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Relation of Cholecystokinin B Receptor Gene Polymorphisms in Helicobacter Pylori Patients with Aand without Hepatitis C Virus

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Abstract

Background: There is no doubt about the association between the cholecystokinin B receptor (CCKBR) gene and susceptibility to helicobacter pylori (H. pylori) infection. CCKBR gene encodes a G-protein-coupled receptor for gastrin and cholecystokinin (CCK). Several genetic studies have demonstrated an implication of CCK in the feedback control of gastrin release and gastric acid secretion in healthy subjects. previous studies in patients infected with hepatitis C virus (HCV) refer to the bad effect of H. pylori infection on the course of liver injury, especially extensive fibrosis.

Objectives: We aimed to investigate whether there is an association between the CCKBR (rs2929180 and rs1800843) single nucleotide polymorphisms (SNPs) and the susceptibility to H. pylori infection in patients with and without HCV.

Subjects and methods: The current work was carried out on 195 subjects. They were classified into three groups: Group I: 65 H. pylori patients with HCV, Group II: 65 H. pylori patients without HCV, and 65 age- and gender-matched healthy controls. All subjects underwent genotyping of CCKBR (rs2929180 and rs1800843) by real-time PCR.

Results: The frequency of TT in CCKBR (rs2929180) was higher in H. pylori patients with HCV than in H. pylori patients without HCV and controls (23.1% vs. 7.7% and 0%). The frequency of the CC and CA of CCKBR (rs1800843) was higher in H. pylori patients with HCV than in H. Pylori patients without HCV and controls (13.8% vs. 4.6% and 1.5%) and (32.3% vs. 20% and 12.3%) respectively.

Conclusion: CCKBR (rs2929180 and rs1800843) gene polymorphisms play a role in determining the degree of H. Pylori infection occurrence in HCV patients.

Keywords: H. pylori; HCV; CCKBR.

1. Introduction

H. pylori is a microaerophile. In 1982, it was described by Marshal and Warren for the first time. These Gram-negative short rods bacterium is resistant to the gastric acid activity **[1]**.

The percentage of infection by these bacteria in developed countries may reach 50%, while in developing countries, it may be detected in about 90%, as *H. pylori* is distributed through the faecal–oral route by drinking water and tainted food as a result of poor hygiene, insufficient nutrition, and geographical variances **[2]**.

In *H. pylori* infection, proinflammatory cytokines IL-1, -2, -4, -6, -8, -10, -17, interferon-β, and TNF-α increase in the gastric mucosa and systemic circulation **[3]**. *H. pylori* cause chronic atrophic gastritis, metaplasia, dysplasia, and gastric carcinoma **[4]**.

H. pylori may also potentiate extra gastric organ disturbances, exacerbating the diseases of cardiovascular system or metabolic diseases, and deteriorating normal function of the liver, especially in patients with cirrhosis **[5,6]**. Several studies have provided evidence that *H. pylori* involved in the pathogenesis of some liver diseases **[7]**.

CCKBR gene encodes a G-protein-coupled receptor for both CCK and gastrin. These are regulatory peptides of the brain and gastrointestinal tract **[8]**.

There is no doubt about the association between the CCKBR gene and susceptibility to *H. pylori* infection. This gene is a therapeutic-effect target gene for a peptic ulcer drug, proglumide, which inhibits gastrointestinal motility and reduces gastric acid secretion **[8].**

In our study, we assessed the association of CCKBR (rs2929180 and rs1800843) SNPs with H. pylori infection and HCV severity.

2. Subjects and Mmethods

Subjects:

This case-controlled study was carried out in Chemistry Department, Faculty of Science, and Medical Biochemistry & Molecular Biology Department, Faculty of Medicine, Menofia University. All patients were recruited from the Endoscopy

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Unit of Internal, Menofia University Hospital. A total number of 195 subjects, 65 *H. pylori* patients with HCV, 65 *H. pylori* patients without HCV, and 65 age- and gender-matched healthy controls were enrolled in this study from January 2022 till January 2023. Informed consent was obtained from all study participants and/or their legal guardians. The study design was approved by the appropriate ethics review board, followed the tenets of the Declaration of Helsinki, and was approved by the ethics committee of the Faculty of Medicine, Menofia University.

