Original Article

Molecular and Genetic Study for Detection of *Helicobacter pylori* Virulence Genes among Patients with Diffuse Large B-Cell Lymphoma

Eman Abid Fahad Alhasnawi[1], Wasan Abdulelah Bakir[2]

[1]Department of Medical Laboratories, Medical Technical Institute, Middle Technical University, Baghdad, Iraq, [2]Department of Microbiology, Collage of Medicine, Al-Mustansyriah University, Baghdad, Iraq

Abstract

Background: The bacteria *Helicobacter pylori* (Hp) has supposed to confirm an oncogenic consequence with the evolution of malignancy events in stomach, effectiveness of Hp consider a top risk factor of gastric-diffuse large B-cell lymphoma (DLBCL). **Aims:** the aim of this study was to observe the correlation between virulence factors, especially cytotoxin association gene A (CagA) and the progress of gastric DLBCL. **Materials and Methods:** Biopsy samples (50) involved in this study (28 were men, and 22 women, with a mean age up to 57.64 years), among gastric-DLBCL patients, with (25) control (were 14 men and 11 women) without DLBCL, tested at Medical City Teaching Hospital and Teaching Laboratories, study conducted from January 1st to September 30, 2022. Diagnosis was by molecular methods included DNA extraction for Hp by STR-Format kit (BIORON Diagnostics GmbHm In den Rauhweiden 20,67354 Germany), and "real-time polymerase chain reaction" methods using the (ABI PRISM 7700) Sequence Detection System, also applied the primers and probes, for Hp-CagA. **Results:** The mean of age was 55.7 ± 2 in DLBCL, while in control were 61.82 + 6, CagA +ve 66.66 + 2. In gastric DLBCL, the red blood cell count, white blood cell count, platelets count, and hemoglobin were all decline in DLBCL, not in the control group, lactate dehydrogenase levels in gastric DLBCL patients were rise (322 U/L), while less in control, while albumin (322g/dl) was less in gastric DLBCL patients. Hp 16S rRNA was detected relating to CagA variety, 66% (33/50) were CagA positive with highly significant in 23 patients with DLBCL ($P = 0.004$), and 10 in control patients. CagA gene expression was highly expression in gastric DLBCL patients with range up to (+), 8 (++), and 12 (+++), while in the control group was 5 (+), 2 (++), and 3 (+++), respectively. **Conclusions:** This present study fixed high frequency of CagA gene in gastric DLBCL patients. Moreover, confirmed this gene is significantly associated with gastric DLBCL in patients with Hp. Moreover, genotype EPIYA-ABC motif is related with the high risk of gastric DLBCL.

Keywords: Cytotoxin association gene A, diffuse large B-cell lymphoma, *Helicobacter pylori* bacteria, virulence genes

Introduction

The progressive disease "Diffuse large B-cell lymphoma (DLBCL)" is a blood malignancy derivative from the mature B cells inside the germinal-center response to specific antigen,[1] rises from lymphoid rather than myeloid cells,[2] is categorized as a kind of "Non-Hodgkin's lymphoma."[3] These tumor cells demonstrate a diffuse growth pattern.[4] Activated B-cell (ABC) subtype outstanding unclassified.[5] Some altered transcription factors control the change of B cells in the germinal center reaction.[6] There are many pathogens as risk factors that induce transformation to DLBCL, such as *Helicobacter pylori (*Hp), which arises in the stomach, but gastric lymphomas.[7] Hence, gastritis produced by Hp causes infiltration of plasma cells with lymphocytes[8] gastric lymphoma often can series after the extra-nodal marginal zone-B cell lymphoma (MALT lymphoma) into DLBCL,[9] which has a heterogeneous disorder with conventionally treated by systematic chemotherapy.[10] A significant role of a positive Hp result related with the early stage of gastric-DLBCL through Hp eradication (HPE).[9] The

Access this article online

Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/mj.mj_6_23

Address for correspondence: Ms. Eman Abid Fahad Alhasnawi, Medical Technical Institute, Middle Technical University, Baghdad, Iraq. E-mail: ealhasnawi@gmail.com

Submitted: 11-Jan-2023 **Revised:** 05-Feb-2023 **Accepted:** 07-Feb-2023 **Published:** 07-Aug-2023

How to cite this article: Alhasnawi EA, Bakir WA. Molecular and genetic study for detection of *Helicobacter pylori* virulence genes among patients with diffuse large B-cell lymphoma. Mustansiriya Med J 2023;22:106-12.

