacilli are mostly mobile, catalase and oxidase positive, capable of rapidly hydrolyzing urea, and require a microaerobic atmosphere to grow. This bacterium has a high affinity for the cells of the gastric mucosa, probably as a consequence of its ability to bind to a specific receptor that has been linked to the Lewis-type receptors of blood groups. It colonizes only the surface of the gastric mucosa without invading the tissues. It is not found in mucinous cells or in areas of intestinal metaplasia.

Variability in gene order is a unique feature of *H. pylori* compared to other well-studied gram-negative bacteria (Fangrat, 2006).

Mechanisms of pathogenicity of *H. pylori*

Although the reservoir of this bacterium is not yet well known, it is known that the infection occurs orally. In such a way that when an *H. pylori* inoculum reaches the gastric cavity, it crosses the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

mucus layer and is placed in close contact with the epithelial cells of the gastric mucosa, triggering an inflammatory reaction at this level called acute gastritis that is fundamentally characterized by an infiltrate of polymorphonuclear cells and that is clinically translated by a difficult-to-diagnose symptomatology consisting of a dyspeptic syndrome characterized by nausea, gastric heaviness, epigastric discomfort, belching, etc., which usually lasts approximately 5-7 days. This clinical picture was already described at the beginning of the 20thcentury by Olsen.(De Falco, 2015)

The pathogenesis of *H. pylori* in gastric carcinogenesis

The virulence of *H. pylori*, host genetic vulnerability, and a gastric environment susceptible to the development of gastric cancer all play a role in the development of gastric carcinogenesis in individuals infected with *H. pylori*(Meng, 2017).

When the host's intracellular signalling pathways are disrupted by *H. pylori*, it can lead to cancerous transformation. CagA (cytokine-associated gene A) and its pathogenicity island (cag PAI), vacA (virulence-associated gene A) are among the most important bacterial pathogens (vacuolar cytotoxin A). The cag PAI and the cagA C. difficile virulence factor cag PAI, which is 40 kb long and contains 27-31 genes, is the most well understood H.pylori component. CagA, the island's final gene, is highly immunogenic. More severe inflammation, gastric atrophy, and a larger risk of gastric adenocarcinoma have been related to cagA-positive strains (cagA-PAI) than cagA-negative strains. This protein is a powerful inhibitor of T cells in vitro, as well as having a number of other functions, such as forming pores in membranes, releasing cytochrome C from mitochondria, leading to apoptosis, and binding cell membranes to receptors that result in an inflammatory response. More than half of the vacA genes, which encode a porin that attaches to the epithelium through an interaction with protein tyrosine phosphatase, are expressed in all *H. pylori* strains(Kim, 2011).

An important cytokine in the host's defence response to infection and damage, Interleukin-1B (IL-1B) is a powerful pro-inflammatory agent. Furthermore, IL-1B is the most investigated and characterised member of the IL-1 family. T cells, endothelial cells, and fibroblasts all generate and emit this cytokine, despite the fact that most investigations have concentrated on monocytes and macrophages (Kalsoom, 2020).

To note, most macrophages, phagocytic cells in many tissues, are produced from stem cells of the granulocyte-monocytic lineage in the hemopoietic marrow, where they are exposed to cytokines and mature. Cellular cytokines are expressed on the membrane of stem cells during their transformation into macrophages. They digest waste items from tissues and remove apoptotic cells, but their primary job is to phagocytose invading foreign agents. As a result, it is critical for the growth of tissues and the maintenance of homeostasis, as well as for immunity(Figueiredo, 2002).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

cytokines are produced by macrophages, which are exposed to inflammatory stimuli and produce cytokines themselves. Cells from other parts of the innate immune system, however, also produce these molecules. Chemokines, leukocytes, prostaglandins, and complement are also released by macrophages. They all work together to open up blood vessels and bring in inflammation-inducing cells. Since there are so many different stimuli, they can be categorised into pathogen-associated molecular patterns (PAMPs) and damage-associated molecular pattern molecules (DAMPs) (DAMPs). Aside from PAMPs like LPS, LTA or flagellin, pathogenic microbes are also detected through DAMPs such S100 proteins, which are secreted by injured cells as an endogenous signalling ligand. PAMP and DAMP molecules can both contribute to the release of IL-1B in the context of bacterial infections, which are associated with tissue destruction and the release of intracellular components.

