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Polymorphic analysis of interleukin IL-1β in patients with a diagnosis of Helicobacter pylori in Tabasco, Mexico

Análisis polimórfico de interleucina IL-1β en pacientes con diagnóstico de *Helicobacter pylori*, en Tabasco, México

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Abstract

Introduction: Studies on the infection of Helicobacter pylori and the IL-1B gene play an important role within inflammation and its proinflammatory properties that promote defense against pathogens. Polymorphisms have been related to a higher production of IL-1β associated with hypochlorhydria and cancer development under H. pylori infection, so the objective of this study was to evaluate polymorphism in the +3954 region (IL- 1B C> T) of the interleukin IL1β gene.
Method: The blood samples were donated and collected to be processed for the determination of H. pylori, by means of the Urease Test (Proindusquim), in the city of Villahermosa Tabasco, Mexico, separating positive and negative samples (control), the positive samples were analyzed by PCR and enzymatic digestion for polymorphic analysis in the +3954 region (IL-1B C> T).
Results: Two hundred and forty-five samples of 78 cases of H. pylori infection were analyzed, 49 were men (62.8%) and 29 women (37.17%), with a mean age of 58.5 years (21-77 years). Genotypic and allelic frequencies observed, 45 individuals were normal homozygotes (CC), 12 rare homozygotes (TT) and 21 heterozygotes (CT), in the control group it was found that 74 individuals were normal homozygotes (CC), 32 were rare homozygotes (TT) and 61 were heterozygous (CT).
Conclusion: This study shows an association between the IL-1β + 3954 (C> T) interleukin polymorphism and the predisposition of the population carrying the homozygous CC genotype infected with H. pylori to develop cancer.

Keywords: Helicobacter pylori, Interleucin, PCR, Polymorphism.

Resumen

Introducción. Los estudios sobre la infección de *Helicobacter pylori* y el gen de IL-1B juega un importante rol dentro de la inflamación y sus propiedades proinflamatorias que promueven a la defensa contra patógenos. Los polimorfismos se han relacionados con una mayor producción de IL-1β asociada a cuadros de hipoclorhidria y desarrollo de cáncer bajo la infección de *H. pylori*, por lo que el objetivo de este estudio fue evaluar el polimorfismo en la región +3954 (IL-1B C>T) del gen interleucina IL1β.
Método. Las muestras de sangre fueron donadas y colectadas para ser procesadas para la determinación de *H. pylori*, mediante Test de ureasa (Proindusquim), en la ciudad de Villahermosa Tabasco, México, separando muestras positivas y negativas (control), las muestras positivas fueron analizadas mediante PCR y digestión enzimática para el análisis polimórfico en la región +3954 (IL-1B C>T).
Resultados. Se analizaron 245 muestras 78 casos de infección por *H. pylori*, 49 fueron hombres (62.8%) y 29 mujeres (37.17%), con edad media de 58.5 años (21-77 años). Las frecuencias genotípicas y alélicas observadas, 45 individuos fueron homocigotos normales (CC), 12 homocigotos raros (TT) y 21 heterocigotos (CT), en el grupo de controles se encontró que 74 individuos eran homocigotos normales (CC), 32 eran homocigotos raros (TT) y 61 fueron heterocigotos (CT).
Conclusión. Este estudio muestran una asociación entre el polimorfismo de interleucina IL-1β+3954(C>T) y la predisposición de la población portador del genotipo homocigoto CC infectado con *H. pylori* desarrolle cáncer.

Palabras clave: *Helicobacter pylori, Interleucina, PCR, Polimorfismo*.

