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Journal of Bioscience and Applied Research
www.jbaar.org

Synergistic infection of *Helicobacter pylori* with IL-Iβ Gene Polymorphism Among the Liver Transplant Recipients

Running title: Helicobacter pylori Infection among the liver transplant recipients

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DOI: 10.21608/jbaar.2023.245416.1022

Abstract

Background and Objective: Patients receiving liver transplantation are more likely to develop a wide range of infectious complications such as *Helicobacter pylori* (*H. pylori*). This study aims to assess the prevalence of *H. pylori* in individuals receiving liver transplants through the lymph nodes. Moreover, to investigate the relationship between the polymorphisms in the IL-1β gene and the *H. pylori* infection.

Methods: A total of 43 liver-transplanted patients were selected. They performed a history interview and physical and biochemical examination. The UreA gene and the virulent gene CagA were molecularly screened from paraffin-embedded tissue, additionally, the polymorphism of IL-1β was performed by Polymerase Chain Reaction-Restriction length polymorphism (PCR-RFLP).

Results: all 23(53.5%) patients showed *H. pylori* infection with UreA gene detection. Out of them 8 (34.8%) were positive for the CagA virulence gene. The infected group with *H.pylori* showed a significant decrease in albumin (P<0.05). In the case of CagA-positive patients showed a significant decrease in albumin, urine creatinine, HB, platelets, total calcium, and AT III and an increase in urea, creatinine, uric acid, iron, INR, K, TSH, LDH, triglyceride, lipase, and amylase. Analysis of IL-1β (C+3954T), the TT genotype is considered a protective factor, while the CT genotype might be a risk factor for infection. Concerning IL-1β (T-31C) in the case of CagA positive C allele might be protective from infection with CagA-positive strain.

Received: October 9, 2023. Accepted: December 19, 2023. Published: December 28, 2023

Conclusion: Based on these results, *H. pylori* appears to have a significant impact on the progression of liver transplanted patients with an interference role of IL-1β polymorphism.

Keywords: *H. pylori*; Liver transplantation; IL-1β polymorphism; Lymph nodes.

Introduction

Liver diseases, both acute and chronic, are significant global sources of morbidity and mortality. The most prevalent non-neoplastic cause of mortality among gastrointestinal tract diseases currently is liver cirrhosis **(1,2).** The "decompensated phase" of cirrhosis, which is more advanced and marked by the emergence of ascites, portal hypertension gastrointestinal hemorrhage, encephalopathy, and jaundice, ranges from the relatively stable and usually asymptomatic "compensated phase" **(3).** The most severe acute decompensation of cirrhosis is likely acute-on-chronic liver failure (ACLF), which is defined as acute decompensation of chronic liver disease accompanied by (multiple) organ failures, despite developments in pathophysiology and the best available therapy **(4).** Liver transplantation (LT) is the most effective and fundamental form of treatment for patients who have unfavorable side effects from internal medicine and artificial livers **(5).**

One of the most significant clinical issues in patients with decompensated liver cirrhosis, particularly in those who are hospitalized, is bacterial infections, which are linked to high rates of mortality and morbidity **(6,7).** About 20–35% of patients in certain series and 20–60% of patients in other series of cirrhotic patients have infections at the time of admission or acquire them while they are hospitalized **(8).** On the other hand, even though dissemination through the lymphatic system is occasionally proposed as a possible channel that aids in deeper microbial invasion, it is often regarded as being unimportant due to ineffective tissue absorption and efficient lymph node filtering **(9).** As a result, lymphatics are frequently vaguely and sparsely mentioned in the literature on bacterial infections. Instead, the perspectives on invasion are dominated by studies on direct microbial disruption of epithelial and vascular endothelial cell layers **(10,11).** Cirrhotic patients experience a steady, gradual rise in capillary filtration, which is predominantly brought on by an increase in hydrostatic pressure. This helps to increase the amount of lymph produced, which leads to compensatory lymphatic responses such as an increase in the number and size of lymphatic vessels, which helps the interstitial fluid drain more effectively **(12).** Increased lymphatic flow restricts edema and reduces tissue dendritic cell retention when pathogens like *Helicobacter pylori* are present, whereas lymphostasis can result in persistent inflammation **(13).**

Helicobacter pylori (H.pylori), a gram-negative, spiral-shaped opportunistic infection, is frequently linked to gastritis, peptic ulcers, and other gastrointestinal disorders **(14).** Around 50% of the people in developed countries were thought to have *H. Pylori* infections, whereas 90% of those in underdeveloped nations appeared to have this bacterium **(15).** Researchers from all over the world are interested in the part that *H. pylori* plays in the development of liver conditions including liver cirrhosis. A curious finding is that *H. pylori* has also been linked to the etiology of hepatic encephalopathy, a common complication that affects between 30 and 70% of people with liver cirrhosis **(16,17).** In addition to causing localized inflammation due to its cytopathic effect, *H. pylori* infection also leads to a generalized rise in the number of pro-inflammatory cytokines **(18,19).** Two key single nucleotide polymorphisms (SNPs) were detected in the IL-1β gene (IL-1β T-31C and IL-1β C3954T), where they were associated with *H. pylori* infection **(20,21).**

