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ORIGINAL ARTICLE

The impact of the nucleotide-binding oligomerization domain 1 (NOD 1) gene polymorphism on *Helicobacter Pylori* induced chronic gastritis in hepatic patients.

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Key words:
H.pylori, Chronic gastritis, Hepatic patients, NOD 1 gene polymorphism, Gastric outcomes (endoscopic &pathological findings)

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ABSTRACT

Background*: Chronic infection with* Helicobacter pylori *(*H.pylori *) causes atrophic and even gastric metaplastic changes, and it has a well-known link to peptic ulceration. Nucleotide-binding oligomerization domain-containing protein 1 (NOD1) is a protein receptor that is presented by the NOD1 gene. It distinguishes H.pylori bacterial molecules and enhances an immune response* ***Objectives:*** *to describe the relation between the NOD1 gene (rs2075820) polymorphism and H.pylori infection in hepatic and non hepatic patients with chronic gastritis, study its impact on the degree of chronic gastritis in H.pylori positive individuals, and to examine the effect of* H. pylori *on clinical, endoscopic and histopathological findings and child paugh scoring in hepatic patients.* ***Methodology:*** *Gastric tissue samples were taken from selected 200 patients with chronic gastritis, either hepatic or non hepatic. Rapid urease test and pathological findings classified them into H.pylori infected and non infected patients. Genotyping of NOD 1 was studied using polymerase chain reaction /restriction fragment length polymorphism (PCR–RFLP) method.* ***Results:*** *A significant higher frequency of AA genotype, and the A allele of NOD1 gene in H.pylori +ve patients, either hepatic; (58%)-(73%) or non hepatic;(62%)-(78%) as compared to H.pylori –ve patients,(P <0.001). A highly significant relation between NOD1 genotypes and endoscopic findings, where most of H.pylori infected patients with AA genotype had more peptic ulcer, antral erosion, gastric prolapse, esophageal varices and esophageal hiatus hernia compared to patients with GA and GG genotypes, (P<0.001). No significant impact of* H.pylori *on signs of liver affection and child paugh scoring in hepatic patients.* ***Conclusions:*** *In NOD1 gene polymorphism, AA genotype and A allele have significantly implicated in H.pylori infection susceptibility and progression. While GG genotype and G allele have a protective effect against H.pylori infection.*

INTRODUCTION

H.pylori is a micro aerophilic Gram negative fastidious human pathogen. It is known as one of the greatest serious chronic bacterial infections worldwide.[1] *H.pylori* infection is responsible for gastritis, peptic ulcers, gastric mucosa associated lymphoid tissue lymphoma and considered a predisposing factor for the progression to gastric adenocarcinoma.[2]Recent studies in patients infected with hepatitis B virus (HBV), hepatitis C virus HCV, hepatocellular carcinoma (HCC) and patients with chronic noninfectious liver conditions refer to the bad effect of *H.pylori* infection on the course of liver injury, especially extensive fibrosis.[3] *H.pylori* infection in people with liver cirrhosis is really dangerous and may significantly affect liver function leading to hyperammonemia, increased portal pressure, and exposure to esophageal varices.[4]

(NOD1) is a member of the Nod-like receptors, which is expressed in the cytoplasm of antigen presenting cells and gastric epithelial cells and is related to recognition of gram negative bacteria. [5] Stimulation of epithelial cells of stomach with NOD1 ligands leads to release of pro inflammatory cytokines and NOD1 play an important role in host defense against mucosal infection with *H.pylori* infection.[6]

The rs2075820 SNP was selected for the coding sequence of the NOD1 gene in exon 3 as it was earlier known to encode an altered protein (E266K) in the nucleotide-binding domain changing a glutamic acid residue, suggesting an essential functional consequence of the mutation. The change of negatively charged glutamine to positively charged lysine may cause an extreme alteration in the structure or regulation of the NOD1 protein that changes the reaction to *H.pylori* or the nature of inflammatory pathways.[6]