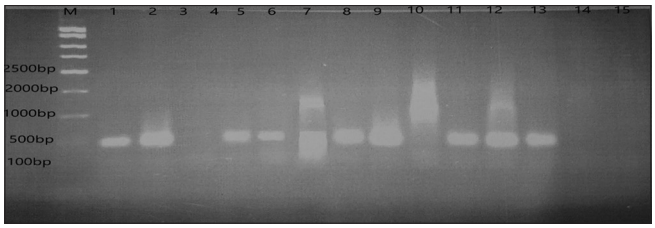

Figure 1: Graph of agarose gel electrophoresis, showed that PCR analysis of CagA DNA with M-marker of (2500-100) bp, tracks (1, 2, 5–13) showed some positive samples. CagA: Cytoxin association gene A, PCA: Polymerase chain reaction

CagA can stimulate pro-inflammatory responses polyclonal activation of B cells and genetic unpredictability,[10,11] so the CagA induces gastric precancerous damage. In current study fixed the suggested relation within *CagA* gene position and gastric-DLBCL lymphoma and it role in the progress of disease from "MALT lymphoma" into DLBCL.

Materials and Methods

Study design

A cross-sectional study for the detection of Hp by CagA-gene, the one of furthermost important virulence genes included estimating with association to CagA sequence of (EPIYA motifs), and the consequence of gastric DLBCL. The study involved compare results of both cases: gastric and duodenal ulcers, gastritis groups as a control with gastric tumor. Specimens are obtained from patients with present DLBCL, who were previously diagnosed with Hp infection. Molecular methods were used for the detection of the virulence genes and genotyping, also the study included a comparison between age, sex, and some of blood parameters distribution with patients.[11]

Patients and samples

Biopsy samples from fifty patients (50) involved in this study (28) were men, and (22) were women with mean age up to 57.64, who were suffer from gastric DLBCL with previous confirmed diagnosis with Hp, and 25 patients, included 12 (8 men and 4 women; mean age: 64.83 years) with chronic gastritis, 8 (5 men, with 3 women had a mean age of (59.25), others had gastric ulcer, and 5 patients with duodenal ulcer included 4 men, 1 woman with mean age 61 years, and 25 subjects without DLBCL with previous confirmed diagnosis with Hp, as control were 14 men, and 11 women, with mean age 61.82 + 6. All patients were diagnosed of primary gastric DLBCL at Medical City Teaching Hospital and National Center of Teaching Laboratories, and other private clinics, conducted from January 1st to September 30, 2022, had accomplished. Pathological biopsy samples were gained from the endoscopic and surgical methods, the identification was built by the "World Health Organization" aimed at blood malignancy disorders. All malignant tumors were analyzed as gastric DLBCL.

Molecular methods

DNA extraction

DNA had extracted from tissues using a RealLine Hp Str-Format kit (BIORON Diagnostics GmbHm in den Rauhweiden 20-67354 Romerberg Germany) and the total DNA had a very small amount of Hp DNA content. Proteinase K (PK) (1.1 ml) storage buffer was added to proteinase K tube and resuspended by vortexing. Proteinase K (10 mg/ml) solutions were stored at 4°C. Extraction protocol was made by carefully applying the solutions to the center of the membrane, and the tip of the micropipette was not touched the spin column membrane.[12]

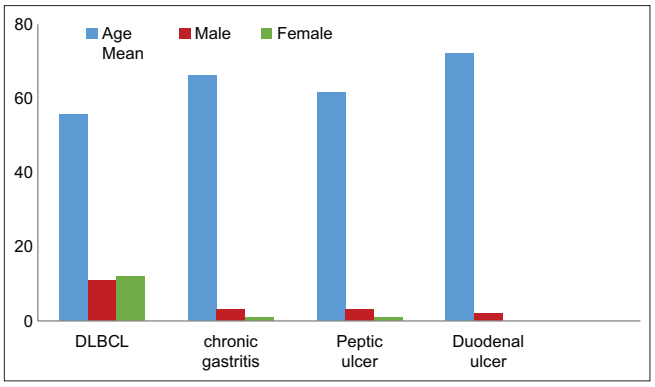

Figure 2: Prevalence of CagA genotypes according to age and sex. CagA: Cytoxin association gene A