The inclusion criteria for patients were as follows: 1) age ranging between 15 and 70 years old; 2) patients diagnosed with H. pylori infection by stool and breath tests.

The exclusion criteria for patients were as follows: 1) patients with a history of hypertension (HTN), diabetes mellitus (DM), cardiovascular diseases, and old stroke; 2) patients with a current inflammatory or autoimmune disease; 3) patients with recent surgery, trauma or heart attack; 4) patients with a personal history of cancer; 5) patients with a personal history of kidney diseases; 6) pregnant patients.

Methods:

Sample Collection:

Under complete aseptic conditions and after overnight fasting, five milliliters (ml) of venous blood were withdrawn by venipuncture and were processed as follows: 3 ml was transferred into a plain tube, left to clot for 15 min, and centrifuged for 10 min at 4000 rpm. The obtained serum was stored at -80°C until the lipid profile was performed. The remaining 2 ml of blood was placed into EDTA-containing tubes for DNA extraction and genotyping of CCKBR (rs2929180 and rs1800843) SNPs by using the TaqMan allelic discrimination assay technique.

Genotyping of CCKBR (rs2929180 and rs1800843) SNPs:

1-DNA extraction:

DNA was extracted from whole blood by a GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania. cat# K0721) following the manufacturer's protocol. DNA concentration, quality, and purity were assessed using a Nanophotometer N-60 (Implen, Germany).

2-Real-time PCR:

CCKBR (rs2929180 and rs1800843) gene polymorphisms were genotyped using an allelic discrimination assay by real-time PCR using a TaqMan probe (Applied Biosystems, Foster City, USA). Master Mix II (2x), primers and probes were supplied by Thermo Fisher Scientific. The probe sequence was labelled with [VIC/FAM] fluorescent dyes. The sequences of specific primers were as follows: **CCKBR (rs2929180):** forward primer: 5´CGCAGCGTGAGCAGGTGGAGCCGCG 3´ and reverse primer: 5´TGGGAGCCCGCCGGGTCGAGCTGAG 3´. **CCKBR (rs1800843):** forward primer: 5´ CTGGACCACGTGAGCAAAATCTGGG 3´ and reverse primer 5´ GAGGCGGAGCTTTGGAGGGCGACGG 3´. Then, 12.5 µl of master mix was added to 1.25 µl of primer/probe mix and 6.25 µl of DNase-free water. Five microliters of genomic DNA extract for every sample and 5 µl of DNase-free water for the negative control reaction were applied. The cycling conditions were adjusted as follows: initial denaturation was performed at 95°C for 10 minutes, followed by 50 cycles of denaturation at 95°C for 15 seconds and 60°C for 1 min for annealing and extension. Analysis of data was completed by a real-time PCR Instrument, Applied Biosystems®7500, software version 2.0.1.

Statistical Analysis:

Analysis was achieved using version 20.0 of the IBM SPSS software package (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. We used the Kolmogorov–Smirnov test to verify the normality of the distribution. Quantitative data were described using range (minimum and maximum), mean and standard deviation, or median. We used the chi-square test for categorical variables to compare different groups. We used the F test (ANOVA) for normally distributed quantitative variables to compare more than two groups and the post hoc test (Tukey) for pairwise comparisons. We used the Mann–Whitney test for abnormally distributed quantitative variables to compare the two studied groups. We used the Kruskal–Wallis test for abnormally distributed quantitative variables to compare more than two groups, and the post hoc test (Dunn's test for multiple comparisons) was used for pairwise comparisons between each pair of groups. The population of the studied sample was explored to find its equilibrium with the Hardy-Weinberg equation. A P value of $\leq$ 0.05 was regarded as statistically significant.