This study aims to assess the prevalence of extra gastric translocation of *H. pylori* in the enlarged

lymph nodes of seropositive ascites patients with decompensated end-stage liver disease. Moreover, to investigate the relationship between the polymorphisms in the IL-1β gene (IL-1β T-31C and IL-1β C3954T) and the *H. pylori* infection.

Patients and Methods

Study design and Patient enrollment

Between April 2019 and May 2021, patients were recruited from the liver transplantation program of (NHTMRI) at the National Hepatology and Tropical Medicine Research Institute and participated in this retrospective cross-section pilot study. We prospectively enrolled 43 patients, and data on the patient's clinical, radiological, demographic, hematological, and biochemical findings were assessed both at the start of the study and throughout the follow-up periods. This study included patients with liver cirrhosis who were above the age of 20. The study did not include patients who had a history of chronic diseases like diabetes, kidney problems, or hematologic disorders.

Samples collection

Slices of the 4 µm thick, paraffin-embedded lymph node tissue blocks were inserted in sterile 1.5 ml centrifuge tubes after being treated in formalin. A distinct microtome knife was used for each sample to avoid cross-contamination. Fresh venous blood samples (10 mL) were taken from all patients after an overnight fast. Five mL were taken without any anticoagulants for serum testing, 2.5 mL were taken with EDTA for complete blood count (CBC), and 2.5 mL were taken with citrate for coagulation profile analysis. Following that, the serum samples were separated into aliquots and stored at 20°C pending further analysis. The samples were then taken right away to the lab for examination.

Serologic Laboratory analysis

All serum samples used in this study were subjected to a variety of laboratory tests, including those for the liver (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, and albumin), kidneys (urea, creatinine, ureic aid, Creatinine clearance, GFR, and urine creatinine), blood sugar profile (fasting blood sugar (FBS), postprandial blood sugar (PPBS), and glycated hemoglobin (HbA1C)], lipid profile [cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL)], as well as enzymes amylase, lipase, lactate dehydrogenase (LDH), and homocysteine, tested using an automated Cobas c 111 analyzer by Roche Diagnostics. Modular Cobas 6000 C501 systems were used to test the iron profile (iron, Ferritin, Transferrin, and Total Iron-Binding Capacity, or TIBC), Thyroid profile [TSH, T3, T4] tested by Anylab F1 Thyroid Machine and electrolytes [Sodium (Na), Potassium (K), Total and ionized calcium] tested on ST-200 Plus Electrolyte. On a Cobas t 511 coagulation analyzer, the coagulation profile (prothrombin time (PT), INR, fibrinogen, AT III, protein C, and protein S) of all citrated plasma samples was evaluated. Additionally, the CBC of the EDTA blood samples was performed using the Sysmex Xn-550 Cell Counter and blood types. By using the Cobas 6000 (e 601 module), RT-PCR for HCV RNA and HBV DNA, and the hepatitis B surface antigen (HBsAg), anti-HCV antibodies were identified.

Serum levels assessment of AFP, CEA, and CA 19-9

According to the manufacturer's instructions, the ARCHITECT i2000sr automated immunoassay analyzer (Abbott Diagnostics, Abbott Park, IL, USA) was used to measure the serum levels of the markers Alpha-fetoprotein (AFP), Carcinoembryonic antigen (CEA), and CA19-9.

DNA Extraction

Using Thermo Scientific ™ K0171 following the manufacturer's instructions, DNA was extracted from Formalin-fixed paraffin-embedded (FFPE) enlarged lymph node tissues by deparaffinization, rehydration, and homogenization as described before by Yun et al., 2014 **(22).** Until usage, all extracted DNA was kept in a -20°C freezer. Using NanoDropTM 2000/2000c (Thermo Fisher

Scientific, Waltham, MA, USA), DNA from tissues was examined for purity and concentration.

Detection of *H. pylori*

Nested PCR (n-PCR) for *H. pylori* targeting the UreA gene was performed initially for infection screening, then to confirm *H. pylori* species by detecting the virulence gene (CagA). When the amplified PCR products were electrophoresed on a 1.5% agarose gel, they revealed a 200-bp fragment of the UreA and a 550-bp fragment of the cagA. The amplified DNA was stained with ethidium bromide and examined on a gel while being compared to a 100bp DNA ladder (Fermentas, Thermo Fisher Scientific Inc.).