The real-time polymerase chain reaction method

The polymerase chain reaction (PCR) results are all done by real-time technique by the (ABI PRISM 7700) Sequence Detection System [Figure 1]. Moreover, PCR made within (50-ll) mixture-reaction cover (25/ll) of Universal PCR TaqMan, Master Mix by the Applied Biosystems, there was 200–800 nM for each primer, with the length of 400–600 nM for probe, and 5/ll for each DNA section, with about 10 fold of sequential diluting, ordinary Hp-DNA up to 0.1:106 fg, besides dual distilled water within 0.2 ml Micro Amp of photosensitive tubes from Applied Biosystem. The stage of thermal cycling situations included the primary denaturation phase at 95°C for 10 minutes with 50 cycles, at 95°C for 15 seconds, and at 60°C for 1 min. Then, several yields of PCR were scanned by (3%) "agarose gel electrophoresis" method.[13]

Preparation of agarose gel

Agarose gel was prepared by the (w/v) solution ratio, and the agarose concentration often depends on the DNA bulks, and the fragmentation to be separated, and most of these gels reaching 0.7%–2%. Then, the capacity of the buffer must not be larger than (1/3) of the volume of the fit flask.[14]

One % agarose gel was prepared in 1X TBE buffer and heated by hot magnetic stirrer until all crystals disappeared.

Then, the gel was placed within the cast device, and adhesive tape, for gel made a decay.

The melted agarose was poured to the gel decay. Then, established at room temperature, and the gel material was placed in a box, so, it was wrapped within a plastic wrap and then stored at (4°C) pending usage.

After cooling, 4 µL of ethidium bromide was added for 100 ml of gel solution, and then poured the gel a tray, fixed the comb at the right point, then left until hardening.

Transferred into electrophoresis device, which controlled by (1× TBE) buffer solution that was used in agarose gel preparing.[12]

Gel electrophoresis

Important stage for complete the PCR assess for following:

The DNA sections were set by mixing of 5 µl DNA sample per 3 µl of dye filling and dejected in the micropipette.

The electrophoresis was run, power at 70 Volt for 1.5 h, and the DNA bands, had seen by ultraviolet and transilluminator, then photographed by a digital apparatus.[14]

Statistical analysis

Data were statistically analyzed using the SPSS program (version 26 (IBM Corp, Armonk, NY) and statistically, the data have been evaluated through the use of Microsoft Program, also the specific group differences have been defined through the use of mean, standard deviation, percentiles, *P* value-Chi-square, standard deviation, and indicated of significant differences.[14]

Institution of sequencing

The primers and probes used for the detection of the CagA of Hp bacteria were considered using the "Primer Express ver.1.5" by Applied Biosystems/CA-USA). Moreover, the arrangement of the amino acid sequence of 30 regions of the CagA-gene of strain: 26695. Previously reported that (NCTC-116375) EPIYA-motifs, marks of SHP-2. The EPIYA-motifs are intricate within the interface of the CagA and SHP-2.

The first and second of EPIYA-motifs that included "(EPIYA-A) and (EPIYA-B)" presented basically in totally proteins of CagA, while lasting third EPIYA motifs of "(EPIYA-C)" that made by replication of EPIYA of sequence with 34-amino acid.[14]

Then, the sequence of (34) amino acids with various numbers, about 1 to 3 in furthermost CagA-proteins, selected were the specific CagA (SHP-2) sequence (WSS),[12] and the strain (26695) with a single-WSS, which is thus, is familiar as the A-B-C type, 11637-CagA categorized equally at the A-B-C-C-C type.[14]

The amino acid of the sequence of "East-Asian CagA (ESS)" within the consistent district (ESS) covers a JSR area, holds an EPIYA motif, selected "(EPIYA–D)" which has a single ESS, and thus, classified as the "(A-B-D) type", were not found.[14]

Results

Study contributed with blood tests

Table 1 included the age mean: which was 57.64 ± 1 in DLBCL patients, sex: was mostly in male gender (8) while in female (4), and also included blood parameters and biochemical tests, such as C-reactive protein (CRP), blood cell counts (red blood cell [RBC] and white blood cell [WBC]), the platelets count (PLTs), and hemoglobin (Hb) [Figure 2].

