RESEARCH ARTICLE

Estimating the Incidence Frequency of *Helicobacter pylori* Co-infection with Hepatitis C Virus among Thalassemia Individual in Wasit Governorate of Iraq

Safaa Z. M. Al-Saidi*, Abdulameer M. Ghareeb

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq

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ABSTRACT

Objective(s): *Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium that plays a significant role in developing gastrointestinal diseases such as peptic ulcers. *H. pylori* infection affects more than half of the world's population. This pathogen incidence becomes more effective among thalassemia patients, a common genetic disorder characterized by a point mutation that leads to impaired production of the β-globin chain. Chronic hepatitis C is a chronic disease caused by the hepatitis C virus (HCV) that has significantly increased morbidity and mortality rates in these patients due to liver failure or hepatocellular carcinoma.

Methods: Current study included 90 β-thalassemia patients admitted to the thalassemia hematology center in Al-Kut women and children Hospital, Wasit province, Iraq from September 2020 to November 2021. The patients are divided into two groups (β-thalassemia with HCV group and β-thalassemia without HCV). First and second groups contain 32 and 58 specimens of blood and stool samples, respectively. The patient diagnosed with thalassemia syndrome regularly attended the thalassemia hematology center for transfusion and chelation and follow up on Hb level and iron status**.** This study aimed at serological and molecular diagnosis of co-infection *H. pylori* with Hepatitis C virus (HCV) among thalassemia patients and diagnosis infection rate. Diagnosis was used Serological enzyme-linked immunoassay (ELISA) techniques for *HP-IgG,* Level of ferritin, liver function test will be determined and Nested PCR method targeting the molecular diagnosis will be used to confirm *H. pylori* 23S rRNA using DNA extracted from stool samples.

Results: 90 β-thalassemia patients sample were diagnosed by Serological ELISA techniques for HP-IgG and the result showed 30 samples were positive (18 from the β-thalassemia with HCV group and 12 from the β-thalassemia without HCV)and 60 samples negative for HP-IgG and Nested polymerase chain reaction (PCR) method targeting the molecular diagnosis will be used to confirm *H. pylori* 23S rRNA using DNA extracted from stool for 30 stool samples revealed 13 samples positive (9 from the β-thalassemia with HCV and 4 from β-thalassemia without HCV) while 17 samples were negative.

Conclusions: The predominance of *H. pylori* among patients in Wasit was 43.3%. Levels of ferritin have significant correlation with *H. pylori* in HCV patients. There was a positive correlation between hepatitis C status and high *H. pylori* rate when compared with β-thalassemia without HCV. Splenoctomy was a positive correlation with *H. pylori* in β-thalassemia with HCV. Moreover, these results recommend study about the virulence factor and genes. Other molecular studies are needed for detecting more virulence genes in *H. pylori* bacteria such as real-time PCR and sequencing of *H. pylori* in patients with thalassemia, especially those with high serum ferritin.

Keywords: *Helicobacter pylori,* Hepatitis C, Jhalassenine

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INTRODUCTION

Helicobacter pylori are a gram-negative bacterium that plays a significant role in developing gastrointestinal diseases such as peptic ulcers, low-grade B-cell lymphoma (MALT lymphoma) and gastric cancer.[1] *H. pylori* infection is highly prevalent in human, affecting nearly half of the world's population;

**Author for Correspondence: Safaa.Zqar1200a@ige.uobaghdad.edu.iq*

however, infection remains asymptomatic in the majority of the population.[2] Chronic hepatitis C is a chronic disease that has increased morbidity and mortality rates in these patients significantly due to liver failure or hepatocellular carcinoma. About 80–90% of post-transfusion hepatitis in patients who received blood transfusion prior to routine blood product screening was caused by the hepatitis C virus (HCV) in 1990.[3] The hepatitis C virus is an effective blood-borne agent that causes liver disease around the world has infected more than 170 million people worldwide. HCV is a diverse group of RNA viruses that are currently classified into both subtypes and genotypes 1-7.[4] Moreover, concerning the marked liver iron overload, which is often inevitable in patients on regular blood transfusion, HCV infection has been shown to have a potentiating effect on hepatic fibrogenesis in thalassemia patients.[5] Severe anemia has also been seen as a risk factor for bacterial infections in thalassemia. Other causes such as transfusion-related immunomodulation, iron overload, and certain therapeutic interventions such as iron chelation therapy, central venous catheters and stem cell transplantation, may contribute to infectious complications with resultant morbidity and mortality. The risk of severe bacterial infections in thalassemic patients appears to be high, particularly after splenectomy.[6] Thalassemia is a common genetic disorder characterized by a point mutation in the expression of the globin gene. β-thalassemia is the result of the impaired production of the β-globin chain. This hemoglobinopathy has three main forms: major thalassemia, intermediate thalassemia and minor thalassemia. Clinical manifestations, such as mild microcytic and hypochromic anemia, are fully level-related.[7] Thalassemia syndromes are the main cause of the disease in Mediterranean countries overloading iron.[8] Significant β-thalassemia patients typically have extreme anemia requiring regular blood transfusions relative to beta-middle thalassemia.[9]