Materials and Methods

Materials and Sample collection

Chemical: all the chemicals (Ex: 0.1 M Tris HCl (pH 7.5)/ 1% SDS/proteinase K solution/PCR buffer (50 mM KCl,)/ Tris-HCl, pH 8 .4), $MgCl_2$, Dntp, etc) used in this study purchased from Sigma-Aldrich (USA), Merck (Germany), with 95% to 99% purity in AR grade quality. All reagents and chemicals prepared with double distilled water with pH 7.02

Study design: This study is a case-control studyand this is a prospective cross-sectional descriptive observational study based on the standard clinical practice model.

Study Population; Patients referred on an outpatient basis to the Digestive Endoscopy Unit of the Al Mosul Governorate,who attended outpatient clinics at Ibn Sina Teaching hospital in Mosul,Iraq for gastroscopy, from February 2021 to June 2021 there were a total of 60 patients.Individuals who had a diagnosis of *H. pylori* through urease test.

Those who met the following criteria were excluded:

1. Serious general condition or from the Emergency Service.
2. People who have contraindicated taking biopsies due to coagulation disorders or who are being treated with anticoagulants or antiplatelet agents.
3. Minors. With these criteria and during this time interval, upper gastrointestinal endoscopy was performed in 190 patients.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

Sample (Patients) selection

- A group of 30 people who were infected with *Helicobacter pylori* and were treated but did not recover. A group of 30 people infected with *Helicobacter pylori* was treated and recovered

- For technical reasons, only 3 patients per day could be cultured in the Microbiology Service. The first patients who did not present exclusion criteria were selected.
- To try to make an approximation to the conditions of routine clinical practice, only exclusion criteria were considered.

Control group

To know the prevalence of *H. pylori* infection in our environment and thus confirm that our study population is comparable to the general population, 30 contemporary individuals were included in the study (the data were obtained during the same period of time as the Patient group). These people were randomly selected from among the companions of patients who attended outpatient clinics atIbn Sina Teaching hospital in Mosul, Iraq. To avoid confounding factors, the inclusion criteria in this group were the following:

1. Absence of digestive symptoms at the time the test was performed.
2. Lack of a previous history of peptic ulcer disease.
3. Not having followed any treatment with antibiotics or gastric antisecretors for at least 30 days prior to the test.

The control group was matched for age and sex with the sample group.

Methodology

A blood sample collection: Assesses the presence of antibodies (cells that fight infection) against H. pylori. The screening method used to check for *H. pylori* infection is (urea breath test)

Procedure: Blood will be drawn from a vein in your arm by a health care professional using a small needle. A small amount of blood will be taken after the needle is inserted into a test tube or vial. After collecting venous blood samples, we perform isolation of DNA from venous blood for sampling. Determining the genetic structure to be examined for the interleukin gene (IL-1B).

DNA extraction from blood: The method used to extract DNA from blood was using The ChargeSwitch® gDNA Purification Kit.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

The Invitrogen ChargeSwitch gDNA Tissue Mini (Carlsbad, CA) was put to the test. 5 ml of blood was utilised to extract genomic DNA from a buffy coat. The manufacturer's instructions were followed exactly for the DNA extraction.Finally, the sample was incubated at a temperature of -70º C for 30 minutes and, after this period, centrifuged for 15 minutes at 1500 rpm. The solution was decanted and the pellet left to dry. Then, it was resuspended with a buffer solution (Tris – EDTA) in a volume of 30 □ L and placed in a water bath at 37ºC for one hour. The sample was then stored at a temperature of -20°C.

Polymerase chain reaction using primers to detect the human beta-globin gene for internal control of the reaction: The polymerase chain reaction to detect the human beta globin gene followed the method described byHe et al. (2002). This amplification aimed to analyze the presence and quality of DNA extracted from gastric tissue samples, ensuring an internal quality control of the experiment, which was essential for the safety of the results obtained. Forty cycles of amplification were performed for each sample in a thermocycler ("DNA Thermal Cycler" Perkin Elmer/Cetus, Norwalk, Conn, USA), observing the following temperatures and their respective times: initial denaturation at 94°C for 5 minutes, denaturation at 94º C for 30 seconds, annealing at 55º C for 1 minute, extension at 72º C for 2 minutes, and finally, final extension at 72º C for 8 minutes. Primers PCO3 and PCO4 were used, resulting in a final amplification product of 110 base pairs. Next, the sequence of primers used, which flank a conserved region of the human beta-globin gene, is shown. (Table 1).