NOD1 share in mucosal host defense against *H.pylori* infection by the activation of type I interferon (IFN) signaling pathways.[7] Also NOD1 activation negatively controls caudal type homeobox transcription factor 2 (Cdx2) expression, and inhibits the progress to gastric cancer. Molecules related to NOD1-mediated signaling pathways might be new therapeutic goal for treating chronic gastritis and gastric cancer. [8]

Gene polymorphism of NOD1 has been convoluted in gastric ulcertion in *H.pylori* positive patients and an important association with very high odds ratios has been recently informed for the risk of exposure, progression, and premalignant gastric lesions.[9]

the present study aimed to identify the association between the NOD1 gene (rs2075820) polymorphism and *H.pylori* infection in hepatic and non hepatic patients with chronic gastritis, study its impact on the degree of chronic gastritis in *H.pylori* positive individuals, and to examine the effect of *H.pylori* on clinical, endoscopic and histopathological findings and child paugh scoring in hepatic patients.

METHODOLOGY

This study was achieved during the period from May 2018 to September 2019. Participants were carefully chosen from patients complaining gastritis with hepatic disease admitted to Internal Medicine Endoscopy Unit, National Liver Institute, Menoufia University and patients complaining gastritis without hepatic disease presented to EL Helal Hospital, Sheben El Kom, El Menoufia. The design of the research was approved by the National Liver Institute Ethical committee with No: 00237/2021.

Participants in this research were classified according to diagnosis of *H.pylori* infection by Urease test and pathological findings into the 4 groups. Group1; including hepatic patients with chronic gastritis and positive for *H.pylori* (50 patients divided into 31 HCV, 10 HBV, 5 HCC and 4 fatty liver).Group 2; including hepatic patients with chronic gastritis and negative for *H.pylori* (50 patients divided into 27 HCV, 7 HBV, 11 HCC and 5 fatty liver). Group 3; including non hepatic patients with chronic gastritis and positive for *H.pylori* (50 patients). Group 4; including non hepatic patients with chronic gastritis and negative for *H.pylori* (50 patients).

Diagnosis of chronic liver disease was based on clinical and laboratory data (recorded in the file of each participant) including CBC and liver function tests, and on positive serological findings for HCV and HBV related liver cirrhosis and liver tumor markers for HCC for more than six months. Liver cirrhosis and tumor were diagnosed by liver biopsy or imaging criteria of cirrhosis in ultrasound and computerized tomography (CT), and elevated liver function parameters.

Inclusion criteria:

Patients referred to endoscopy clinic with a appropriate medical history of persistent or recurrent chronic gastric disease with diagnostic workup including through medical examination, upper endoscopy, laboratory tests to diagnose or exclude *H.pylori* infection.

Exclusion criteria:

Patients who underwent sclerotherapy or band ligation of esophageal varices. Patients taking drugs for *H.pylori* infection or for primary prophylaxis of variceal bleeding. Patients with coagulation disorders.

Informed consent was acquired from all participants in this research. The study protocol was approved by the Ethical committee of medical research, National Liver Institute, Menoufia University.

Samples collection and processing:

Six gastric biopsies; 3 antral and 3 corpal were taken from each participant. A set of one antral and one corpal biopsies were collected for rapid urease test, which was done immediately inside Endoscopy Unit. The result was obtained within 2 hours. The basis of this test is the ability of *H.pylori* to secrete the urease enzyme, which catalyzes the change of urea to ammonia that raises the pH of the medium, and changes the color of the ring surrounding biopsy spot from yellow (-ve) to red (+ve) [10] as shown in figure 1.

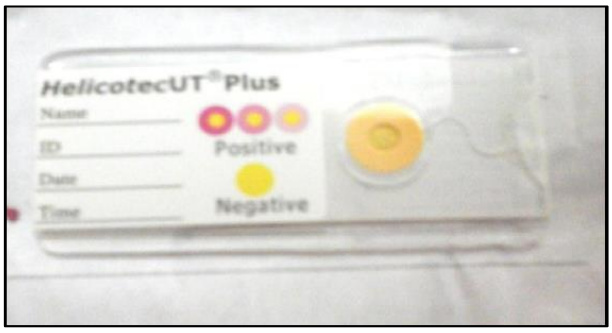

Fig.1: Rapid urease (CLO) test

The second set was dispatched in 10% buffered formalin, and frozen for histopathological examination using Hematoxyline-Eosin (H&E) and Giemsa stains,[11] as in figure 2.