Genotyping of IL-1β Polymorphisms

Restriction fragment length polymorphisms in the polymerase chain reaction were used to genotype the IL-1β T-31C and C3954T SNPs (PCR-RFLP). **Table 1** lists the PCR conditions and utilized primer. *AluI* and *TaqI* restriction enzymes from Thermo Scientific were used to digest the PCR products of the IL-1β T-31C (rs1143627) and C+3954T (rs1143634) polymorphisms, respectively. Ten µl of the PCR product were used for restriction digestion (37 °C for 2 h), and the DNA fragments were represented by electrophoresis on a 2.5% agarose gel and visualization under UV light following ethidium staining.

Table 1: The sequences of reverse and forward primers and PCR conditions

Primer name	Sequence (5'-3')	Gene	Reference
2F2	ATATTATGGAAGAAGCGAGAGC	UreA	**Sasaki et al., 1999 (23)**
2R2	ATGGAAGTGTGAGCCGATTTG		
2F3	CATGAAGTGGGTATTGAAGC		
2R3	AAGTGTTGAGCCGATTTGAACCG		
CagA F1	GGAACCCTAGTC AGTAATGGGTT	CagA	**Hirai et al., 2009 (24)**
CagA R1	GCTTTAGCTTCTGATACCGCTTGA		
CagA F2	CCAATAACAATAATAATGGACTCAA		
CagA R2	AATTCTTGTTCCCTTGAAAGCCC		
-31T/C F	AGAAGCTTCCACCAATAC TC	*IL-1β*	**Shakhatreh et al., 2020 (25)**
-31T/C R	ACCACCTAGTTGTAAGGA		
+3954C/T F	GTTGTCATCAGACTTTGACC	*IL-1β*	**Hefler et al., 2001 (26)**
+3954C/T R	TTCAGTTCATATGGACCAGA		

Statistical analysis

Version 28 of the Statistical Package for Social Sciences (SPSS) was used to analyze the data. Data were presented as means ± standard deviation (SD). The Mann-Whitney test was used to assess the significance of differences in non-parametric continuous variables, and the independent t-test was performed to compare two independent groups of *H. pylori* infection. The significance of the difference between categorical variables was evaluated using the Chi-square test. The risk associated with each group was determined using the odds ratio (OR) and 95% confidence intervals (CI). When comparing the strength and significance of associations between parametric and non-parametric variables, Spearman's correlations were used instead of Pearson's. The correlation coefficient indicated the power of the association, and a statistically significant difference was detected when P <0.05.

Results

Patient's baseline characteristics

Among the 43 LT recipients, 37 (86%) were men, and 6 (14%) were women with a mean age was 46.53± 15.1 years, ranging from 20 to 85 years. When *H. pylori* infection was examined in the studied cases, 23 (53.5%) were positive, whereas 20 individuals (46.5%) showed negative and were consequently deemed uninfected. The baseline characteristics of age, gender, and biochemical profiles were comparable in infected and non-infected individuals, as shown in **Table 2**. When biochemical parameters and *H. pylori* infection were analyzed, albumin, Na, decreased significantly in the infected group ($P<0.05$). **Figure 1** presents the tissue histological features in the presence of *H. pylori*.

Table 2: Comparison of sociodemographic and clinical parameters according to *H.pylori* infection

Variables	*H.pylori* positive (n=23)	*H.pylori* Negative (n=20)	*P* value
Sociodemographic parameters			
Age	48.74 ± 15.87	44.32 ± 14.25	0.352
Sex (M/F)	19/4	18/2	0.787
Liver functions			
ALT	49.09 ± 38.55	68.05 ± 95.81	0.388
AST	85.30 ± 61.75	103.09 ± 136.14	0.576
Total Bilirubin	4.53 ± 4.49	2.76 ± 2.34	0.122
Direct Bilirubin	2.34 ± 3.68	1.33 ± 1.53	0.259
Albumin	2.72 ± 0.50	3.44 ± 1.34	0.021*
Kidney Functions			
Urea	29.36 ± 8.99	29.65 ± 10.19	0.923
Creatinine	0.81 ± 0.22	0.94 ± 0.30	0.105
Uric acid	5.27 ± 1.64	5.75 ± 2.26	0.425
Creatinine clearance	97.49 ± 40.99	85.56 ± 43.11	0.358