Table 1– Primers for human beta-globin gene

First	Sequence (5' - 3`)	Sense
PCO3	CCG CAA CTG TGT TCA CGA GGC	sense
PCO4	CGC CGG CAT CTA CGT TGC ACC	antisense

Visualization of DNA in amplified fragments

To assess the positivity of *Helicobacter pylori* DNA in the amplified fragments, after the three amplification reactions carried out for each sample (detection, urease C gene and urease B gene), the reaction products were submitted to electrophoresis in a horizontal vat, containing gel of 2% agarose stained with ethidium bromide. After electrophoresis, each gel was observed under ultraviolet light. In the positive samples, fragments of 345 base pairs in the detection reaction, 798 base pairs in the urease C gene region and 759 base pairs in the urease B gene region were observed, comparing to the ladder (molecular marker).Each gel was photographed using the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

Polaroid□ □ system.

Genotyping for the amplified regions of the urease C and urease B genes –Restriction Fragment Length Polymorphism

Analysis of fragments of the urease C and urease B genes obtained by nested PCR and PCR, respectively, demonstrate that the genomic structure of *H. pylori* is highly diverse when the restriction enzymes Mbo I and Hae III are used.Therefore, after the amplification of the bacterial DNA in the urease C and urease B gene regions, the products obtained were submitted to enzymatic digestion. The enzyme Mbo I was used for the products obtained in the amplification of the urease C gene region, and the Hae III enzyme for the amplified products of the urease B gene region. The total volume for each reaction, regardless of the amplified region and the enzyme used, was 20.0 □ L, being 7.0 □ L of the product of nested PCR (in the case of the urease C gene) or PCR (in the case of the urease B gene), 2.0 □ L of the specific buffer for each enzyme, 0.5 □ L of the enzyme (Mbo I for the urease C gene amplification product and Hae III for the urease B gene amplification product) and 11.5 □ L of distilled water. Then, the samples were vortexed and placed in a bath at a temperature of 37^{o} C, where they were left for 18 hours, in order to allow the digestion of the nested PCR and PCR products.After this period, the fragments obtained from the digestion were visualized under ultraviolet light in a 1% agarose gel stained with ethidium bromide and photographed in a Polaroid□ system.

SNP selection, polymorphism genotyping and data analysis

The SNPs of the IL-1B interleukin gene were searched in the NCBI (National Center for Biotechnology Information) database in articles published through the keywords "Gastric+cancer+IL-1beta+and+SNP", where the polymorphisms rs16944 and rs1143627 were associated with the development of gastric cancer in different populations.

Thus, the two polymorphisms IL-1BF1 (rs16944) and IL-1BE1 (rs1143627) of the SNP type in the proinflammatory cytokine gene IL-1B were genotyped using probes labeled with fluorophore VIC/FAM (Real Time PCR, Life Technologies, CA, USA) and samples genotyped according to the manufacturer's protocol recommendations.

Subsequently, the molecular analysis of the two SNPs was performed by Real-Time PCR with TaqMan® probes (Applied Biosystems®, Foster City, California, USA) using the 7500 Real-Time PCR System (Applied Biosystems) equipment. The TaqMan system uses a set of primers and a fluorescent probe to enable detection of a specific product as it builds up during reaction cycles. The protocol uses 3.5 µL of Master Mix, 0.157 µL of TaqMan probe, 3.325 µL of water and 1.0 µL

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

of DNA. The final mix was amplified with the following program: 10′ at 95°C, 40 cycles of 15″ at 92°C, and 1′ at 60°C. Therefore, the Biostatistics analyzes were carried out using the SPSS v.20.0 program (SPSS, Chicago, IL, USA) following the interpretation of the data. The genomic DNA was isolated from biopsies included in paraffin, prior to treatment with xylene, using the QIA-amp tissue Kit (Qiagen) and from fresh endoscopic biopsies using the Wizard Genomic DNA Purification kit (Promega), followed by each case the manufacturer's recommendations. Assignment of genotypes. The determination of polymorphisms was carried out by chain reaction of the polymerase and restriction fragment length polymorphisms (PCR-RFLP) for IL-1B-511 and IL-1B+3954, and PCR for IL-1RN, according to conditions previously described 16, and summarized in Table 10. The PCR products and their digestion were analyzed by electrophoresis in 2% agarosa gels, stained with ethidium bromide and visualized with UV light. Additionally, PCR amplifications of a 268 bp fragment of the human ß-globin gene17 were carried out to ensure the quality of the DNA samples obtained from the paraffin-embedded biopsies.