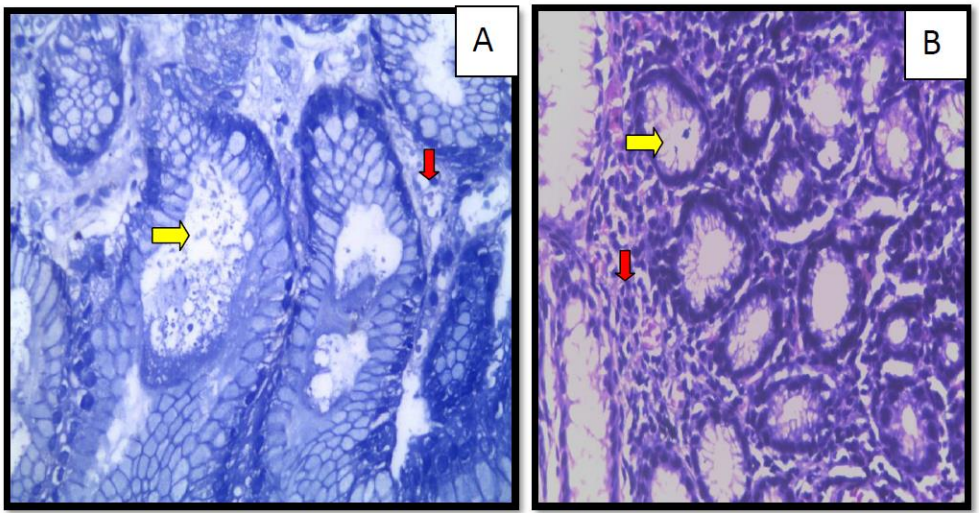

Fig. 2: *H.pylori* detection by histopathology staining methods. Numerous *H.pylori* bacilli were observed in the lumen of a gastric pit (yellow arrow) with predominant lymphocytosis (red arrow)by Giemsa stain (x400). (B) *H.pylori* bacilli seen by H&E stain in the lumen of a chronic inflamed gastric tissue (yellow arrow) with predominant leukocytosis (red arrow) (x400).

The third set for PCR was transferred immediately to the Microbiology laboratory into sterile tubes containing Brain Heart Infusion broth supplemented by 30% sterile glycerol, and stored at -80^{O}C till testing.

Molecular detection of NOD 1 genotypes

Genomic DNA was extracted from all frozen gastric tissue samples using Quick-g DNA ™ Mini prep Kit, USA following the manufacturer's instructions. Thermocycler PCR amplification of NOD 1 gene alleles, using the following primer with Amplicon bp:170/397.[7] 5′-TTAGCACCCCTGGCCAAGG- 3′ 5′-CTTACTCCTTGGAGGCCATG-3′ The amplification reaction was performed on a final volume of 50 µl containing 25µl of 2×Master Mix primer, 1.0 µM of forward and reverse primers, 20 µg of Template DNA and 3 µl of Water nuclease -free. The thermocycler conditions were 3 minutes at 94˚C, followed by amplification for 30 cycles by denaturation at 94˚C for 30 seconds, annealing at 65˚C for 30 seconds and extension at 72˚C for 1 minute in each cycle and a final extension at 72˚C for 10 minutes.[12]

PCR cycles analysis

(RFLP) analysis for NOD1 genotypes, where the PCR product was exposed to i-Star Taq DNA polymerase enzyme (Thermo scientific, USA) by adding 10 µl PCR reaction mixture, 18 µl nuclease free water, 2 µl 10 x buffer and 1.5 µl i-Star Taq DNA polymerase restriction enzyme then incubated at 65°C for 8 hours in a 20 µl volume. The digests were analyzed by electrophoresis in a 3% agarose gel. Wild-type DNA (GG type) is visible as a double- band 209 bp and 170 bp; the mutated DNA (AA type) is visible as a single 379-bp band, whereas heterozygotes give three bands (GA),[12] as in Fig 3.