3. Results

Demographic data of *H. pylori* patients with and without HCV and healthy controls: There was a nonsignificant difference between *H. pylori* patients with and without HCV and healthy controls regarding gender (P=0.273) and age (P=0.143). There was a significant difference between *H. pylori* patients with and without HCV and healthy controls regarding body mass index (BMI) (P=0.003) **(Table 1).**

Lipid profile of *H. pylori* patients with and without HCV and healthy controls: There was a significant difference between *H. pylori* patients with HCV versus *H. pylori* patients without HCV and healthy controls regarding increased total cholesterol (P1<0.001, P2<0.001), increased TG (P1<0.001, P2<0.001), increased LDL (P1<0.001, P2<0.001) and decreased HDL (P1<0.001, P2<0.001) **(Table 2).**

CCKBR (rs2929180 and rs1800843) gene polymorphisms of *H. pylori* patients with and without HCV and healthy controls: There was a significant difference regarding the genotype frequency and allelic distribution of CCKBR (rs2929180) between *H. pylori* patients with and without HCV and healthy controls (P<0.001). The frequency of the TT genotype was significantly higher in *H. pylori* patients with HCV (23.1%) than in *H. pylori* patients without HCV (7.7%) and healthy

controls (0%). There was a significant difference regarding the genotype frequency and allelic distribution of CCKBR (rs1800843) between *H. pylori* patients with and without HCV and healthy controls (MCp=0.001*). The frequency of the CA and AA genotypes was significantly higher in *H. pylori* patients with HCV (13.8%, 32.3% respectively) than in *H. pylori* patients without HCV (4.6%, 20% respectively) and healthy controls (1.5%, 20% respectively). **(Table 3)**.

Table 1: Demographic data of *H. pylori* patients with and without HCV and healthy controls:

	Group I (n = 65)	**Group II (n = 65)**	**Control (n = 65)**	**Test of Sig.**	**p**
Gender					
Male	22 (33.8%)	26 (40%)	31 (47.7%)	$\chi 2$= 2.596	0.273
Female	43 (66.2%)	39 (60%)	34 (52.3%)		
Age (years)					
Mean ± SD.	38.2 ± 13.2	36.5 ± 11.4	33.9 ± 8.5	H= 3.885	0.143
Median (Min. – Max.)	39 (15 – 70)	35 (18 – 70)	32 (22 – 53)		
BMI (kg/m^2)					
Mean ± SD.	30.5 ± 4.6	29.6 ± 3.7	27.7 ± 1.9	H= 11.782*	0.003*
Median (Min. – Max.)	32 (23.8 – 39)	29.3 (22.1 – 36)	26.6 (22.6 – 31.3)		
Sig. bet. grps.	p1=0.474, p2=0.001*, p3=0.011*				

Group I: *H. pylori* patients with HCV **Group II:** *H. pylori* patients without HCV
SD: **Standard deviation** χ^2: **Chi square test**
H: H for **Kruskal–Wallis test,** Pairwise comparison bet. each 2-group analysis was performed using **a post hoc test (Dunn's test for multiple comparisons)**
*: Statistically significant at $p \leq 0.05$
P1: p value for comparing *H. pylori* patients with and without HCV
P2: p value for comparing *H. pylori* patients with HCV and healthy controls
P3: p value for comparing *H. pylori* patients without HCV and healthy controls
BMI: Body mass index

Table 2: Lipid profile of *H. pylori* patients with and without HCV and healthy controls:

	Group I (n = 65)	**Group II (n = 65)**	**Control (n = 65)**	**Test of Sig.**	**p**
Total Cholesterol(mg/dl)					
Mean ± SD.	206.6 ± 48.8	158.6 ± 22.4	155.6 ± 13.8	H= 48.737	<0.001*
Median (Min. – Max.)	208 (121- 300)	156 (118.8 – 263)	155 (129.0 – 181.6)		
Sig. bet. grps.	p_1<0.001*, p_2<0.001*, p_3=0.670				
TG (mg/dl)					
Mean ± SD.	145.1 ± 53.8	115.4 ± 21.9	116.1 ± 19.4	H= 17.009	<0.001*
Median (Min. – Max.)	140 (46 –268)	109 (92 – 195)	110 (95 – 160)		
Sig. bet. grps.	p_1<0.001*, p_2=0.001*, p_3=0.545				
LDL (mg/dl)					
Mean ± SD.	126.2 ± 46.6	90.6 ± 22.2	86.7 ± 14.5	H= 54.691	<0.001*
Median (Min. – Max.)	111 (26 –214)	87 (49.4 – 178)	87 (60 – 116.1)		
Sig. bet. grps.	p_1<0.001*, p_2<0.001*, p_3=0.444				
HDL (mg/dl)					
Mean ± SD.	40.3 ± 7.8	47.2 ± 4.1	46.8 ± 3.3	F= 32.562*	<0.001*
Median (Min. – Max.)	39 (30 – 78)	48 (34 – 55)	46 (40 – 52)		
Sig. bet. grps.	p_1<0.001*, p_2<0.001*, p_3=0.914				

Group I: *H. pylori* patients with HCV **Group II:** *H. pylori* patients without HCV
SD: **Standard deviation** χ^2: **Chi square test**
H: H for **Kruskal–Wallis test,** Pairwise comparison bet. each 2-group analysis was performed using **a post hoc test (Dunn's test for multiple comparisons)**
*: Statistically significant at $p \leq 0.05$
P1: p value for comparing *H. pylori* patients with and without HCV
P2: p value for comparing *H. pylori* patients with HCV and healthy controls
P3: p value for comparing *H. pylori* patients without HCV and healthy controls
TG: Triglyceride **HDL:** High-density lipoprotein **LDL:** Low-density lipoprotein

Table 3: CCKBR (rs2929180 and rs1800843) gene polymorphisms of *H. pylori* patients with and without HCV and healthy controls:

	Group I (n = 65)	**Group II (n = 65)**	**Control (n = 65)**	**OR_1 (LL – UL 95% C.I)**	**p_1**	**OR_2 (LL– UL 95% C.I)**	**p_2**	**OR3 (LL – UL 95% C.I)**	**p_3**
rs2929180									
GG	26 (40.0%)	37 (56.9%)	43 (66.2%)		1.000		1.000		1.000
GT	24 (36.9%)	23 (35.4%)	22 (33.8%)	1.485(0.69 – 3.18)	0.308	1.804(0.85 – 3.84)	0.126	1.215 (0.59 – 2.52)	0.602
TT	15 (23.1%)	5 (7.7%)	0 (0.0%)	4.269(1.38 – 13.21)	0.012*	–	–	-	–
$^{HW}p_0$	0.053	0.595	0.101						
Allele									
G	76 (58.5%)	97 (74.6%)	108 (83.1%)		1.000		1.000		1.000
T	54 (41.5%)	33 (25.4%)	22 (16.9%)	2.085(1.23 – 3.53)	0.006*	3.488(1.96 – 6.21)	<0.001*	1.670 (0.91 – 3.06)	0.097
rs1800843									
CC	35 (53.8%)	49 (75.4%)	56 (86.2%)		1.000		1.000		1.000
CA	21 (32.3%)	13 (20.0%)	8 (12.3%)	2.262 (1.0 – 5.11)	0.049*	4.200(1.68 – 10.51)	0.002*	1.857 (0.711 – 4.85)	0.207
AA	9 (13.8%)	3 (4.6%)	1 (1.5%)	4.200(1.06 – 16.64)	0.041*	14.40(1.75 – 118.6)	0.013*	3.429 (0.35 – 34.04)	0.293
$^{HW}p_0$	0.063	0.109	0.282						
Allele									
C	91 (70.0%)	111 (85.4%)	120 (92.3%)		1.000		1.000		1.000
A	39 (30.0%)	19 (14.6%)	10 (7.7%)	2.503(1.35 – 4.63)	0.003*	5.142(2.44 – 10.85)	<0.001*	2.054 (0.915 – 4.61)	0.080