Table 2:The study of IL-1B and IL-1RN polymorphisms, PCR primers and conditions were employed.

Polymorphism	Initiators	PCR conditions, restriction enzymes and allele definition*
IL-1BF1 (rs16944)	511F: 5'-TGCCAGTGACCGGATCGC-3' 511R: 5'-GTGTACGAACTCGCCACTT-3'	3 min at 94°C, 35 (40) cycles of 45 (60) sec at 94°C, 45 (90) sec at 57°C, 30 (90) sec at 72°C, followed by 10 min at 72°C; PCR-RFLP (AvaI); Allele: 106 bp and 199 bp; T allele: 305 bp.
IL-1BE1 (rs1143627)	3954F: 5'-GATCTCGCACACGGTGACC-3' 3954R: 5'-TTCAGCAGCTCGAGGAGA-3'	3 min at 94°C, 35 (40) cycles of 45 (60) sec at 94°C, 45 (90) sec at 57°C, 30 (90) sec at 72°C, followed by 10 min at 72°C; PCR-RFLP (TaqI); Allele: 135 bp and 114 bp; T allele: 249 bp.
IL-1RN	RNF: 5'-CTGACCGAGACCCTTA-3'	3 min at 94°C, 35 (40) cycles of 30

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

	RNR: 5'-TCGTCGTGTGAATGTAA-3'	(60) sec at 94°C, 30 (90) sec at 57°C, 20 (90) sec at 72°C, followed by 10 min at 72° Ç; PCR; allele 1= 410 bp (four repetitions), allele 2= 240 bp (from repetitions),

Afterwards, PCR reactions were carried out that amplify different blanks of the *H. pylori* genome (H3-H4: 298 bp and Hp1-Hpx2: 150 bp). The assay was considered positive when at least one of the PCR products was obtained.

Results and Discussion

Results

Table 3 shows the clinical data of patients selected for the current study. Clinical information from patients shows that 72.9% were smokers and gastric cancer patients are 56.6%. Among individuals with data for *H. pylori* infection, 98.33% had no history of infection and 14.7% did. For the histopathological type, 100% were diagnosed with gastric adenocarcinoma. Among the 20 individuals for the Lauren classification, 63% were of the diffuse type and 37% of the intestinal type.

Table 3. Clinical data of patients selected for the current study.

Characteristics	**Sample (n)**	**%**
smoking (n=64)		
Yes	47	73
No	17	27
Chemotherapy (n=35)		
Yes	3	8.8
No	32	91
Gastric Cancer (n=60)		
Yes	34	56.6
No	26	43.3
***H. pylori* infection (n=60)**		
No	59	98.3
Yes	1	1.7

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

Histological Type(n=60)		
adenocarcinoma	60	100
Lauren's rating (n=20)		
Diffuse	13	63
Intestinal	7	37
CA family history (n=26)		
Yes	14	52
No	12	48

Sera from 90 individuals infected with *H. pylori* were evaluated by immunoblotting. Regarding gender, (65) 72 % were male. Mean age was 57.75 ± 11.997years and ranged from 17 to 86 years.Of the 90 infected individuals, 25 (28%) were female patients with gastric cancer (PC), Mean age was 60 ± 15 years and ranged from 22 to 86 years.

In Table 4, demographic variables related to sex, age, genetic ancestry (Iranian based; German Based & Iraq) in case and control groups. No of case group 50 individuals (20 are P30NR & 30 are P30R) were male and 20 female (5 are P30NR & 15 are P30R) where as in control group 20 male and 10 female, there were significant differences between the groups (p = 0.0263). For Age, no of case-to-medium group was 57.75/ 56.27 years for P30NR &P30R and the control group was 53.75 (p = 0.0519). For genetic ancestry, case group in two groups (P30NR & P30R), mean forum: 0.425/0.401 for Iranian Based; 0.275/0.225 for German Based; 0.299/0.264 for Iraq. No group controls mean forum: 0.5468 for Iranian Based; 0.2005 German Based; 0.287034 for Iraq. There are significant differences between the groups for the Iranian Based ancestral ties with (p = 0.00519) and Iraqis (p = 0.00172).

Table 4. There are demographic variations in two case and control groups.