GFR		84.99 ± 17.63	88.70 ± 17.41	0.492
urine creatinine		113.58 ± 116.63	69.52 ± 38.29	0.114
Hematological parameters				
Blood groups	O+	10 (43.6%)	4 (20%)	0.09
	O-	0 (0%)	2 (10%)	
	A+	4 (17.5%)	6 (30%)	
	A-	1 (4.3%)	1 (5%)	
	B+	6 (26%)	2 (10%)	
	B-	1 (4.3%)	2 (10%)	
	AB+	1 (4.3%)	3 (15%)	
HB		11.35 ± 2.73	12.32 ± 1.85	0.189
TLC		4.93 ± 2.07	5.22 ± 2.82	0.701
PLT		109.4 ± 78.31	129.1 ± 113.79	0.507
Coagulation profile				
PT		19.57 ± 4.78	17.29 ± 3.37	0.082
INR		1.52 ± 0.35	1.49 ± 0.29	0.807
Fibrinogen		249.52 ±128.59	214.95 ± 84.71	0.312
AT III		46.13 ± 22.09	59.5 ± 32.12	0.115
Protein C		44.15 ± 26.78	47.26 ± 29.69	0.719
Protein S		64.11 ± 20.89	70.11 ± 22.69	0.373
Iron profile				
Iron		105.05 ± 39.69	114.13 ± 94.34	0.691
Ferritin		441.11 ± 628.24	237.47 ± 195.28	0.182
Transferrin		75.35 ± 81.17	62.84 ± 55.06	0.564
TIBC		240.57 ± 90.42	249.9 ± 56.31	0.692
Electrolytes				
Na		133.04 ± 5.22	136.90 ± 2.97	0.005*
K		4.03 ± 0.66	4.36 ± 0.69	0.123
Calcium Total		8.57 ± 0.70	8.63 ± 0.67	0.776
Calcium Ionized		3.45 ± 1.83	9.33 ± 26.81	0.299
Blood glucose profile				
FBS		97.09 ± 27.66	101.75 ± 41.82	0.664
PPBS		165.74 ± 90.27	154.05 ± 82.07	0.661
HbA1C		5.12 ± 1.53	5.42 ± 0.97	0.452
Lipid profile				

Cholesterol	134.0 ± 52.77	125.65 ± 37.87	0.559
Triglyceride	91.17 ± 56.82	92.15 ± 45.49	0.951
HDL	48.87 ± 55.66	37.9 ± 13.29	0.395
LDL	83.65 ± 48.69	70.3 ± 31.84	0.301
VLDL	17.30 ± 10.99	15.85 ± 7.26	0.479
Thyroid profile			
TSH	2.21 ± 2.13	1.84 ± 0.99	0.488
T3	2.38 ± 0.42	2.38 ± 0.52	0.992
T4	1.14 ± 0.26	1.51 ± 1.66	0.299
Enzymes			
LDH	222.83 ± 88.42	229.83 ± 138.15	0.842
Lipase	69.55 ± 37.29	72.35 ± 39.17	0.813
Amylase	98.30 ± 50.62	89.45± 21.09	0.306
Homocysteine	14.87 ± 3.98	13.59 ± 3.79	0.288

*Significant difference.

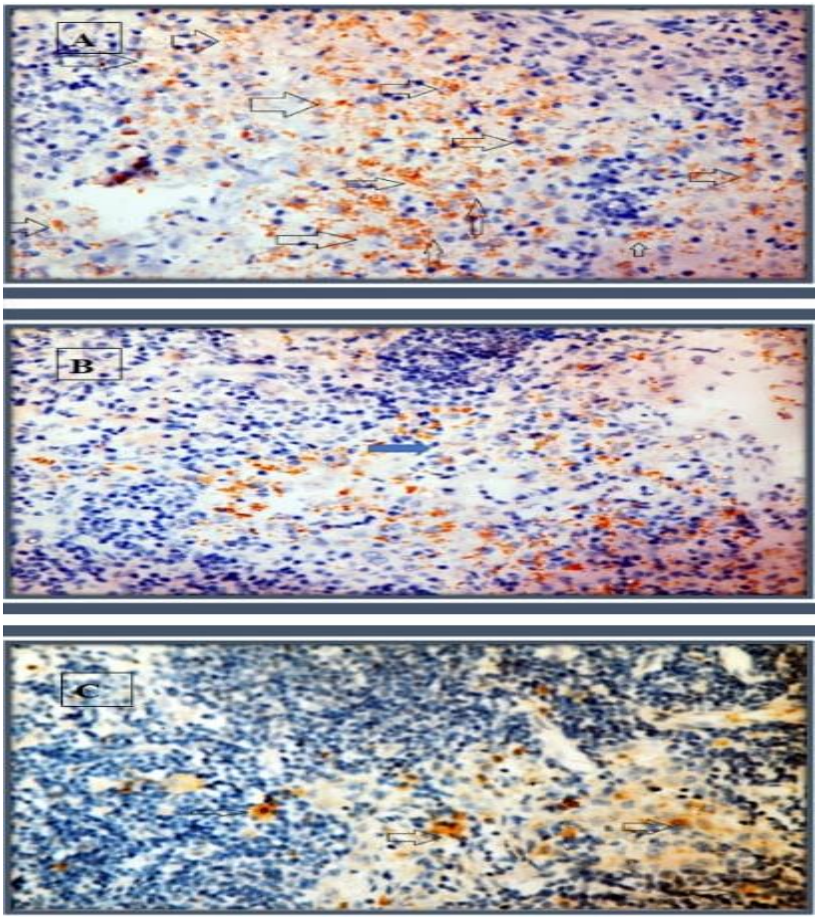

Figure 1: Immunohistochemical staining with anti-Helicobacter polyclonal antibodies of a node showing granular cytoplasmic brown staining in the paracortical area (arrow) by (original magnification X400).