Group I: *H. pylori* patients with HCV **Group II:** *H. pylori* patients without HCV
OR: Odds ratio OR1: Odds ratio for Group I and Group II
OR2: Odds ratio for Group I and Control OR3: Odds ratio for Group II and Control
p: p value for Univariate regression analysis for comparing with the reference genotype
CI: Confidence interval LL: Lower limit UL: Upper Limit
HWp0: p value for Chi square for goodness of fit for Hardy-Weinberg equilibrium
(If P < 0.05 – not consistent with HWE.)
*: Statistically significant at p ≤ 0.05

There were figures indicating comparisons the three studied groups according to rs2929180 and rs1800843 (**Figures 1,2**).

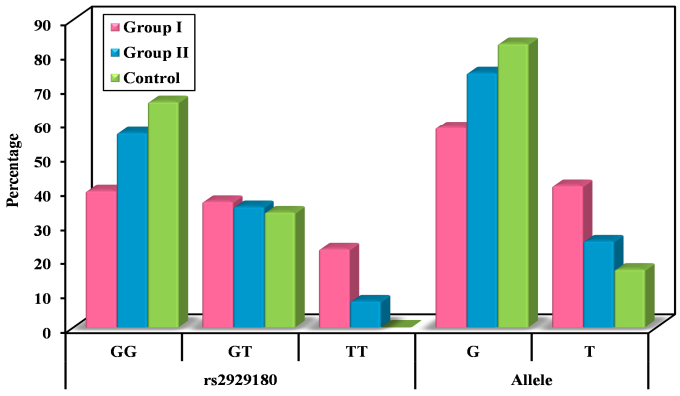

Figure 1: Comparison between the three studied groups according to rs2929180
GG, GT& TT: GG, GT and TT genotypes of rs2929180. G & T: G and T allele

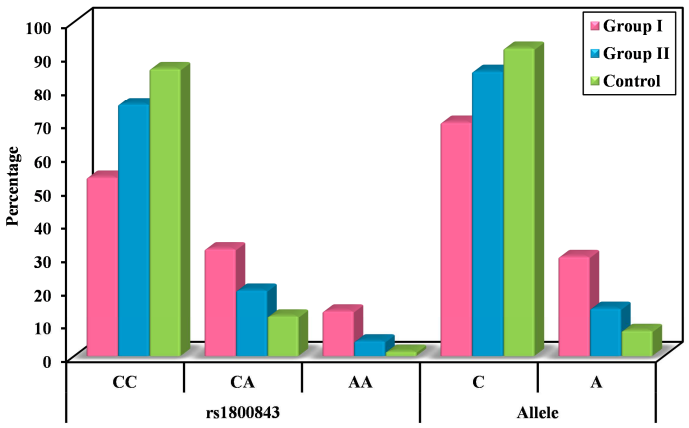

Figure 2: Comparison between the three studied groups according to rs1800843
CC, CA & AA: CC, CA and AA genotypes of rs1800843. C & A: C and A allele

Relation between CCKBR (rs2929180) gene polymorphism and gender and lipid profile in *H. pylori* patients with HCV: Regarding the relation of CCKBR (rs2929180) genotype distribution in *H. pylori* patients with HCV, there was a significant association of higher total cholesterol (P<0.001) and LDL (P<0.001) in TT genotype compared to the GG and GT genotypes. There was no significant association regarding other parameters **(Table 4).**