Variables	P30NR	P30R	P30C	***p-value***
Gender (M/F)*	30 (20/10)	30 (15/15)	(20/10)	**0.0023**
Age#(Years)	57.75 ± 11.997	56.27 ± 11.20	53.75±12.349	0.00519
Genetic Ancestry*				
Iranian based	0.425 ± 0.113	0.401 ± 0.024	0.5468±0.1414	**0.00518**
German Based	0.275 ± 0.110	0.225 ± 0.104	0.2005 ± 0.128	**0.00172**
Iraq	0.299 ± 0.108	0.264 ± 0.112	0.287±0.150	0.077

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

Note: P30NR (Helicobacter pylori and were treated but did **N**ot **R**ecover);

P30R Helicobacter pylori and were treated but **R**ecovered; P30C (Control)

*values expressed as mean ± SD (standard deviation).

Significance determined by Fisher's exact test.

#values expressed as mean ± SD (standard deviation).

Significance determined by Mann Whitney test.

Nested PCR for detection of DNA from *H. pylori* bacteria

The standardization of the nested-type polymerase chain reaction for detection of *H. pylori* DNA was performed without complications, with the only need being to adjust the quantities of reagents used so that unspecific bands were not amplified. To confirm the results, the nested reaction was performed in duplicate for all samples, using a positive control and a negative control for bacterial DNA.

The amplification program, with temperatures, reaction cycles and reagent concentrations, is described in the item "Methods".

Amplification occurred in 90 DNA samples extracted from blood sample obtained by biopsy from the total number of patients present in this study.

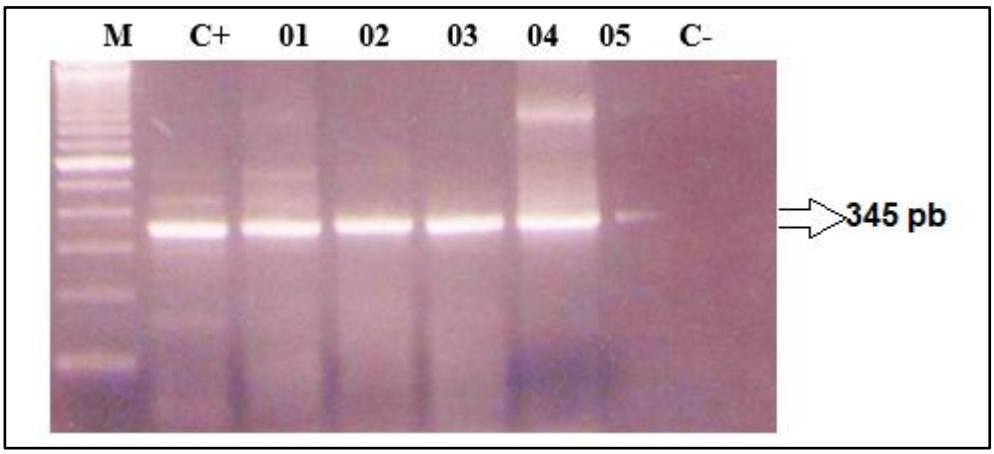

Figure 1 - After electrophoresis on a 2% agarose gel stained with ethidium bromide, the fragments were analysed directly. *H. pylori* detection (345 bp).

M — molecular weight marker for a 100-bp ladder. Positive control (C).

Patients with a score of 1 to 5 are considered to be positive. Negative control (C)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

Nested PCR for amplification of the urease C gene region of *H. pylori*

Amplification of the *H. pylori* urease C gene region was performed for 90 DNA samples, but with some difficulties, with the need for repeated reactions, as the final amplification showed low intensity fragments, providing a final genotyping many inappropriate times. Despite this, standardization was successfully carried out, as described in the item "Methods" of the present work, being possible, consequently, the typing of the obtained products. The best results were obtained with a 1:5 dilution of the primers (ureC 1, ureC 2 and ureC 3). (figure 2).