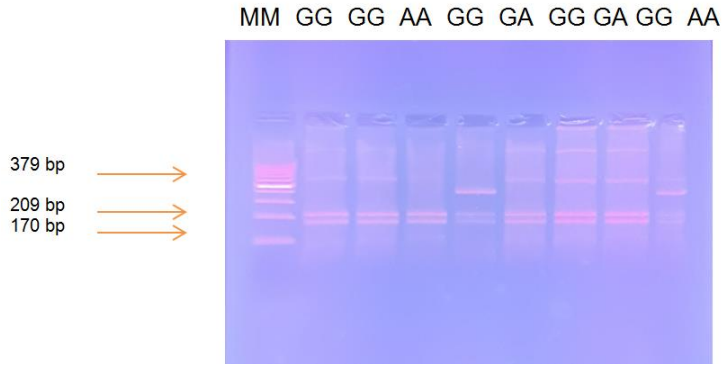

Fig. 3: Three distinguishable genotypes of G796A NOD1polymorphism: double bands of 209 bp and 170 bp in GG homozygote, single 379-bp band in AA homozygote mutant, and all three bands in GA heterozygote. (MM; molecularweight marker).

Statistical analysis:

Analysis of data was done using SPSS (Statistical Package for Social Science) program, (version 20; SPSS Inc, Chicago, IL). Fisher's exact test or $\chi 2$ was used to compare qualitative variables. Calculation of odds ratios and 95% confidence intervals was done using logistic regression analysis for risk estimation. P values less than 0.05 were considered significant.

RESULTS

This study included selected 200 patients complaining of chronic gastritis. They were categorized into four groups. Baseline demographic data of the studied groups are shown in table (1).

Table 1: Demographic data of the studied groups

Demographic data		Hepatic				Non Hepatic				X^2	P-value	
		G1: *H. pylori +Ve* (No=50)		G2: *H .pylori - Ve* (No=50)		G3: *H. pylori+Ve* (No=50)		G4: *H.pylori - ve* (No=50)				
		No	%	No	%	No		No	%			
Age (Mean± SD)		41.1±13.8		39.9±10.6		41.3±13.4		41.4±14.3		*F = 0.14	0.9	
Gender	**Male**	27	54	36	72	20	40	30	60	10.8	0.013*	*P1=0.06 P2=0.16 P3=0.5 P4=0.002* P5=0.5 P6=0.045**
	Female	23	46	14	28	30	60	20	40			
Residence	**Urban**	32	64	34	68	37	74	36	72	1.39	0.71	
	Rural	18	36	16	32	13	26	14	28			
Socioeconomic status	**Low**	8	16	1	2	10	20	11	22	21.2	0.002*	*P1=0.004* P2=0.86 P3=0.68 P4<0.001** P5<0.001** P6=0.9*
	Moderate	34	68	29	58	33	66	33	66			
	High	8	16	20	40	7	14	6	12			
Diabetes	**Positive**	19	38	23	46	17	34	15	30	3	0.39	
	Negative	31	62	27	54	33	66	35	70			
Hypertension	**Positive**	23	46	24	48	25	50	22	44	0.4	0.9	
	Negative	27	54	26	52	25	50	28	56			
Hepatic disease	**HCV**	31	62	27	54	0	0	0	0	0.65	0,5	
	HBV	10	20	7	14	0	0	0	0			
	HCC	5	10	11	22	0	0	0	0			
	Fatty liver	4	8	5	10	0	0	0	0			

***F**=one way ANOVA test . * = significant p value . ** = high significant p value .

P1: Comparison between group 1 and group 2. **P2:** Comparison between group 1 and group 3.

P3: Comparison between group 1 and group 4. **P4:** Comparison between group 2 and group 3.

P5: Comparison between group 2 and group 4. **P6:** Comparison between group 3 and group 4.

Table (2) illustrates that, there was a highly significant difference between hepatic patients in group 1&2 and non hepatic patients in group 3&4 regarding liver functions, as hepatic patients had higher AST, AIT and Total bilirubin in comparison to non hepatic patients. While Albumin was significantly lower in hepatic patients compared to non hepatic patients. But there was no significant difference between *H.pylori* +ve and *H.pylori* -ve patients regarding liver function tests.