Study contributed with biochemical tests

Results within Table 2 showed values of lactate dehydrogenase (LDH) and albumin, comparable with mean age and sex, in study patients with DLBCL and control

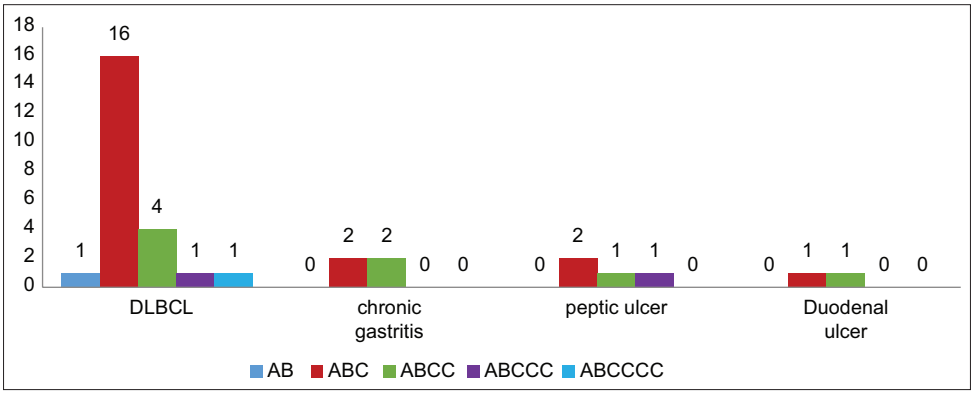

Figure 3: Prevalence of CagA genotypes according to mutated genes. CagA: Cytoxin association gene A

Table 1: Values contributed with blood and biochemical tests

n	Patients	Age mean (years)	Sex		CRP (mg/L)	Mean of some of blood parameters/µL			
			Male	Female		PLT (thousands)	RBC (million)	WBC (thousands)	Hb (g/dL)
50	Gastric DLBCL	57.64±1	28	22	38.4	110	3.5	3.1	10.1
12	Chronic gastritis[#]	64.83±3	8	4	14.1	280	6.1	4.2	13.2
	P	0.03	0.001		0.0001	0.002	0.003	0.001	0.006

[#]Control. CRP: C-reactive protein, PLT: Platelets count, RBC: Red blood cell count, WBC: White blood cell count, Hb: Hemoglobin, DLBCL: Diffuse large B-cell lymphoma

patients. The excessive stage discovered in DLBCL patients showed CRP with 38.4 mg/L changed into the excessive stage in peptic ulcer (mg/L), the levels of CRP are circulating and quietly raised during chronic inflammatory illnesses and cancers. PLT was 110000 cell/cm, RBC: 3.5 million, WBC: 3100 cell/cm, Hb: 10.1 g/dl, LDH: 382 U/L, and albumin was 2.1 g/dl.

The gene (cytotoxin association gene A) reacting according to diffuse large B-cell lymphoma group and control

Table 3 explain the distribution of the studied gene (CagA) reacting according to the DLBCL group and control group included with significance result, the results showed that the DLBCL group appeared highly significant outcomes included the gastric DLBCL and control groups with detection of CagA positive with its load (+, ++, and +++) presence within biopsies by PCR method and agarose gel electrophoresis test, involved comparison by age and sex.

Cytotoxin association gene A gene and scientific features according to gene mutations

Hp-*CagA* possesses five EPIYA motifs, the membrane targeting signal within Hp virulence factor CagA among mammalian cells, which can be ability hold of tyrosine-phosphorylation (SHP-2). "Moreover, these EPIYA-motifs are involved within the interaction of CagA" within "SHP-2". Hence, 1st with 2nd "EPIYA motifs (which distinctive EPIYA A and EPIYA B, respectively)" were found within nearly "all CagA proteins," while within the last, "three EPIYA-motifs" distinctive "EPIYA C" have been doubling series, as in western type CagA (WSS). Hence, in this Table 4, the most result that appeared was ABC type 48%, then ABCC (12%), and both ABCCC and ABCCCC have (3%) for each. Most results were in the female gender with an age mean of 55.7 ± 2 years [Figure 3].

The cytotoxin association gene A genotypes

Result of CagA genotypes as within Table 5 showed differences among positive and negative Hp CagA in gastric DLBCL

Table 2: Blood parameters and biochemical tests

n	Patients	Age mean (years)	Sex		CRP (mg/L)	Mean of some of blood parameters/μL				LDH levels U/L	Albumin (g/dL)
			Male	Female		PLT (thousands)	RBC (million)	WBC (thousands)	Hb (g/dL)		
50	Gastric DLBCL	57.64±1	28	22	38.4	110	3.5	3.1	10.1	322	2.1
8	Peptic ulcer#	59.25±2	5	3	22.3	300	4.4	5.1	11.3	240	2.8
5	Duodenal ulcer#	61.40±4	4	1	18.5	320	5.2	5.5	11.2	211	3.2
	P	0.03	0.001		0.0001	0.002	0.003	0.001	0.006	0.0001	0.002

#Control. CRP: C-reactive protein, LDH: Lactate dehydrogenase, PLT: Platelets count, RBC: Red blood cell count, WBC: White blood cell count, Hb: Hemoglobin, DLBCL: Diffuse large B-cell lymphoma