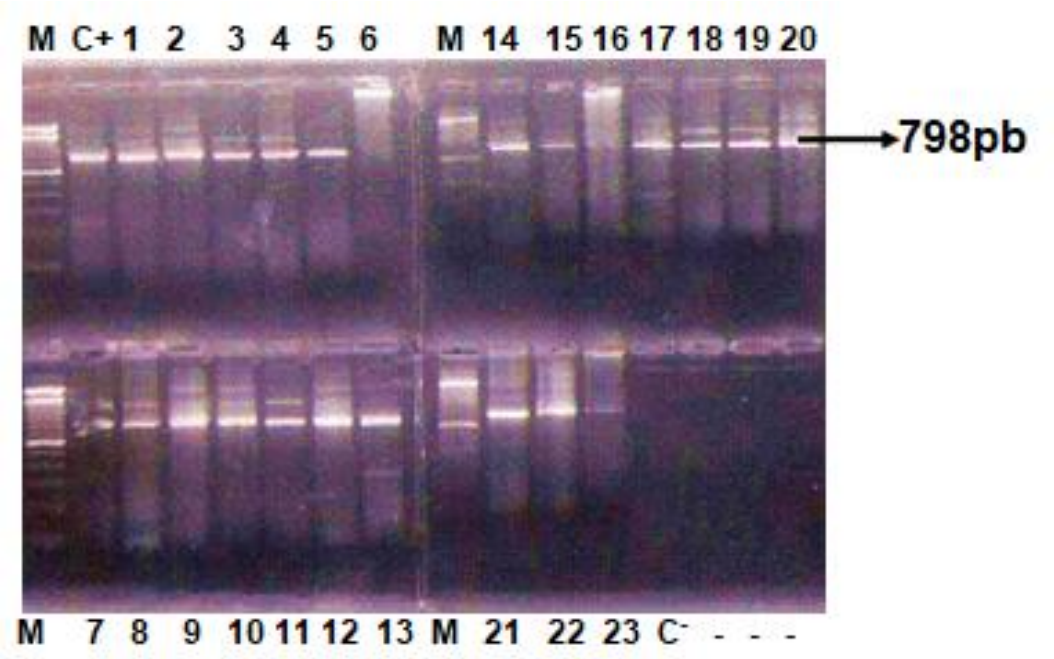

Figure 2 – Direct analysis of the fragments obtained after electrophoresis on a 2% agarose gel stained with ethidium bromide. Amplification of the *H. pylori* urease C gene region (798 bp). M - Ladder molecular weight marker 100 bp. C+ positive control. 1 to 23 – positive samples. C- Negative control.

PCR for the amplification of the urease B gene region of *H. pylori*

Amplification of the *H. pylori* urease B gene region also occurred in all 90 DNA samples, and the standardization of the reaction was performed according to the schemes already described in the item "Methods". The best results were obtained with a 1:5 dilution of the primers (ureB 1 and ureB 2). (figure 3).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

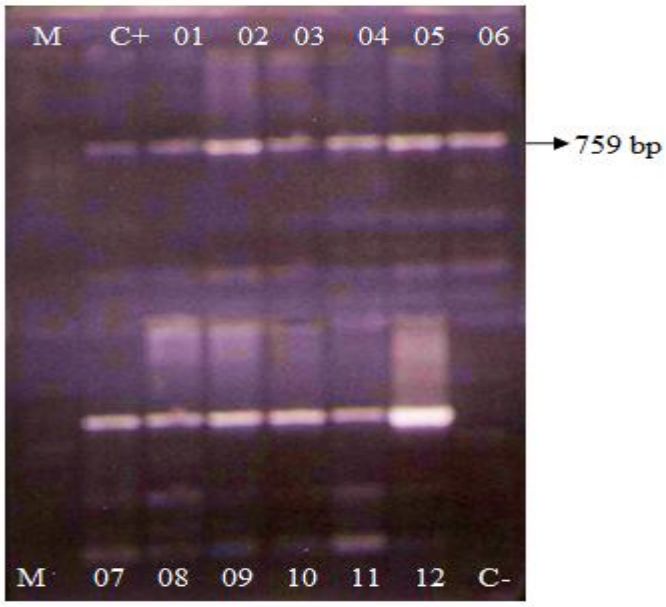

Figure 3 – After electrophoresis on a 2 percent agarose gel stained with ethidium bromide, direct examination of the fragments obtained. The *H. pylori* urease B gene region has been amplified (759 bp). Molecular weight marker 100 bp. Positive control is C+ Samples 1 through 12 are considered positive. C - a reversal of control.

Discussion

Helicobacter pylori affects more than half of the human population(Leja, 2016) and is found in all regions of the world. The World Health Organization classifies *H. pylori* as a Class I carcinogen since it is the primary cause of various gastrointestinal disorders, including chronic gastritis, peptic ulcer, gastric cancer, and gastric MALT lymphoma(Hunt, 2011).. Although the transmission mechanisms are not fully established, it is known that this occurs mainly in childhood, both in developed and developing countries. However, there is evidence that in developed countries the rates of contamination by *H. pylori* are decreasing, causing a decrease in the rates of peptic ulcer cases and even gastric cancer, although there seems to be an increase in the rates of cases of gastric reflux disease and oesophageal cancer. This information led some researchers, such as Sharma, et al. (2003)and Grande, et al (2008) to the hypothesis that the presence of the bacteria in the gastric tract can protect some individuals against the development of certain diseases. Infection with *H. pylori* is universally distributed and has a high prevalence rate. Consequently, an accurate, reliable diagnosis that can be used routinely for the identification and differentiation of *H. pylori* strains is very important. The availability of these methods for monitoring the specific treatment is also essential. In this diagnosis, the determination of the bacterial strain present in each individual is extremely important, not only for the identification of the most prevalent strains in a certain region