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Introduction

Gastric cancer is one of the most common neoplasms present today, since it has 870,000 new cases worldwide, with Helicobacter pylori infection being one of the causes that triggers this disease, becoming the second cause of death (1) . In this sense, the International Agency for Research on Cancer (IARC) has designated Helicobacter pylori as a Type I carcinogen in humans (2), for which molecular studies have been carried out to understand the etiology and diagnosis of the disease through polymorphic analysis (3,4). Recently, certain genetic polymorphisms such as interleukin 1β have been associated with functional differences in the effect of this proinflammatory cytosine on gastric secretion in individuals with gastric cancer (5, 6, 7, 8, 9). These polymorphisms are found in a frequency lower than 1% of the population, and the most common are SNPs or single nucleotide polymorphisms (10). The term interleukin refers to those cytosines that act in communication between leukocytes (11). IL-1 was originally described as "endogenous pyrogen" due to its ability to provoke a febrile reaction in

rabbits. The interleukin family consists of two pro-inflammatory cytokines IL-1α and IL-1β, the genes of most of the members of the IL-1 superfamily are located on the long arm of chromosome 2, in the 2q12-q21 region. both cytokines are produced by monocytes, macrophages, and epithelial cells. Both exhibit similar biological characteristics that include the host response to microbial invasions, inflammation, and tissue damage (12, 13). Some of the cytokines that participate as mediators of the inflammatory response against H. pylori have been associated with an increased risk of developing precancerous gastric lesions and gastric cancer (14, 15,16). There is growing interest in the study of genetic polymorphisms as causes of cancer development. Chen et al. They found that SNPs in the promoter region have functional activity (17). Biallelic polymorphisms have been associated with an increased risk of gastric cancer and are C-T base transitions: 3954 (C> T), whose number indicates their position from the initial translation codon.

The polymorphisms have been related to an increased production of IL1β associated with hypochlorhydria and cancer development under H. pylori infection. The main advantage of studying polymorphisms is that any type of DNA can be used, it is an inexpensive technique that allows obtaining immediate results, allowing discrimination between homozygous or heterozygous individuals due to a single nucleotide polymorphism (18). The objective of this study was to evaluate the polymorphisms in the region +3954 (IL-1B C> T) of the interleukin IL1β gene for the diagnosis of Helicobacter pylori and its predisposition to gastric cancer by PCR in a sample of the population of the central municipality, Villahermosa tabasco.

Material and methods

Obtaining samples

Two hundred and forty-five blood samples were collected donated by the "Maximiliano Dorantes" public health center, in the municipality of the center of the city of Villahermosa, Tabasco, during 2019, as part of the project: Association between IL-genes 1 alpha 1L-1 beta, TNF alpha in periodontitis in a Tabasco population, the same project was approved by the ethics committee of the institution of the Division of Health Sciences of the Universidad Juárez Autónoma de Tabasco (UJAT), later the samples were sent to the Molecular Biology Laboratory of the Multidisciplinary Academic Division of Jalpa de Méndez for subsequent biochemical processing and DNA analysis.

Biochemical analysis

The identification of Helicobacter pylori was carried out using the urease kit (Proindusquim ®, REQUIMEC, Quito, Ecuador) according to the protocol established by the supplier, from centrifuged serum, by the laboratory of patients with positive detection of Helicobacter pylori, as well as negative samples and control samples (healthy individuals).

Genomic DNA extraction from blood

Genomic DNA extraction was performed from blood samples by the chloroform-phenol method (19). The extracted DNA was quantified with a NanoDrop One spectrophotometer (Thermo Scientific, Ohio USA) and the integrity of DNA was confirmed on a 2% agarose gel stained with ethidium bromide. The genotype of each individual was determined by the Polymerase Chain Reaction (PCR) method using restriction enzymes using a Proflex Applied Biosystems ™ thermocycler (C.A. USA). The results were verified on horizontal 2% agarose gels with a 100 bp molecular marker (Invitrogen (R) Tech-Line NY, USA) for the detection of the different bands.