Effect of CagA gene on biochemical profiles

Fifteen infected patients (65.2%) did not test positive for CagA and were therefore believed to be infected by "less virulent" *H. pylori* strains. In contrast, 8 (34.8%) who tested positive for CagA among the infected individuals were colonized by strains that expressed this marker of increased pathogenicity. The baseline characteristics of age, gender, and biochemical parameters showed a significant association between infections with CagA strain and younger age, decreased albumin urine creatinine, HB, platelets, total calcium, and AT III. On the other hand, urea, creatinine, uric acid, iron, INR, K, TSH, LDH, triglyceride, lipase, and amylase increased in infected than non-infected patients, as shown in **Table 3**.

Table 3: Comparison of sociodemographic and clinical parameters according to *CagA*

Variables		*H.pylori* CagA+ (n=8)	*H.pylori* CagA– (n=15)	*P* value
Sociodemographic parameters				
Age		40.63 ± 12.12	53.07± 15.41	0.007*
Sex (M/F)		8/0	11/4	0.091
Liver functions				
ALT		52.88 ± 41.73	47.07 ± 35.19	0.619
AST		89.0 ± 66.25	83.33 ± 56.93	0.763
Total Bilirubin		3.03 ± 2.02	5.33 ± 5.05	0.088
Direct Bilirubin		1.43 ± 1.09	2.83 ± 4.31	0.210
Albumin		2.52 ± 0.63	2.82 ± 0.36	0.045*
Kidney Functions				
Urea		35.63 ± 5.96	26.27 ± 8.05	0.0002*
Creatinine		0.98 ± 0.15	0.73 ± 0.18	P < 0.0001*
Uric acid		6.34 ± 1.46	4.71 ± 1.38	0.0005*
Creatinine clearance		97.94 ± 30.32	97.27 ± 44.44	0.957
GFR		84.13 ± 15.14	85.45 ± 18.25	0.806
urine creatinine		62.28 ± 21.69	140.93 ± 132.47	0.023*
Hematological parameters				
Blood groups	O+	2 (25%)	8 (53.34)	0.235
	O-	0 (0%)	0 (0%)	
	A+	2 (25%)	2 (13.33%)	
	A-	1 (12.5%)	0 (0%)	
	B+	1 (12.5%)	5 (33.33)	
	B-	1 (12.5%)	0 (0%)	
	AB+	1 (12.5%)	0 (0%)	
HB		10.04 ± 2.75	12.05 ± 2.35	0.012*
TLC		4.24 ± 1.26	5.29 ± 2.24	0.091
PLT		80.0 ± 23.69	125.07 ± 89.38	0.050*
Coagulation profile				
PT		20.63 ± 3.27	19.01 ± 5.19	0.264

INR	1.67 ± 0.28	1.44 ± 0.35	0.028*
Fibrinogen	204.5 ± 85.91	273.53 ± 136.59	0.073
AT III	37.75 ± 20.66	50.6 ± 20.75	0.050*
Protein C	42.25 ± 23.89	45.16 ± 27.27	0.721
Protein S	66.75 ± 12.14	62.71 ± 23.57	0.526
Iron profile			
Iron	92.87 ± 48.57	154.0 ± 132.72	0.002*
Ferritin	278.38 ± 253.9	527.91 ± 723.07	0.189
Transferrin	76.88 ± 52.04	74.53 ± 90.65	0.924
TIBC	248.13 ± 41.36	236.54 ± 105.04	0.674
Electrolytes			
Na	133.38 ± 5.39	132.87 ± 4.94	0.748
K	4.3 ± 0.73	3.89 ± 0.55	0.037*
Calcium Total	8.16 ± 0.68	8.79 ± 0.59	0.002*
Calcium Ionized	3.71 ± 1.63	3.31 ± 1.86	0.473
Blood glucose profile			
FBS	91.63 ± 15.39	100.0 ± 31.16	0.319
PPBS	159.38 ± 67.63	169.13 ± 97.36	0.723
HbA1C	5.05 ± 0.49	5.15 ± 1.82	0.831
Lipid profile			
Cholesterol	141.6 ± 56.15	119.75 ± 37.9	0.171
Triglyceride	103.47 ± 63.75	68.13 ± 21.08	0.037*
HDL	49.0 ± 36.17	48.8 ± 62.19	0.991
LDL	69.5 ± 28.23	91.2 ± 53.74	0.140
VLDL	19.2 ± 12.52	13.75 ± 4.35	0.100
Thyroid profile			
TSH	3.23 ± 2.99	1.66 ± 0.99	0.011*
T3	2.44 ± 0.49	2.34 ± 0.37	0.440
T4	1.05 ± 0.23	1.19 ± 0.26	0.077
Enzymes			
LDH	271.75 ± 88.24	196.73 ± 73.18	0.003*
Lipase	88.63 ± 40.22	58.64 ± 28.9	0.005*
Amylase	122.88 ± 61.73	85.2 ± 35.11	0.011*
Homocysteine	13.8 ± 4.12	15.44 ± 3.64	0.171