Table 3: Comparison *Helicobacter pylori*-cytotoxin association gene A results in study patients with control

n	Patients	Age mean	Sex		*CagA*, *n* (%)	CagA level				Method of diagnosis
			Male	Female		*CagA*−	*CagA*+	*CagA*++	*CagA*+++	
50	DLBCL	57.64	11	12	23 (46)	27	3	8	12	PCR
12	Chronic gastritis*	64.83	3	1	4 (33)	8	3	1	0	PCR
8	Peptic ulcer*	59.25	3	1	4 (50)	4	2	0	2	PCR
	P	0.07	0.001	0.002	0.004	0.003	0.04	0.002	0.001	
	CC, *P*				0.610, 0.008 (HS)*			0.420, 0.001 (HS)*		

*HS at (*P*<0.01); (CC: Test based on a CC). CC: Contingency coefficient, HS: Highly significant, PCR: Polymerase chain reaction, DLBCL: Diffuse large B-cell lymphoma, *CagA*: Cytoxin association gene A

Table 4: Result of cytoxin association gene A genotypes among positive *Helicobacter pylori* cytoxin association gene A patients by real time-polymerase chain reaction and sequencing

n=33	Patients	Age mean (years)	Sex		*CagA* genotypes*				
			Male	Female	AB, *n* (%)	ABC, *n* (%)	ABCC, *n* (%)	ABCCC, *n* (%)	ABCCCC, *n* (%)
23	DLBCL	55.7±2	11	12	1 (3)	16 (4)	4 (12)	1 (3)	1 (3)
4	Chronic gastritis	66.2±2	3	1	0	2 (6)	2 (6)	0	0
4	Peptic ulcer	61.5±1	3	1	0	2 (6)	1 (3)	1 (3)	0
2	Duodenal ulcer	72.0±1	2	0	0	1 (3)	1 (3)	0	0
	Total		19	14	1 (3)	21 (64)	8 (24)	2 (6)	1 (3)

*AB, ABC, ABCC, ABCCC, ABCCCC: the sequences of amino acids in EPIYA-motif related to *CagA* genotypes. DLBCL: Diffuse large B-cell lymphoma, *CagA*: Cytoxin association gene A

Table 5: Result of cytoxin association gene A genotypes among positive and negative *Helicobacter pylori* cytoxin association gene A patients by real-time-polymerase chain reaction (*n*=33)

	CagA genotypes					CS, *P*#
	AB, *n* (%)	ABC, *n* (%)	ABCC, *n* (%)	ABCCC, *n* (%)	ABCCCC, *n* (%)	
DLBCL (*n*=23) positive						
Positive	1 (3)	16 (48)	4 (12)	1 (3)	1 (3)	0.534, 0.001 (HS)
Negative	32 (97)	17 (52)	29 (88)	32 (97)	32 (97)	
Control* (*n*=10) positive						
Positive	0	5 (15)	4 (120	1 (3)	0	
Negative	33 (100)	28 (85)	29 (88)	32 (97)	0	
Total						
Positive	1 (3)	21 (64)	8 (24)	2 (6)	1 (3)	
Negative	32 (97)	12 (37)	25 (76)	31 (94)	32 (97)	

*Control: Chronic gastritis, Peptic ulcer and Duodenum ulcer. #CS/P: HS at (P<0.01); (CC: Test based on a CC). CC: Contingency coefficient, HS: Highly significant, DLBCL: Diffuse large B-cell lymphoma, *CagA*: Cytoxin association gene A, CS: Chi square test

patients and control. These results showed high difference in results of positive CagA between DLBCL patients and control, especially in the ABC genotype with highly significant at $P < 0.01$.

Discussion

Results according to some of blood parameters and biochemical tests

In gastric DLBCL blood borders of "RBC count", "WBC", PLTs, and Hb range were all decline in levels, less than their values in the control group (chronic gastritis, gastric ulcer, and duodenum ulcer),[2] this results agreement with.[3] Furthermore, LDH levels in gastric DLBCL patients were (322 U/L), while less in the control group.[7] Furthermore, albumin (322 g/dl) in gastric DLBCL patients was less in the level than in control (approximately similar to the finding of a previous study 7 and 15).[7,15] All differences in levels of blood and biochemical parameters between gastric DLBCL patients and control group patients were significant.