Table 2: Laboratory investigations of the studied groups

Laboratory investigations	Hepatic		Non Hepatic		K	P- value	
	G1: *H. pylori +Ve* (No=50)	G2: *H. pylori - Ve* (No=50)	G3:, *H.pylori +Ve* (No=50)	G4: *H. pylori – Ve* (No=50)			
AST (IU/L) Mean ±SD	**77.58±10.18**	**77.92±14.82**	**24.70±4.12**	**24.92±6.29**	**144.2**	**<0.001****	***P1=0.99 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6=0.99***
ALT (IU/L) Mean ±SD	**81.02±9.56**	**82.62±10.88**	**27.58±9.80**	**26.90±10.17**	**149.4**	**<0.001****	***P1=0.86 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6=0.98***
Albumin (mg/dl) Mean ±SD	**2.26±0.6**	**2.60±0.45**	**4.27± 0.7**	**4.42±0.59**	**149.3**	**<0.001****	***P1=0.024 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6=0.56***
Total bilirubin (mg/dl) Mean ±SD	**1.51±.22**	**1.59±.23**	**0.46±.18**	**0.49±.15**	**151.6**	**<0.001****	***P1=0.25 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6=0.9***

**** = high significant p value .**

P1: Comparison between group 1 and group 2. **P2:** Comparison between group 1 and group 3.
P3: Comparison between group 1 and group 4. **P4:** Comparison between group 2 and group 3.
P5: Comparison between group 2 and group 4. **P6:** Comparison between group 3 and group 4.

No significant impact of *H.pylori* on clinical features and child paugh scoring in hepatic patients.

According to Modified Sidney scoring of histopathological findings in *H.pylori* induced gastritis, the score less than 33% of mononuclear infiltration, neutrophilic activity, atrophy, and *H.pylori* density indicates mild histopathological findings. The score between 33% and 66% of mononuclear infiltration, neutrophilic activity, atrophy, and *H.pylori* density indicates moderate histopathological findings. the score more than 66% of mononuclear infiltration, neutrophilic activity, atrophy, and *H.pylori* density indicates severe histopathological findings.[13]

Table (3) shows that, there was a highly significant difference between *H.pylori +ve* patients in groups (1&3) and *H.pylori -ve* patients in groups (2&4) regarding histopathological findings, as *H.pylori* infected patients had severe mononuclear infiltration, more neutrophilic activity, more atrophy and *H.pylori* density as compared to non infected patients. While there was no significant difference between hepatic & non hepatic patients in histopathological examination.

Table 3: Comparison between the studied groups according to histopathological findings.

Histopathological findings		Hepatic				Non Hepatic				X^2	P- value	
		G1: *H. pylori +Ve* (No=50)		G2: *H. pylori -Ve* (No=50)		G3: *H. pylori +Ve* (No=50)		G4: *H. pylori –Ve* (No=50)				
		No	%	No	%	No	%	No	%			
Mononuclear Cell infiltration	absent	0	0%	25	50%	0	0%	22	44%	21.2	<0.001*	*P1=0.004**
	mild	1	2%	12	24%	2	4%	10	20%			*P2=0.86*
												P3=0.68
	moderate	6	12%	7	14%	8	16%	18	36%			*P4<0.001***
	severe	43	86%	6	12%	40	80%	0	0%			*P5<0.001***
												P6=0.9
Neutrophilic Activity	absent	0	0%	38	76%	0	0%	36	72%	109.1	<0.001*	*P1<0.001***
	mild	27	54%	8	16%	22	44%	10	20%			*P2=0.02 **
	moderate	10	20%	2	4%	9	18%	2	4%			*P3<0.001***
												*P4<0.001***
	severe	13	26%	2	4%	19	38%	2	2%			*P5=0.3*
												*P6<0.001***
Atrophy	absent	0	0.0%	13	26%	0	0.0%	12	24%			
	mild	21	42%	12	24%	20	40%	24	48%			
	moderate	21	42%	6	12%	22	44%	5	10%			
	severe	8	16%	9	18%	8	16%	9	18%	2.26	0.52	
H.pylori density	absent	0	0%	50	100%	0	0%	50	100%	149.3	<0.001*	*P1=0.024**
	mild	29	58%	-	-	25	50%	-	-			*P2<0.001***
	moderate	10	20%	-	-	12	24%	-	-			*P3<0.001***
	severe	11	22%	-	-	13	26%	-	-			*P4<0.001***
												*P5<0.001***
												P6=0.56