*Significant difference.

Association between *H.pylori* infection and tumor markers

Table 4 illustrates the levels of AFP, CEA, and CA19.9 were performed to analyze the difference in tumor marker levels between the *H. pylori* (+) and *H. pylori* (−) groups, Also, the association with CagA status. The levels of AFP in subjects in the *H. pylori* infection group were significantly lower than those in the *H. pylori* (−) group, while CEA was significantly higher than those in the *H. pylori* (−) group. Subjects showed no significant differences in the levels of the CA19.9 between two groups (P>0.05). On the other hand, subjects with CagA (+) and Cag A (-) showed no significant differences in the levels of the studied tumor markers (P>0.05).

Association between IL-1β gene Polymorphisms and *H.pylori* infection

The distribution of IL-1β genotypes and alleles (+3954 C/T and -31 T/C) is summarized in **Table 5**. According to the analysis of IL-1β (+3954 C/T), TT genotype was significantly lower in the uninfected group than infected (OR= 0.124, CI=0.027-0.580, P=0.008), while the CT genotype was higher in infected than uninfected group, but insignificantly (P<0.05). In addition, infected patients' CC genotype and C allele frequencies were slightly higher than those of *H. pylori*-infected patients (OR=1.242, 1.391 respectively). Concerning IL-1β (-31 T/C), the analysis demonstrated no statistical significance in the distribution of all genotypes or alleles between the infected and non-infected groups. However, in case of CagA positive showed a significantly low frequency of the C allele, which might be a protective from infection with CagA positive strain (OR= 0.268 CI= 0.086-0.831, P=0.022).

Haplotype

Haplotype analysis of IL-1β C3954T and T-31C SNPs is shown in **Table 6**. Among all examined haplotypes, the TT haplotype was significantly lower in infected than uninfected 26% vs. 47.5%, while CagA positive showed an insignificant increase in the TT haplotype 37.5% vs. 16.7% (P >0.05).

Table 4: Association between *H.pylori* infection and tumor markers

Variables	*H.pylori* positive (n=23)	*H.pylori* Negative (n=20)	*P* value	*H.pylori* CagA+ (n=8)	*H.pylori CagA*- (n=15)	*P* value
Tumor markers						
AFP	4.87 ± 4.31	51.55 ± 119.29	0.009*	4.91± 3.54	4.84 ± 4.67	0.958
CEA	79.5 ± 2.91	12.49 ± 44.41	P < 0.0001*	3.53 ± 2.32	3.42 ± 3.18	0.902
CA 19.9	44.07 ± 98.80	43.69 ± 69.44	0.983	61.75 ± 107.52	34.63 ±92.45	0.375

Table 5: Genotype distribution and allelic frequency of IL-1β (+3954 C/T and -31 T/C) in *H.pylori* infected and non-infected subjects.