Table 4: Relation between CCKBR (rs2929180) gene polymorphism and gender and lipid profile in *H. pylori* patients with HCV (n= 65):

	rs2929180			**Test of Sig.**	**p**
	GG (n = 26)	**TG (n = 24)**	**TT (n = 15)**		
Gender					
Male	10 (38.5%)	8 (33.3%)	4 (26.7%)	χ^2= 0.595	0.742
Female	16 (61.5%)	16 (66.7%)	11 (73.3%)		
Total Cholesterol(mg/dl)					
Mean ± SD.	179.7 ± 42.6	208.2 ± 45	250.6 ± 29.6	H= 20.677*	<0.001*
Median (Min. – Max.)	192 (121 – 280)	208 (125 – 300)	252 (201 – 300)		
TG (mg/dl)					
Mean ± SD.	144.8 ± 54.3	146.4 ± 54.4	143.7 ± 55.8	H= 0.211	0.900
Median (Min. – Max.)	140.5 (46 – 268)	140 (58 – 268)	137 (61 – 250)		
LDL (mg/dl)					
Mean ± SD.	110.5 ± 36.9	118.6 ± 48.8	165.5 ± 37.0	H= 16.656*	<0.001*
Median (Min. – Max.)	101 (26 – 200)	111 (26 – 200)	176 (110 – 214)		
HDL (mg/dl)					
Mean ± SD.	41.9 ± 11.3	38.8 ± 4.08	40 ± 3.93	F=0.972	0.384
Median (Min. – Max.)	40 (33 – 78)	39 (30 – 45)	39 (34 – 46)		

SD: **Standard deviation**
F: F **for one-way ANOVA test** H: H for **Kruskal–Wallis test**
χ2: **Chi square test**
p: p value for comparison between the studied categories
*: Statistically significant at $p \leq 0.05$
TG: Triglyceride
HDL: High-density lipoprotein **LDL:** Low-density lipoprotein

Relation between CCKBR (rs1800843) gene polymorphism and gender and lipid profile in *H. pylori* patients with HCV (n= 65): Regarding the relation of CCKBR (rs1800843) genotype distribution in *H. pylori* patients with HCV, there was a

significant association of higher total cholesterol (P<0.001) and LDL (P<0.001) in CA and AA genotypes compared to the CC genotype. There was no significant association regarding other parameters **(Table 5).**

Table 5: Relation between CCKBR (rs1800843) gene polymorphism and gender and lipid profile in *H. pylori* patients with HCV (n= 65):

	rs1800843			**Test of Sig.**	**p**
	CC (n = 35)	**CA (n = 21)**	**AA (n = 9)**		
Gender					
Male	12 (34.3%)	8 (38.1%)	2 (22.2%)	χ^2= 0.715	0.699
Female	23 (65.7%)	13 (61.9%)	7 (77.8%)		
Total Cholesterol (mg/dl)					
Mean ± SD.	181.1 ± 36.1	233.19 ± 48.7	243.3 ± 36.5	H= 21.436*	<0.001*
Median (Min. – Max.)	196 (121 – 252)	252 (121 – 300)	240 (210 – 300)		
TG (mg/dl)					
Mean ± SD.	136.9 ± 46.6	165.1 ± 66.6	130.4 ± 35.9	H= 3.028	0.220
Median (Min. – Max.)	140 (46 – 268)	153 (46 – 268)	140 (61 – 195)		
LDL (mg/dl)					
Mean ± SD.	103.9 ± 38.1	151.3 ± 42.7	154.3 ± 44.0	H= 16.555*	<0.001*
Median (Min. – Max.)	102 (26 – 200)	167 (72 – 214)	167 (110 – 214)		
HDL(mg/dl)					
Mean ± SD.	39.9 ± 7.75	41.3 ± 9.36	39.6 ± 2.88	F=0.240	0.788
Median (Min. – Max.)	40 (30 – 78)	40 (33 – 78)	39 (36 – 46)		

SD: **Standard deviation**
F: F **for one-way ANOVA test** H: H for **Kruskal–Wallis test**
χ2: **Chi square test**
p: p value for comparison between the studied categories
*: Statistically significant at $p \leq 0.05$
TG: Triglyceride
HDL: High-density lipoprotein **LDL:** Low-density lipoprotein

Relation between CCKBR (rs1800843) gene polymorphism and gender and lipid profile in *H. pylori* patients without HCV (n= 65): Regarding the relation of CCKBR (rs1800843) genotype distribution in *H. pylori* patients without HCV, there was a significant association of female gender (MCp=0.031), higher total cholesterol (P<0.001) and LDL (P<0.001) in CA and AA genotypes compared to the CC genotype. There was no significant association regarding other parameters **(Table 6).**