Age and sex

The mean age in this study was 55.7 ± 2 year in DLBCL, while the mean age in control was 61.82 + 6 year, then in CagA +ve (66.66 + 2) mostly in duodenal ulcer were 72.0 ± 1, this result appears that younger age was in gastric DLBCL in this study.[3] The males with gastric DLBCL shared in this study were 28, while females were 22 which is refer to the prevalence appearance of this disease among male patients rather than in females,[14] and this outcome is related with genotyping variation in infection ability, and need a specific study for prove that.[15]

Confirmation of the organization

This study identified the Hp CagA gene, showed characteristic CagA presence, all the Hp-positive products were amplified, and few from CagA samples of negative infections by PCR method. Furthermore, these results were obtained by agarose gel electrophoresis,[12] real-time PCR was used. Furthermore, nucleotide sequences of the result were checked with western-CagA PCR products. In the sequence among the strains with F32 sequence of (92-bp) was identified.[6,8] Moreover, compared with the sequence of western-CagA of strains-26695 and 080 of 92-bp sequencing were extremely fit with the western-CagA sequence.[16]

Cytotoxin association gene A gene expression and clinical features according to gene mutations

All of the patients with positive-Hp, scanned with CagA, as a (16S rRNA). Relating to CagA variety, 66% (33/50) were CagA-positive, the study detected a highly significant affiliation between the incident of CagA-gene and gastric-DLBCL in 23 patients (P = 0.004) while were in 10 control patients.[17] Moreover, all of them were western CagA-positive. CagA gene expression was highly expression in gastric DLBCL patients with range up to (+), 8 (++), and 12 (+++), while in the control group were 5(+), 2(++), and 3(+++), respectively. The differences between gastric DLBCL results and the control group in gene presence with highly significant, also in gene expression was in high expression in gastric DLBCL. "Newly, the molecular studies appeared that the carrying of CagA toxin within the gastric epithelial cells will phosphorylated by the Src-kinases of EPIYA-motifs."[17] The induction of proinflammatory response through the B-cell signaling pathway and stimulation of gastric DLBCL.[18]

The frequency of gastric DLBCL is high significant in this study, with the presence of variance of Hp within control patients,[2] and there are great differences in the sequence that discriminate the CagA gene sectors among them. The study described that the evaluations of inflammation were active of gastritis among DLBCL patients,[18] and also significant differences were found in patients of control with chronic gastritis, peptic ulcer, and duodenal ulcer who appeared with the Hp CagA-positive strains, rather than in gastritis patients with strains of CagA-negative.[19]

Furthermore, it was confirmed that the CagA-specific sequence, advises clear (SHP-2) mandatory with alteration events more than sequence made in control patients,[20] and this implication

that the probable of CagA toward distract host cell roles as a virulence influence is greater than in control patient, and these results supposed the endemic circulation of Hp bacteria has a high CagA proteins virulence can increase the induction of gastric-DLBCL within some patients of control[7] and thus, the CagA-positive strains in DLBCL patients may be virulent in the control CagA-positive strains.[6] Hence, it will be essential to differentiate between control and DLBCL type CagA to accelerate clinical measures where many strains of Hp are diverse existing.[20] Hence, in this study used an extremely "sensitive real-time PCR" method, for detect CagA-gene of the Hp-DNA samples within the gastric tissue biopsies.[12] This method was established by TaqMan procedure with fluorescence that needs less phases by PCR compare with the conventional PCR procedures and shortening of techniques. Moreover, using these primers, groups were as well potential for demonstration of the outcomes obtained by agarose gel electrophoresis, by PCR yields,[11] then the sensitivity appeared with "real-time PCR method", more than within outcomes from "agarose gel electrophoresis", that likewise equal to identify of 16S-rRNA of the Hp bacteria and compare the result with the western CagA-gene within paraffin blocked tissues of DNA samples. Moreover, the samples, also have a host DNA, with very small concentrations of Hp-DNA. As the extracted DNA of the host samples is essential, it can observe the CagA typing, without needing for Hp culturing.[18]

The most importance of CagA-gene, it acts as a virulence factor in the genome of Hp that is positioned with the termination of section-I, within the pathogenicity islands. And is smaller than other genes, inside the bacterial genome, transporting horizontally among bacteria strains.[21]

Built on many studies, many strains of Hp can transmit the CagA-gene and are considered further virulent factors in this pathogen, so, the incidence of strains with CagA-positive, still with a high frequency of gastric malignancy growth. The presence findings confirmed of CagA is intentionally related with gastric DLBCL.[13]

Clinical features according to sequencing of gene mutations

Many cases detected with variable genotypes in DLBCL patients were AB 3%, ABC 48%, ABCC 12%, ABCCC 3%, and ABCCCC 3%, while in control groups were AB 0%, ABC 15%, ABCC 12%, ABCCC 3%, and ABCCCC 0%.