SNPs	*H. Pylori* + n=23	*H.Pylori* - n=20	OR(95%CI)	*P* valu	CagA+ n= **8**	CagA- n=**15**	OR(95%CI)	*P* value
IL-1β								
+3954 C/T (Allele and genotypes)								
C	32 (69.6%)	20 (50%)	1.391 (0.847-2.284)	0.19	12 (75%)	21(70%)	1.071 (0.554-2.073)	0.838
T	14 (30.4%)	20 (50%)	0.609 (0.345-1.075)	0.08	4 (25%)	9 (30%)	0.833 (0.326-2.127)	0.703
CC	10 (43.5%)	7 (35%)	1.242 (0.556-2.775)	0.59	4 (50%)	6 (40%)	1.250 (0.424-3.684)	0.686
CT	12 (52.2%)	6 (30%)	1.739 (0.772-3.918)	0.18	4 (50%)	9 (60%)	0.833 (0.297-2.335)	0.729
TT	1 (4.3%)	7 (35%)	0.124 (0.027-0.580)	0.00	0 (0%)	0 (0%)	1.848 (0.035-97.501)	0.761
CT and TT	**13 (56.5%)**	**13 (65%)**	**0.869 (0.436-1.732)**	**0.69**	**4 (50%)**	**9 (60%)**	**0.833 (0.297-2.335)**	**0.729**
-31 T/C (Allele and Genotypes)								
T	30 (65.2%)	30 (75%)	0.869 (0.545-1.387)	0.55	14	16 (53.3%)	1.641 (0.844-3.188)	0.144
C	16 (34.8%)	10 (25%)	1.391 (0.738-2.623)	0.30	2 (12.5%)	14 (46.7%)	0.268 (0.086-0.831)	0.022
TT	21(47.8%)	12 (60%)	0.797 (0.389-1.633)	0.53	6 (75%)	5 (33.3%)	2.250 (0.799-6.338)	0.125
TC	8 (34.8%)	6 (30%)	1.159 (0.491-2.740)	0.73	2 (25%)	6 (40%)	0.625 (0.173-2.257)	0.473
CC	4 (17.4%)	2 (10%)	1.739 (0.487-6.211)	0.39	0 (0%)	4 (26.7%)	0.109 (0.006-2.005)	0.136
TC and CC	**12 (52.2%)**	**8 (40%)**	**1.304 (0.609-2.793)**	**0.49**	**2 (25%)**	**10 (66.7%)**	**0.375 (0.109-1.287)**	0.119

Table 6: Analysis of IL-1β haplotypes of the examined polymorphisms

Haplotype	*H.pylori* +	*H.pylori*-	OR (95% CI)	*P* value	CagA+	CagA-	*OR (95% CI)*	*P* value
CC	32.6	22.5	1.449 (0.751-2.795)	0.478	25.0	33.3	0.750 (0.297-1.892)	0.542
TT	26.0	47.5	0.549 (0.304-0.993)	0.047*	37.5	16.7	2.250 (0.877-5.775)	0.092
CT	26.0	15.0	1.739 (0.817-3.700)	0.151	25.0	30.0	0.833(0.326- 2.126)	0.703
TC	17.4	15.0	1.159 (0.518-2.596)	0.969	12.5	20.0	0.625 (0.186-2.096)	0.446

Discussion

Numerous gastrointestinal and extra-gastrointestinal diseases have been linked to *H. pylori* infection **(27,28).** According to recent studies **(29,30)**, *H. pylori* may play a role in the etiopathogenesis of certain liver diseases. Its DNA has also been found in the liver tissue of individuals with hepatocellular carcinoma and chronic liver diseases. This study's objective was to assess the impact of H. pylori in people receiving liver transplants because they have end-stage liver disease. Furthermore, to study the association between *IL-1β* gene polymorphisms (*IL-1β* T-31C and - *IL-1β* C3954T) and the *H. pylori* infection.

The present study reported that 53.5% of LT recipients had *H. pylori* infection, which was consistent with a longitudinal study in Japan that found an *H. pylori* infection rate of 50% two weeks before LT **(31).** In contrast to our study's findings, earlier investigations found that only 18.6% of LT recipients had *H. pylori* **(32).** This phenomenon suggested that *H. pylori* infections may recur often

in LT recipients; the average annual recurrence rate of *H. pylori* infections after eradication therapy is 4.3% (95% CI, 4%–5%) globally **(33)**, and it varies from place to place, associated with local socioeconomic levels and the local *H. pylori* infection prevalence.

Our findings demonstrated a statistically significant decrease in albumin, Na with *H. pylori* infection (P<0.05), but not for the other baseline or biochemical parameters. This finding is in line with **Liu et al., (2022)** who reported that *H. pylori* infection was strongly linked to decreased serum albumin levels in the Chinese population **(34).** Additionally, *H. pylori* infection may be a driving factor in the development of hypernatremia in elderly people **(35)**.

In comparison to cagA-negative patients, *H. pylori* CagA-positive individuals displayed significantly decreased levels of albumin, urinary creatinine, HB, platelets, total calcium, iron, and AT III. These findings support the findings of **Caliskan et al. (2014)** who suggested that infection with *H. pylori* may help to minimize proteinuria **(36).** According to **Xu et al. (2017)**, the individuals they evaluated had lower hemoglobin levels and a greater correlation between anemia and *H. Pylori* infection **(37).** Infection with *H. pylori* is becoming a more common cause of immunological thrombocytopenia (ITP), according to **Lee et al. (2020)**, and **Kishore et al., (2021)** found that participants with the infection had considerably lower mean serum iron levels **(38,39).**

To the best of our knowledge, this work was the first to document a link between *H. pylori* CagA and AT III. On the other hand, CagA-positive patients had significantly higher levels of urea, creatinine, uric acid, INR, K, TSH, LDH, triglyceride, lipase, and amylase than CagA-negative patients. The results of the study are in line with those of **Lu et al. (2018)** who found a link between *H. pylori* infection and uric acid **(40). Ndebi et al. (2018)** confirm a potential link between *H. pylori* infection, lipid profile, and uric acid **(41),** and **Milutin et al., (2014)** found that patients with *H. pylori* infection secrete more pancreatic enzymes **(42).** An increase in pancreatic bicarbonate production and stimulation of ductal epithelial cell proliferation brought on by an *H. pylori* infection with antral predomination may be a factor in the disease.