Polymerase chain reaction (PCR)

For the study of this polymorphism the region +3954 (IL-1B C> T) of the interleukin gene was amplified with 249 base pairs (bp) corresponding to a region exon 5 of the IL-1β gene. A final PCR volume of 25 µL was obtained for each sample, which is made up of 11.7 µL of MilliQ H2O, 2.5 µL of 10X buffer, 0.8 µL of MgCl2, 0.5 µL of dNTPs (dideoxynucleotides triphosphate at 200 mM each), 2.5 µL of 5'-GTTGTCATCAGACTTTGACC-3 'primer (0.2 µM), 2.5 µL of 5'-TTCAGTTCATATGGACCAGA-3' primer (0.2 µM), both from Invitrogen, plus 0.5 µL of Invitrogen Taq polymerase and 4 µL of DNA (200 ng per reaction) (20). The tubes were placed in the Proflex Applied Biosystems ™ thermal cycler (CA USA) for amplification under the following protocol: 94 ° C for 10 min., 95 ° C for 30 sec., 58.0 ° C for 30 sec., 72 ° C. for

45 sec., performing 35 cycles (20). The resulting fragments were observed using a 2% agarose gel injecting the samples and the 100 bp molecular weight marker (Invitrogen Tech-Line NY, USA), later they were visualized in an ENDURO Gel Documentation System Labnet International, Inc. transilluminator. (NJ USA).

Digestion of PCR products IL-1β + 3954 polymorphism (C> T)

The IL-1β + 3954 polymorphism corresponds to a Single Nucleotide Polymorphism (SNP) located in exon 5 of the Interleukin 1β gene, at position +3954 and corresponds to a change from cytosine to thymine (C> T) that is associated with increased production of cytosine. Genotype recognition and development is given by digestion with the TaqI enzyme (Invitrogen (R) Tech-Line NY, USA), using 10 µL of the PCR product, 2 µL of 10X Buffer, 0.8 µL of TaqI enzyme (10 U / µL) and 7.8 µL of H2O for a final volume of 20 µL. The digestion was incubated for 3 hours at 65 ° C, and the samples were visualized on a 2% agarose gel, using the 100 bp molecular weight marker (Invitrogen Tech-Line NY, USA). The homozygous CC genotype was determined by observing 2 bands resulting from digestion (135 bp and 114 bp), the homozygous TT genotype was confirmed by observing a single 249 bp band and the heterozygous CT genotype was determined by observing 3 bands (249 bp , 135bp and 114bp).

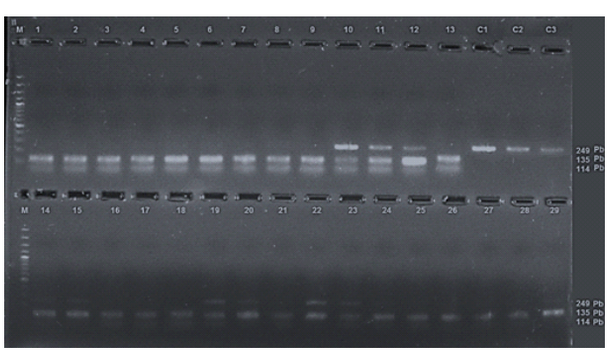

Figure 1. Agarose gel at 2% showing the PCR products, homozygous genotype TT (249 bp), homozygous genotype CC (135 and 114 bp), M: 100 bp molecular marker, numbers of H. pylori positive patients and controls (C), n = 245.

Statistical analysis

For data analysis, Statistica v. 7.5, by means of which Chi-square independence tests were performed to determine the possible relationship between the different host genotypes and the presence of H. pylori, taking a statistically significant value p≤ 0.05, under the Hardy- Weinberg equilibrium for each of the polymorphic positions.

Results

This research analyzed a total of 245 samples of which 130 were men and 115 women, of both 78 cases of Helicobacter pylori infection were detected and 167 individuals who were negative in the detection of H. pylori were considered controls in this investigation. The mean age of the individuals with H. pylori infection at the time of diagnosis was 58.5 ± 12.3 years, whose age range varied between 21 and 77 years with an average of 57 years. The mean age of the control individuals was 50.3 ± 11.6 years, whose age range varied between 23 and 85 years with an average of 51 years. Of the total of 245 individuals analyzed in the study, 130 were men (53%), while 115 were women (46.9%). The 78 infected cases, 49 were men (62.8%) and 29 women (37.17%). The 167 controls were divided into 92 men (55.08%) and 75 women (44.91%).