Hp detected in these samples with AB-type CagA, that which has not to contain WSS sequence either hereditary or by deletion,[7] "almost all Hp strains with CagA positive among western countries, carry EPIYA-motifs A, B, and one or more replications of C, whereas EPIYA-motifs in East-Asian countries are typically ABD."[12]

The study also established that C-motifs repeating, persuade high prevalence decease, and in a study, also showed that this motif be able to phosphorylate SHP-2, therefore, this motif represented a risk factor of induction a gastric DLBCL.[8]

The incidence of EPI-X sequences found within ABCC, ABCCC, and ABCCCC is related with the higher propensity of SHP-2.[10] From many strains, the CagA-genotypes documented from everywhere around the world, contain "(AB), (ABC), (ABCC), (ABCCC), (ABCCCC), and (ABD)."

In this study, both ABC and ABCC genotypes are the established manifestation of the gastric ulcer as well as gastric DLBCL, conferring with an investigation, the ABCC genotype was typically related with induction of gastric-DLBCL.[22]

Correspondingly, many studies revealed a significant connection with infection by the various EPIYA-C strains and gastric tumors as DLBCL.[10] Conferring to these results, the "(EPIYA-ABCCC)" present is significantly linked to the gastric DLBCL.[16] Furthermore, the routine of lifestyle and ethnicity also can rise the occurrence of this type of DLBCL.[8] The arrangement of the sequence nucleotide in the 30 section of the western CagA-gene among the strain (26695).[11]

Each sequence of 92-bp, fits at the locus of 2824 in CagA, within the strain (26695) open-reading frame, according to the recorded strain at accession number as a position (2812) of the (F32-CagA) strain open reading frame at accession number in GenBank as: "(AF202972)."[20] The frequency of positive CagA-Hp infection, diverse in the result (50%) were among chronic gastritis,[15] this study advocates that the (real-time PCR), may deliver a very sensitive valuation of a CagA category through Hp within the gastric tissue biopsies,[9] so, this could offer valuable and simple with highly sensitive method, for diagnosis of CagA type, by real-time PCR.

Positive results appeared of Hp in gastric-DLBCL, and mainly with the CagA manifestation confirm that infection with Hp correlated to pathogenically and clinically and different from DLBCL unrelated to Hp, and positive CagA belongings to the DLBCL, lean toward a minor clinical stage, and local position.[14]

Conclusions

This study fixed a high incidence of a CagA-gene within gastric DLBCL disease. Moreover, the confirmed appearance of this gene as significantly associated with a frequency occurrence of a gastric DLBCL among patients who were previously infected with Hp.

The genotype EPIYA-ABC motif was linked with a high risk of the induction of gastric-DLBCL within patients who joined this study and also detected a high significant correlation with EPIYA-ABCC and gastric-DLBCL among those patients.

Study results appeared the expression of Hp strains) EPIYA-ABCC (of must be reflected a suitable indicator for the development of main infections that affect and induce gastric DLBCL in untreated chronic infected patients.

Recommendations

All the patients after HPE without PCR detection test must be immediately have treated by typical chemotherapy to avoid biological transformation activities.