*** = significant p value. ** = high significant p value**

P1: Comparison between group 1 and group 2. **P2:** Comparison between group 1 and group 3.
P3: Comparison between group 1 and group 4. **P4:** Comparison between group 2 and group 3.
P5: Comparison between group 2 and group 4. **P6:** Comparison between group 3 and group 4.

Table (4) shows that, among hepatic patients, AA genotype of NOD1 gene was the most dominant genotype in *H.pylori +ve* patients (group1) as compared to *H.pylori –ve* patients (group 2). While genotypes GA followed by GG, were predominant in *H.pylori –ve* patients as compared to *H.pylori +ve* patients.

As regard NOD1 alleles, the A allele was the greatest predominant in *H.pylori* infected patients (73%) compared to *H.pylori* non infected patient, where G allele was prevalent in *H.pylori –ve* patients (74%). All of these differences were of highly significant importance.

Table 4: Association between NOD1 gene polymorphism and *H. pylori* infection in hepatic patients with chronic gastritis

		G1:Hepatic, *H. pylori +Ve* (No=50)		G2:Hepatic, *H. pylori –Ve* (No=50)		X^2	P- value
		No	%	No	%		
NOD1 genotypes	AA	29	58.0%	0	0.0%	*42.8*	<0.001**
	GA	15	30.0%	26	52.0%		
	GG	6	12.0%	24	48.0%		
NOD1 allele	A	73	73%	26	26%	*44.2*	<0.001**
	G	27	27%	74	74%		

**** = high significant p value**.

Table (5) shows that, Among non hepatic patients, AA genotype of NOD1 gene was the most prevalent genotype in *H.pylori +ve* patients (group 3) as compared to *H.pylori –ve* patients(group 4). While genotypes GA followed by GG, were predominant in *H.pylori –ve* patients as compared to *H.pylori +ve* patients.

As regard NOD1 alleles, the A allele was the most predominant in *H.pylori* infected patients (78%) compared to *H.pylori* non infected patients, where G allele was dominant (64%). All of these differences were of highly significant importance.

Table 5: Association between NOD1 gene polymorphism and *H.pylori* infection in non hepatic patients with chronic gastritis.

		G3:Non Hepatic, *H. pylori +Ve* (No=50)		G4:Non Hepatic, *H. pylori –Ve* (No=50)		X^2	P- value
		No	%	No	%		
NOD1 genotypes	AA	31	62.0%	2	4.0%	*39.7*	<0.001**
	GA	16	32.0%	32	64.0%		
	GG	3	6.0%	16	32.0%		
NOD1 alleles	A	78	78%	36	36%	*36.0*	<0.001**

**** = high significant p value.**

There was a highly significant relation between NOD1 genotypes and endoscopic findings. Where most of *H.pylori* infected patients with AA genotype had more peptic ulcer, antral erosion, gastric prolapse, esophageal varices and esophageal hiatus hernia. While *H.pylori* infected patients with GG genotype had less severe findings; gastritis or even apparent normal mucosa. While there was no significant relation between NOD1 genotypes and pathological findings, as illustrated in table (6).