Genotyping determination (PCR)

The genotype frequencies of the control population and the affected population were calculated, which was obtained by dividing the number of individuals with each genotype (homozygous dominant, heterozygous, homozygous recessive) for the total number of individuals. To obtain equilibrium in the studied population, the sum of the genotype frequencies for a single locus must be equal to 1.00. The expected frequencies were obtained from the allelic frequencies using the Hardy-Weinberg equation, as well as the Odds ratio test (20). To determine if there were significant differences between the affected individuals, the control, and the polymorphisms, a Chi-square test (x2) was performed in the Statistica v software. 7.5.

Restriction enzyme digestion

The digestion of the 249 bp PCR products with the restriction enzyme TaqI under the conditions described in the Materials and Methods section presents one of the three possible genotypes, which are explained below: Homozygous CC genotype: This genotype is observed as two separate bands,

135 and 114 bp, homozygous genotype TT: This genotype shows the presence of a single band (249 bp) that corresponds to the absence of recognizable sites for the enzyme in both chains of the amplified product and heterozygous CT or TC genotype : This genotype is observed as 3 individual bands on the gel: total length of the amplicon (249 bp) and the products of its total digestion with the enzyme (135 and 114 bp).

Allele frequency

The distribution of genotypic and allelic frequencies is observed in Figure 2. Of the group of infected cases, 45 individuals were normal homozygous (CC), 12 were rare homozygous (TT) and 21 individuals were heterozygous (TC). In the control group it was found that 74 individuals were normal homozygous (CC), 32 were rare homozygous (TT) and 61 were heterozygous (TC). Regarding the frequency of the C allele in those affected it was 0.87 and in the control group it was 0.82, while the frequency of the T allele in affected was 0.13 and in controls 0.18. Through Chi-square analysis, values were obtained that show that both samples are in Hardy-Weinberg equilibrium and that there are no significant differences (Table 1). The Odds ratio test was applied to know the relative risk that a specific genotype or allele is related to the development of gastric cancer in the presence of Helicobacter pylori. The values obtained for the homozygous CC genotype would represent a 3 times greater risk of suffering from cancer in an individual.

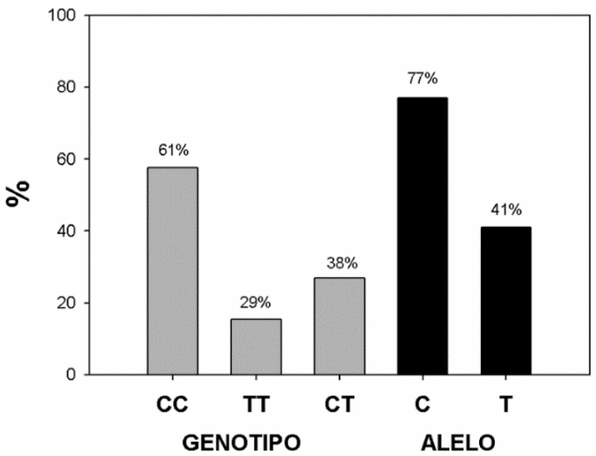

Figure 2. Genotype percentages and allele distribution. n = 245, Gray: genotypes, Black: alleles.

Table 1. Hardy-Weinberg analysis of the IL-1β +3954 polymorphism (C> T)

Group	Genotype	Number of individuals	%	Number of alleles	Genotypic frequencies	Expected frequencies	Allele frequencies	X^2 HW	p
Infected individuals	CC	45	57.69	87	0.75	0.72	0.83	1.34	0.21
	TT	12	15.38	5	0.02	0.01	0.15		
	CT	21	26.92	25	0.17	0.15			
	Total	78							
Control individuals	CC	74	44.31	96	0.65	0.61	0.79	2.03	0.12
	TT	32	19.16	6	0.09	0.06	0.14		
	CT	61	36.52	31	0.21	0.26			
	Total	167							

Values expressed in genotypic (%) and allelic frequencies, non-significant chi square (Hardy-Weinberg) values (P ‹0.5) n = 245.