Histological tests for sections from the biopsies must be estimated conferring to the molecular methods, with endoscopic and ultrasound required for early-stage detection, and PCR method confirmation must be following by two complemented biopsies.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Flinn IW, Erter J, Daniel DB, Mace JR, Berdeja JG. Phase II study of bendamustine and ofatumumab in elderly patients with newly diagnosed diffuse large B-cell lymphoma who are poor candidates for R-CHOP chemotherapy. Oncologist 2019;24:1035-e623.
2. Bagheri V, Memar B, Momtazi AA, Sahebkar A, Gholamin M, Abbaszadegan MR. Cytokine networks and their association with *Helicobacter pylori* infection in gastric carcinoma. J Cell Physiol 2018;233:2791-803.
3. De Falco M, Lucariello A, Iaquinto S, Esposito V, Guerra G, De Luca A. Molecular mechanisms of *Helicobacter pylori* pathogenesis. J Cell Physiol 2015;230:1702-7.
4. Keikha M, Karbalaei M. Correlation between the geographical origin of *Helicobacter pylori* homB-positive strains and their clinical outcomes: A systematic review and meta-analysis. BMC Gastroenterol 2021;21:181.
5. Floch P, Mégraud F, Lehours P. *Helicobacter pylori* strains and gastric MALT lymphoma. Toxins (Basel) 2017;9:132.
6. Talebi Bezmin Abadi A, Ghasemzadeh A, Mohabati Mobarez A. Low frequency of CagA-positive *Helicobacter pylori* strains isolated from Iranian patients with MALT lymphoma. Intern Emerg Med 2013;8:49-53.
7. Flümann R, Rehkämper T, Nieper P, Pfeiffer P, Holzem A, Klein S, *et al.* An autochthonous mouse model of Myd88- and BCL2-driven diffuse large B-cell lymphoma reveals actionable molecular vulnerabilities. Blood Cancer Discov 2021;2:70-91.
8. Kuo SH, Yeh KH, Chen LT, Lin CW, Hsu PN, Hsu C, *et al. Helicobacter pylori*-related diffuse large B-cell lymphoma of the stomach: A distinct entity with lower aggressiveness and higher chemosensitivity. Blood Cancer J 2014;4:e220.
9. Chen CY, Wang FY, Wan HJ, Jin XX, Wei J, Wang ZK, *et al.* Amino acid polymorphisms flanking the EPIYA-A motif of *Helicobacter pylori* CagA C-terminal region is associated with gastric cancer in East China: Experience from a single center. J Dig Dis 2013;14:358-65.
10. Hashinaga M, Suzuki R, Akada J, Matsumoto T, Kido Y, Okimoto T, *et al.* Differences in amino acid frequency in CagA and VacA sequences of *Helicobacter pylori* distinguish gastric cancer from gastric MALT lymphoma. Gut Pathog 2016;8:54.
11. Karbalaei M, Khorshidi M, Sisakht-pour B, Ghazvini K, Farsiani H, Youssefi M, *et al*. What are the effects of IL-1β (rs1143634), IL-17A promoter (rs2275913) and TLR4 (rs4986790) gene polymorphism on the outcomes of infection with *H. pylori* within as Iranian population; a systematic review and meta-analysis. Gene Rep 2020;20:100735.
12. Kuo SH, Chen LT, Lin CW, Wu MS, Hsu PN, Tsai HJ, *et al.* Detection of the *Helicobacter pylori* CagA protein in gastric mucosa-associated lymphoid tissue lymphoma cells: Clinical and biological significance. Blood Cancer J 2013;3:e125.
13. Li Q, Liu J, Gong Y, Yuan Y. Association of CagA EPIYA-D or EPIYA-C phosphorylation sites with peptic ulcer and gastric cancer risks: A meta-analysis. Medicine (Baltimore) 2017;96:e6620.
14. Li S, Young KH, Medeiros LJ. Diffuse large B-cell lymphoma. Pathology 2018;50:74-87.
15. Lind J, Backert S, Hoffmann R, Eichler J, Yamaoka Y, Perez-Perez GI, *et al.* Systematic analysis of phosphotyrosine antibodies recognizing single phosphorylated EPIYA-motifs in CagA of East Asian-type *Helicobacter pylori* strains. BMC Microbiol 2016;16:201.
16. Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. Am J Hematol 2019;94:604-16.
17. Sheikh S, Kuruvilla J. Pembrolizumab for the treatment of diffuse large B-cell lymphoma. Expert Opin Biol Ther 2019;19:1119-26.
18. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7-34.
19. Sougleri IS, Papadakos KS, Zadik MP, Mavri-Vavagianni M, Mentis AF, Sgouras DN. *Helicobacter pylori* CagA Protein Induces Factors Involved in the Epithelial to Mesenchymal Transition (EMT) in Infected Gastric Epithelial Cells in an EPIYA-Phosphorylation-Dependent Manner. New York: Wiley; 2016.
20. Šterbenc A, Jarc E, Poljak M, Homan M. *Helicobacter pylori* virulence genes. World J Gastroenterol 2019;25:4870-84.
21. Yong X, Tang B, Li BS, Xie R, Hu CJ, Luo G, *et al. Helicobacter pylori* virulence factor CagA promotes tumorigenesis of gastric cancer via multiple signaling pathways. Cell Commun Signal 2015;13:30.
22. Youssefi M, Tafaghodi M, Farsiani H, Ghazvini K, Keikha M. *Helicobacter pylori* infection and autoimmune diseases; is there an association with systemic lupus erythematosus, rheumatoid arthritis, autoimmune atrophy gastritis and autoimmune pancreatitis? A systematic review and meta-analysis study. J Microbiol Immunol Infect 2021;54:359-69.