Table 6: Relation between gastric outcome and NOD1 gene polymorphism in *H .pylori* infected patients.

gastric outcome		NOD1 genotypes						X^2	P- value
		AA (No=60)		GA (No=31)		GG (No=9)			
		No	%	No	%	No	%		
Endoscopic findings	Apparent normal mucosa	5	8.3%	8	25.8%	2	22.2%	69.4	<0.001**
	Gastritis	9	15%	8	25.8%	3	33.4%		
	Peptic ulcer	13	21.6%	2	6.4%	1	11.1%		
	Antral erosion	9	15%	3	9.7%	1	11.1%		
	Gastric prolapse	13	21.6%	3	9.7%	1	11.1%		
	Esophageal varices	4	6.8%	2	6.4%	1	11.1%		
	Esophageal hiatus hernia	7	11.7%	5	16.26%	0	0.0%		
Pathological findings	mild	19	31.7%	10	32.2%	3	33.3%	1.519	0.46
	moderate	23	38.3%	18	58%	5	55.5%		
	severe	18	30.0%	3	9.8%	1	11.2%		

**** = high significant p value.**

DISCUSSION

Infection of the stomach with *H.pylori* is an essential risk factor for gastritis, peptic ulcer, and gastric carcinoma. [2] *H.pylori* infection in people with liver cirrhosis is particularly risky and may significantly deteriorate liver function. [14] Although it has been well definite that colonization of *H.pylori* is related to adaptive Th1 responses, the innate immune responses leading to these Th1 responses are poorly defined. The identification of *H.pylori* derived ligands by cytosolic NOD1 encourages several host defense factors, including antimicrobial peptides, cytokines and chemokines.[7] Depending on the vital function of NOD 1 pathway in the *H.pylori* infection, we aimed to evaluate the relation between NOD 1 polymorphism and susceptibility and progression of *H.pylori* in hepatic Egyptian patients.

The present study reported that, there was no significant difference between studied groups regarding age. Mabeku et al,[15] and Megraud et al,[16]had nearly similar results. While Camagro et al,[17]El Khadir et al,[18] and El Shenawy et al,[19] found that *H.pylori* infection was more dominant in adults compared with children. However, Hojsak et al,[20]and Zhu et al,[21]found that, the prevalence of *H. pylori* infection among young age was greater than old age.

As regard gender, this study reported a significant difference among groups with more dominance in male. Mabeku et al,[15] and Zhu et al,[21] had the same findings. While Mwafy et al,[22] found that, the gender of the study patients was of no significant differences.

The present study reported that, there was no significant difference between studied groups regarding residence. Mabeku et al,[15] had nearly the same findings. While Cheng et al,[23] and Amer et al,[24] reported a significant geographic distribution of *H.pylori* infections being more public in rural regions.

In the present study, the socioeconomic status of the studied patients was significantly different among the studied groups and most of patients were of moderate socioeconomic status. Nearly the same results were reported by Attila et al,[25] and Mabeku et al,[15] who found that, the frequency of *H.pylori* infection among patients of the high socioeconomic level was markedly lower than that of moderate and lower levels. This might be linked to the better living and sanitary conditions, with separate bedrooms and towel. While, Zhu et al,[21] and Mwafy et al,[22] reported that, there was no important difference in the prevalence of *H.pylori* in different socioeconomic groups.

The present study reported that, there was no significant difference between studied groups regarding diabetes mellitus or hypertension. Mabeku et al,[15] agreed with our results. While Mahdive et al,[26] reported that *H.pylori* infection was higher in diabetic patients than in non-diabetic patients. MS et al,[27] reported that, *H.pylori* infection had an important association with hypertension.

As regard the underlying liver disease in hepatic patients, Our study showed that, HCV patients were the commonest in *H.pylori* infected & non infected patients in groups (1&2). Hablas et al,[35] had the similar findings. While Okushin et al,[3] found that, there was a strong association between *H.pylori* and HBV infection.

Our study reported that, there was a highly significant difference between hepatic patients in group 1&2 and non hepatic patients in group 3&4 regarding liver functions, as hepatic patients had higher AST, AIT and Total bilirubin compared to non hepatic patients. While Albumin was significantly lower in hepatic patients compared to non hepatic patients. These findings were in agreement with data gained by Łapiński. [28]

Also our study reported non significant difference between *H.pylori* +ve and *H.pylori* -ve patients regarding liver function tests. Hao et al,[29]reached nearly the same results.