Table 6: Relation between CCKBR (rs1800843) gene polymorphism and gender and lipid profile in *H. pylori* patients without **HCV** (n= 65):

	rs1800843			**Test of Sig.**	**p**
	CC (n = 49)	**CA (n = 13)**	**AA (n = 3)**		
Gender					
Male	24 (49%)	2 (15.4%)	0 (0%)	χ^2= 6.427*	MCp= 0.031*
Female	25 (51%)	11 (84.6%)	3 (100%)		
Total Cholesterol(mg/dl)					
Mean ± SD.	151.6 ± 14	176.8 ± 20.9	194.3 ± 59.5	H= 18.662*	<0.001*
Median (Min. – Max.)	150.3(118.8 – 175)	180.1(121.4 – 196.4)	160 (160 – 263)		
TG (mg/dl)					
Mean ± SD.	110.5 ± 16	128.5 ± 26.5	138.3 ± 50.1	H= 5.148	0.076
Median (Min. – Max.)	105 (92 – 154)	120 (92 – 160)	120 (100 – 195)		

LDL (mg/dl)					
Mean ± SD.	83.3 ± 12.6	112.4 ± 24.7	117 ± 52.9	H= 18.600*	<0.001*
Median (Min. – Max.)	81.1(53.8 – 108.6)	116 (49.4 – 140.4)	88 (85 – 178)		
HDL (mg/dl)					
Mean ± SD.	47.4 ± 4.10	46.9 ± 1.82	45.7 ± 10.7	F= 0.279	0.758
Median (Min. – Max.)	48 (37 – 55)	47 (43 – 50)	48 (34 – 55)		

SD: **Standard deviation** MC: **Monte Carlo**
F: F **for one-way ANOVA test** H: H for **Kruskal–Wallis test**
$\chi 2$: **Chi square test**
p: p value for comparison between the studied categories
*: Statistically significant at $p \leq 0.05$
TG: Triglyceride
HDL: High-density lipoprotein **LDL:** Low-density lipoprotein

4. Discussion

It is urgent to provide insights into the association between host genetics and clinical outcomes in *H pylori* and HCV infection. As it is one of the risk factors including environmental factors and bacterial virulence **[9].** *H. pylori* infect around 50% of the population **[10]** and 70% of patients chronically infected with HCV accompanied by *H. pylori* infection **[11]**.

H. pylori can survive in the acidic gastric environment by expressing some acid tolerance genes **[12].** It invades the intestinal mucosa and may increase intestinal permeability and promote bacterial endotoxin to pass through the portal vein and reach the liver **[13]**.

The infection of *H. pylori* is related to hepatic dysfunction. Patients with HCV or HBV have more possibility to get infected with *H. pylori*, compared with patients suffering from primary biliary cirrhosis or autoimmune liver cirrhosis **[14]**.

Colonization of *H. pylori* in the liver happens after transmission of the bacteria directly through the bile ducts or from the stomach through the portal vein. Current studies in patients infected with HCV point to much higher incidence of *H. pylori* or bacterial DNA in the liver tissue causing liver injury, especially exacerbated fibrosis **[15]**.

The present study on patients suffering from *helicobacter pylori* infection with and without hepatitis C virus revealed that there was a significant difference regarding the genotype frequency and allelic distribution of CCKBR (rs2929180) between *H. pylori* patients with and without HCV and healthy controls. The frequency of the TT genotype was significantly higher in *H. Pylori* patients with HCV than in *H. Pylori* patients without HCV and healthy controls. Also, there was a significant difference regarding the genotype frequency and allelic distribution of CCKBR (rs1800843) between *H. pylori* patients with and without HCV and healthy controls. The frequency of the CA and AA genotypes was significantly higher in *H. Pylori* patients with HCV than in *H. Pylori* patients without and healthy controls.