Table 2. Odds ratio analysis for genotypes

Genotype	Number of infected	Control number	Odds ratio	95% IC	p
CT	12	32	0.98	0.275-3.270	0.75
TT	21	61	0.95	0.279-3.250	0.8
CT+TT	33	93	0.96	0.301-3.021	0.81

Odds ratio expressed values adjusted by genotype frequency (95% CI) for the association of the IL-1β +3954 polymorphism with gastric cancer.

Discussion

The results obtained show an association between the polymorphism of interleukin IL-1β + 3954 (C> T) and gastric cancer. Since several studies have identified and related to an increase in the production of interleukin against pathogens, since it is an important pro-inflammatory molecule in signaling and amplifying the response against Helicobacter pylori infection, producing an inflammatory process that can trigger the malignant transformation of the mucosa, through molecular and morphological changes (21, 22). Regarding genotype and allelic frequencies, the results show that the polymorphism, IL-1β + 3954 (C> T), the normal homozygous CC genotype is the one that occurs in the highest percentage (57.69%) of the population. In comparison with the genotypic and allelic frequencies of other populations in the world, the studied population has a behavior similar to that of studies carried out in Central American countries such as Venezuela and Ecuador, observing the prevalence of homozygous TT (Ecuador 53.9%, Venezuela 55%), followed by the heterozygous TC (Ecuador 36%, Venezuela 24%) and finally the normal homozygous CC (Ecuador 10%, Venezuela 21%) (20). The percentages reported in other parts of the world such as India, China and the United States coincide in the highest frequency of homozygous TT. However, the Ecuadorian population shows a high percentage (21.92%) of the homozygous TT genotype than in other countries, even being non-existent in the population. On the other hand, 26.92% of CT heterozygotes were observed, of which the other mentioned populations have a similar frequency (23).

The respective genotypes for the IL +3954 (C> T) polymorphism showed a deviation from equilibrium in the group of affected patients with a x2 HW value of 1.34; $p < 0.05$, but not that of controls (x2 HW = 2.3; $p > 0.05$), which would confirm a possible role of this last polymorphism in the risk of developing cancer (24). In the statistical analysis of Odds ratio, the OR values with 95% CI were calculated with respect to the normal homozygous of each polymorphism studied. This polymorphism is located in a binding site of Ap2, a transcription factor that inhibits gene expression (25). Otero et al. found an elevated risk of gastric cancer associated with the rare homozygous genotype TT of the polymorphism with an OR of 2.6 in the Polish and Scottish population, also finding a significant risk associated with the heterozygous CT genotype (22). Similar data were obtained in other Caucasian populations in the United States and Portugal (21). Studies carried out in Costa Rica indicate the association between the T allele of this polymorphism with the risk of gastric cancer, and in vitro studies have linked the allele with a higher production of cytokine (26). These results are similar to those obtained in populations from China, the United States, European populations and northern Brazil in which the polymorphism IL-1β + 3954 (C> T) had a significant association with gastric carcinogenesis (21, 28).

Conclusion

Thus, in this population evaluated from Villahermosa tabasco, Mexico, it is observed that there is a three times greater risk that an individual carrying the rare homozygous genotype CC infected with Helicobacter pylori will develop cancer. By having two copies of the C allele, the levels of interleukin rise in the face of infection and produce chronic inflammation that generates tissue damage due to so many molecular and morphological changes. This study suggests through this methodology to identify individuals susceptible to Helicobacter pylori disease and their predisposition to gastric cancer, especially in high-risk areas.

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Contribution of the author

All authors participated in the entire research

process.

Interest conflict

We declare that we have no conflict of interes.

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