Our study showed no significant impact of *H.pylori* on clinical features and child paugh scoring in hepatic patients. Identical results were obtained by Hao et al. [29] In contrast to our findings, Łapiński [28]reported that, *H.pylori* infection may significantly worsen liver functions, elevate rate of appearance of jaundice, ascites, splenomegaly and hepatomegaly, and affect child paugh score. That difference may be due to the insufficient sample size, therefore more investigations are needed to explain this implications.

It was demonstrated that, there is a strong relationship between the presence of *H.pylori* infection and gastritis and peptic ulceration. The mechanisms by which *H.pylori* cause mucosal inflammation and damage is that, the bacteria can attack the epithelial cell surface to a narrow degree. Their toxins and lipopolysaccharide may damage the mucosal cells, and the ammonia made by the urease activity may also severely damage the cells.[30]

According to Modified Sydney Scoring of histopathological parameters in *H.pylori* induced gastritis, Our study reported that, there was a highly significant difference between *H.pylori +ve* patients in groups (1&3) and *H.pylori -ve* patients in groups (2&4) in histopathological examination, as *H.pylori* infected patients had severe mononuclear infiltration, more neutrophilic activity, more atrophy and *H.pylori* density as compared to non infected patients. Vakil [31] and Alenezy et al,[32] had nearly identical results. While Subramanian et al,[33] reported that, *H.pylori* density has no significant correlation to severity of pathological findings of gastritis.

As regard effect of hepatic disease on pathology of gastritis, this study found that, there was no major variance between hepatic & non hepatic patients regarding histopathological findings and Bagheri et al,[34] and Hao et al,[29] agreed with us. While Abolfathi et al,[35] and Łapiński[28] suggested that, HBV can significantly increase development of gastric premalignant pathological conditions in *H.pylori* positive patients. Also El-Masry et al,[36] reflected a major increase in the *H.pylori* pathological effect with advancing hepatic lesions, and the recovery of patients with chronic hepatitis C improves *H.pylori* gastritis progression.

In our work, it was found that among both hepatic and non hepatic patients, AA genotype and the A allele of NOD1 gene were the most prevalent genotype and allele in *H.pylori +ve* patient as compared to *H.pylori –ve* patients with high significant difference. Our results were agreed with Ying et al ,[6] who found that the AA homozygote of the (rs2075820) NOD1 gene polymorphism rises the risk of peptic ulceration in *H.pylori* positive hepatic patients. Another report by Kim et al,[7] pointed to that A allele carriers have a noticeable risk of growth of gastric metaplasia and atrophic changes in stomach and prevent eradication in chronic gastritis patients.

In this research, we particularly choosed the rs2075820 SNP to be the coding sequence of the NOD1 gene in exon 3 as it codes an altered protein (E266K) in the nucleotide-binding domain changing a glutamic acid residue, leading to an impending functional effect of the mutation. There is no obvious mechanism by which, the NOD1 polymorphism alters the function of NOD1. That may be due to the change of negatively charged glutamine to positively charged lysine which may cause an extreme change in the structure or regulation of the NOD1 protein and changes the reactivity to *H.pylori* or the nature of inflammatory pathways.

Our study showed a highly significant relation between NOD1 genotypes and endoscopic findings where we found that, most of *H.pylori* infected patients with AA genotype had more peptic ulcer, antral erosion, gastric prolapse, esophageal varices and esophageal hiatus hernia. While *H.pylori* infected patients with GG genotype had less severe findings as gastritis or apparent normal mucosa. As regard pathological findings, most of *H.pylori* infected patients with AA genotype had severe findings with no significant difference. Madkour et al,[37] agreed with our findings. Also Oikawa et al,[38]had nearly similar findings.

However, Kupcinskas et al,[39] found that, there was no association between NOD1 gene polymorphism and atrophic changes of *H.pylori* induced gastritis. Also Li et al,[40] reported that, genetic polymorphism of NOD1 directly affect gastric out come in *H.pylori* patient especially pathological findings.

CONCLUSIONS

In NOD 1 gene polymorphism, AA genotype and A allele have significantly implicated in *H.pylori* infection susceptibility and progression. While GG genotype, and G allele have a protective effect against *H.pylori* infection with no impact of *H.pylori* on signs of liver affection, and child paugh scoring in hepatic patients.

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