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

and in a certain group of patients, but also for the evaluation of patients who were treated for eradication of the microorganism and which, later, were re-infected. In addition, the same individual can be contaminated by more than one strain, one of them being more virulent and, therefore, responsible for the development of gastric cancer. Thus, the present work presents a molecular analysis on the relationship and prevalence of different strains of *H. pylori* obtained from biopsy gastric tissue samples from patients with peptic ulcer or gastritis.

First, nested PCR with primers from an unspecific but conserved region of the *H. pylori* genome was used in order to identify the presence of bacterial DNA in biopsy samples of gastric mucosal fragments. These samples were previously submitted to two routine diagnostic methods used by the Gastrocentro: histology and the urease test. All samples included in our study showed positive results for these tests, confirming the presence of the bacteria. The primers described for this region were described by Pellicano et al. (2016).

In conclusion, our study in this population of Venezuela provides evidence that the homozygous genotype of IL-1RN*2 may play a role in the etiology of GC, with a major contribution of this polymorphism to the development of moderate/bien ADCG differentiated. The potential association of the allelic combination IL-1B-511T–(IL-1B+3954C)–IL-1RN*2 with the nondisposable CG development. On the other hand, additional work is needed with a greater number of samples and implying other variables to deepen the knowledge about the interactions between genes and the environment in the susceptibility to GC.

Not present study, among the individuals diagnosed with gastric cancer (GC), 72.9% Or tobacco is used in different ways around the world. A most common way of using tobacco is or smoking a cigarette that is known to cause various types of cancer including gastric cancer. Various works have shown the direct relationship of the risk of gastric cancer as a tobacco habit, therefore, our results also corroborate these conditions, and the level of consumption may vary between different countries. In a specific study, not from India, or the type of drink consumed, it showed a significant association with the risk and increase in the development of gastric cancer.In our study, there are patients with *H. pylori* infection, 98.3% do not have a history of infection, against 14.7%. *H. pylori* represents the main carcinogen for or development of GC according to the World Health Organization. I know that the inflammation process is a risk factor for many types of cancer. One of the main reasons for chronic inflammation of the stomach and colonization by *H. pylori*. Embora a goal of the population has been infected by *H. pylori*, only 3% are diagnosed with gastric cancer or that can reflect the influence of environmental factors, as well as the genetic background of the host.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3937 - 3952
Received 08 November 2021; Accepted 15 December 2021.

Conclusion

H. pylori infection is recognized as a major public health problem and is very prevalent in Iraq country. It is associated with benign and malignant digestive pathology. There are multiple invasive and non-invasive diagnostic methods. Eradication therapy prevents complications such as digestive bleeding and the development of gastric cancer. There are international recommendations that aim to achieve a minimum eradication rate of 90% with the different schemes, with no preference for one in particular. In our setting, given the rate of resistance to clarithromycin, standard triple therapy should be abandoned, opting for 14-day regimens with high doses of PPIs, combinations of antibiotics and bismuth or, if it is desired to maintain clarithromycin, it should be part of a concomitant quadrotherapy. Once the therapy is completed, eradication must be confirmed and in case of failure to second-line treatment, an evaluation by a specialist should be requested to determine resistance to antibiotics and indicate a personalized scheme based on the result of culture or resistance PCR. Finally, with the emerging evidence regarding the outcomes associated with "massive eradication" in countries with a high prevalence of infection, this option should at least be considered in our environment.The three amplification techniques were standardized and successfully performed for all regions of the *Helicobacter pylori* genome that served as targets for the reactions: nested PCR for bacterial DNA detection, nested PCR for urease C gene and PCR for urease B gene Amplification by nested PCR for detection occurred in a total of 90 samples of gastric ulcer patients obtained by biopsy, samples already positive for the urease test and for histology. Amplification of the urease C gene region by nested PCR was also possible in the 90 samples studied, and the products obtained were analyzed by restriction with the Mbo I enzyme, resulting in 17 genotypes. The most prevalent for cases of gastritis was called UreC Mbo I G, with 110, 120, 250 and 310 base pairs, representing 15.7% of patients with gastritis. For peptic ulcer cases, the most prevalent genotype was called UreC Mbo I E, with 110, 180 and 500 base pairs, representing 24.4% of peptic ulcer cases.