CCKBR receptor gene maps to chromosome 11, encode a 447 amino acid protein act as a receptor in brain and stomach for the brain and gastrointestinal peptide, cholecystokinin **[16]**, which mediates a therapeutic effect for peptic ulcer treatment by reducing acid secretion **[17].** Polymorphisms of CCKBR gene can cause increase of gastric acid secretion and increase susceptibility to H. pylori infection.

The effects of gastric acid that the harsh gastric acid environment causes physiological obstacles to the use and delivery of therapeutic drugs and prevents them from entering the stomach when eradicating H. pylori **[19].** Since *H. pylori* is sensitive to acidic pH, it is challenging to survive in highly acidic environment. It can survive in the superficial mucous layer causing a livable pH in its vicinity by its urease activity **[20]**.

GWAS of peptic ulcer disease, implicating *H. pylori* infection, identify independent and significant loci for peptic ulcer disease at, or near, gene CCKBR **[17].** Also, a meta-analysis demonstrated a positive association between *H. pylori* infection and CHC & a strong correlations of *H. pylori* infection with HCV-related cirrhosis and HCV-related HCC **[21]**. While, a large pancreatic cancer GWAS analysis, Wei and coworkers screened a database of over 3,000 pancreatic cancer patients and found that the ligands CCK or gastrin bind and/or activate the CCK-B receptor, or the splice variant CCK-C receptor was the most significant pathway predicting pancreatic cancer risk. **[22]**.

Meta-analysis study supported that a significant positive association was found between *H. pylori* infection and anxiety **[23].** Also, the CCKBR (rs2941026) heterogenous genotype was associated with anxious personality **[24].**

Recent evidence suggests that activation of CCK receptors in hepatic stellate cells (HSCs) induces fibrosis-associated gene expression, contributing to liver pathology in conditions like metabolic dysfunction-associated steatohepatitis (MASH) and hepatocellular carcinoma (HCC) **[25]**.

Regarding the lipid profile, we found that there was a significant difference between *H. pylori* patients with HCV versus *H. pylori* patients without HCV and healthy controls regarding increased total cholesterol, TG and LDL and decreased HDL.

These results are in accordance with a study of **Drnovsek et al.** who found that, polymorphic CCKBR rs1800843 allele had more visceral fat and a larger waist circumference **[26]**.

Also, a significant difference ($p < 0.001$) was observed of high TG, high total cholesterol, high LDL and low HDL between the patients infected with *H. pylori*-infected and controls **[27].** The dyslipidemia determined in the present study is also consistent with the findings of **Izhari et al. [28],** who demonstrated a higher level of total cholesterol, triglycerides and

LDL and a lower level of HDL in the subjects with persistent *H. pylori* infections as compared to successfully eradicated *H. pylori* study subjects.

This could be due to the involvement of cytokines, especially tumor necrosis factor, which inhibits lipoprotein lipase and enhances free radical generation. This in turn facilitates the oxidation of LDL. Several lines of evidence indicate that chronic infection of gram-negative bacteria involved in the change of lipid profiles through a systemic inflammatory response and secretion of inflammatory cytokines **[29]**.

Finally, the limitations of our study were the small sample size and small number of SNPs. Therefore, it is necessary to conduct further studies with larger sample sizes to assess the possible effect of SNPs on the severity of *H. pylori* infection and HCV.

5. Conclusions

This study demonstrates that CCKBR (rs2929180 and rs1800843) gene polymorphisms play a role in determining the degree of *H. pylori* occurrence in HCV patients especially with dyslipidaemia.

6. Abbreviations

CCKBR: Cholecystokinin B receptor
***H. pylori*:** *Helicobacter pylori*
CCK: Cholecystokinin
HCV: Hepatitis C virus
SNPs: single nucleotide polymorphisms
CDKs: cyclin-dependent kinases
HTN: hypertension
DM: diabetes mellitus.

7. Conflicts of interest

The authors declare that they have no competing financial or nonfinancial interests.

8. Formatting of funding sources

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