In the present study, the levels of AFP in subjects in the *H. pylori* infection group were significantly lower than those in the *H. pylori* (−) group, while CEA was significantly higher than those in the *H. pylori* (−) group. Subjects showed no significant differences in the levels of the CA19.9 between two groups (P>0.05). This result is in line with **Xu et al., (2018), who** reported that a significant positive correlation was found between *H. pylori* infection and CEA values **(43).**

According to the analysis of IL-1β (+3954 C/T), the TT genotype was significantly lower in the uninfected group than infected (OR= 0.124, CI=0.027-0.580, P=0.008), which might be a protective factor from *H. pylori* infection. While CT genotype was higher in infected than uninfected group, but insignificantly (P<0.05). In addition, infected patients' CC genotype and C allele frequencies were slightly higher than those of *H. pylori*-infected patients (OR=1.242, 1.391 respectively). Contrary to the study findings, previous studies reported a lack of association between C3954T SNP and *H. pylori* infection **(44,** 25). Concerning IL-1β (-31T/C), the analysis demonstrated no statistical significance in the distribution of all genotypes or alleles between the infected and non-infected groups. However, cases with positive CagA showed a significantly low frequency of the C allele, which might be protective from infection with CagA positive strain (OR= 0.268 CI= 0.086-0.831, P=0.022). Contrary to this study result, **Shakhatreh et al.,** (**2020**) reported that the TT genotype was higher in *H. pylori-positive* subjects compared to the control ones **(25).**

From haplotype analysis, the present study showed that in four haplotypes (CC, CT, TC, and TT), the frequency of the TT haplotype was

significantly lower in infected than uninfected (26% vs. 47.5%) and the genotypes (CC and CT) were higher in infected than uninfected patients, which might increase the risk of *H. pylori* infection but insignificantly. In the haplotype analysis in the case of the CagA positive strain, the TT haplotype was present in 37.5% of the CagA positive group compared to 16.7% of the Cag A negative group, which might indicate that the CT haplotype is a risk for infection with virulent strain (OR=2.250, CI 0.877-5.775, P=0.092). To the best of our knowledge, it is the first time to study the interaction between IL-1β (T-31C and C+3954T) polymorphisms, in liver transplanted patients.

Conclusion

Our study revealed that the prevalence of *H. pylori* infection was 53.5% in LT recipients. This study demonstrated the role of the lymph node in the infection transmission. Additionally, the interference role of IL-1β with *H. pylori* infection in LT patients.

Ethical Approval and consent to participate: Research Involving Human Participants. The study was conducted following the Declaration of Helsinki. All participants provided written informed consent, and the Ethics Committee of the National Hepatology and Tropical Medicine Research Institute (NHTMRI) approved the study protocol.

Consent for publication: Not Applicable

Availability of data and material: Not Applicable

Competing interests: The authors declare that they have no conflict of interest that affects this study.

Funding: No funding was received for conducting this study.

Authors' contributions: GH, EH, KB, MYN, AA, and AI got the study concept and design, GH, MYN, KB, and MAK did the biochemical analysis, EH did the histopathological analysis, MAS did the clinical examination and diagnosis, AI made data analysis and interpretation, AI and GH do the molecular analysis, and AI, EH, and AA wrote and revised the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

List of Abbreviations

AFP: alpha-fetoprotein
ALT: alanine aminotransferase
AST: aspartate aminotransferase
CagA: Cytoxin-Associated Gene A
CBC: Complete blood count
CEA: carcinoembryonic antigen
FBS: fasting blood sugar
***H. pylori*:** *Helicobacter pylori*
HB: Hemoglobin
HbA1C: glycated hemoglobin
HBsAg: hepatitis B surface antigen
HDL: high-density lipoprotein
IL-1β: Interleukin 1β
INR: International normalized ratio
K: Potassium
LDH: Lactate Dehydrogenase
LDL: low-density lipoprotein
LT: Liver transplantation
Na: Sodium
PCR-RFLP: Polymerase chain reaction-Restriction length polymorphism
PPBS: postprandial blood sugar
PT: prothrombin time
SNPs: single nucleotide polymorphisms
TIBC: Total Iron-Binding Capacity
TSH: Thyroid stimulating hormone.
UreA: Urease A
VLDL: very-low-density lipoprotein

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