References

1. Boyanova, Lyudmila. (2011). Microbiology and characteristics of *H. pylori*. In book: Helicobacter pylori (pp.29-69)Edition: FirstChapter: Microbiology and characteristics of *H. pylori*Publisher: Caister Academic PressEditors: Boyanova L
2. De Falco, Maria & Lucariello, Angela & Iaquinto, Salvatore & Esposito, Vincenzo & Guerra, Germano & Luca, Antonio. (2015). Molecular Mechanisms of Helicobacter Pylori Pathogenesis. Journal of Cellular Physiology. 230. 10.1002/jcp.24933.
3. Fangrat, Katarzyna & Dzierzanowska, D. (2006). Helicobacter pylori: Microbiology and

interactions with gastrointestinal microflora. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 57 Suppl 3. 5-14.

4. Figueiredo, Ceu & Machado, Jose & Pharoah, Paul & Seruca, Raquel & Sousa, Sonia & Carvalho, Ralph & Capelinha, Ana & Quint, Wim & Caldas, Carlos & Doorn, Leen-Jan & Carneiro, Fatima & Sobrinho-Simões, Manuel. (2002). Helicobacter pylori and Interleukin 1 Genotyping: An Opportunity to Identify High-Risk Individuals for Gastric Carcinoma. Journal of the National Cancer Institute. 94. 1680-7. 10.1093/jnci/94.22.1680.
5. Grande, Michele & Cadeddu, Federica & Villa, Massimo & Attinà, Grazia & Muzi, Marco & Nigro, Casimiro & Rulli, Francesco & Farinon, Attilio. (2008). Helicobacter pylori and gastroesophageal reflux disease. World journal of surgical oncology. 6. 74. 10.1186/1477-7819-6-74.
6. He, Qiang & Wang, Jian-Ping & Osato, Michael & Lachman, Lawrence. (2002). Real-Time Quantitative PCR for Detection of Helicobacter pylori. Journal of clinical microbiology. 40. 3720-8. 10.1128/JCM.40.10.3720-3728.2002.
7. Hunt, Richard & Xiao, S & Megraud, Francis & Leon-Barua, R & Bazzoli, Franco & Van der Merwe, Schalk & Coelho, L. & Fock, M & Fedail, S & Cohen, Henry & Malfertheiner, P & Vakil, N & Hamid, Saeed & Goh, Khean-Lee & Wong, B.C.Y & Krabshuis, Justus & Lemair, Ton. (2011). Helicobacter Pylori in Developing Countries. World Gastroenterology Organisation Global Guideline. Journal of gastrointestinal and liver diseases : JGLD. 20. 299-304. 10.1097/MCG.0b013e31820fb8f6.
8. Kalsoom, Furkhanda & Rahman, Sajjad & Mahmood, Muhammad & Zahoor, Tahir. (2020). Association of Interleukin-1B gene Polymorphism with *H. pylori* infected Dyspeptic Gastric Diseases and Healthy Population. Pakistan Journal of Medical Sciences. 36. 10.12669/pjms.36.4.1883.
9. Kim, Sung Soo & Ruiz, Victoria & Carroll, Jaqueline & Moss, Steven. (2011). Helicobacter pylori in the pathogenesis of gastric cancer and gastric lymphoma. Cancer letters. 305. 228-38. 10.1016/j.canlet.2010.07.014.
10. Marshall, Barry. (2016). A Brief History of the Discovery of Helicobacter pylori.In book: Helicobacter pylori (pp.3-15) doi:10.1007/978-4-431-55705-0_1.
11. Pellicano, Rinaldo & Ribaldone, Davide Giuseppe & Fagoonee, Sharmila & Astegiano, Marco & Saracco, Giorgio & Mégraud, Francis. (2016). A 2016 panorama of Helicobacter pylori infection: Key messages for clinicians. Panminerva medica. 58. 304-317.
12. Sharma, Paras & Vakil, N.. (2003). Helicobacter pylori and reflux disease. Alimentary Pharmacology & Therapeutics. 17. 10.1046/j.1365-2036.2003.01428.x.