This study was designed to serological and molecular diagnosis of co-infection *H. pylori* with hepatitis C virus among thalassemia patients and diagnosis infection rate depending on Serological enzyme-linked immunoassay (ELISA) techniques. The level of ferritin and liver function test will be determined and nested polymerase chain reaction (PCR) method targeting the molecular diagnosis will be used to confirm *H. pylori* 23S rRNA using DNA extracted from stool.

Methods

Through the period extending from September 2020 to November 2021, (90) clinical (blood and stool samples), which were divided into two groups: β-thalassemia with HCV and β-thalassemia without HCV, 32 specimens of blood and stool sample were collected from β-thalassemia with HCV group and 58 blood and stool sample were collected from β-thalassemia without HCV group, investigate the association of some factors like type of thalassemia level either. Major or intermediate, age, gender, ferritin level and spleen status with determining the incidence rate of the bacterial viral co-infection in Wasit province was diagnosed as the thalassemia syndrome patient, either for serological and molecular diagnosis of co-infection *H. pylori* with HCV among thalassemia patients and diagnosis infection rate depending on serological –ELISA techniques. The level of ferritin and liver function test will be determined and nested PCR method targeting the molecular diagnosis will be used to confirm *H. pylori* 23S rRNA using DNA extracted from stool. Also, the diagnosis related to β-thalassemia was on the basis of hemoglobin electrophoresis, hematological analysis, clinical presentation, and the iron overload. Ethical verbal and written consent were taken from patients.

Sample Preparation

A 5 mL of the venous blood was acquired through a vein puncture from each one of the β-thalassemia patients included in this work, the blood was almost equally divided into (2 mL) edetic acid (EDTA) tubes, also (3 ml) in the gel tubes (the samples of blood were collected into EDTA tubes and gel tubes were prepared and labeled). A 3 mL blood sample was obtained from each one and clotted at room temperature for 1 hour, then the centrifugation was conducted for a period of five minutes, at 4000 rpm for the serum separation.

The serum was transferred by micropipette, used to determine the following biochemical parameters Gastroesophageal reflux disease (GOT) Glutamic pyruvic transaminase (GPT) Alkaline phosphatase (ALP) Total serum bilirubin (TSB) (iron and ferritin), detecting HCV antibodies. The sera were stored at -20°C until the assay was done. A stool sample was taken from each thalassemic patient to detect *H. pylori* in the stool sample. The stool samples were stored at -20°C until the assay was done. Stool samples were utilized for the extraction and purification of DNA to carry out the molecular diagnosis

Serum Samples Collection

Serum was brought to room temperature to determine the following biochemical parameter (GOT, GPT, ALP and ferritin). Detection of HCV antibodies in this work was done by the hospital laboratory and the detection of HP- IgG by ELISA kit (Cat No.: ED0022HU, 96 Tests: BT LAB Bioassay Technology Laboratory-china).

Principle of the ELISA Test to Detected HP IgG

This kit is based on the qualitative reverse phase enzyme immunoassay technique. The microtiter plate was pre-coated with a target Ag. Positive/negative controls or samples were added to the wells and incubated. Antibody in the samples was bound to the Ag, on the plate. Unbound antibodies were washed away during the washing step.

A Horseradish peroxidase (HRP) conjugated detection antibody was then added and incubated. Unbound HRP has washed away during the washing step. traditional medical practitioners (TMP) substrate was then added and color developed. The reaction was stopped by adding acidic stop solution and color changed into yellow, which can be measured at 450 nm. The O.D of the unknown sample could then be compared to the O.D of the positive and negative control to determine the presence of HP-IgG.

Serological Analyses for Detecting Antibodies to HCV

ELISA for Detecting Antibodies to HCV (Screening Test)

HCV antibody ELISA test kit was regarded as a solid phase qualitative indirect simultaneous EIA to detect IgG antibodies to the HCV in the plasma or in the human serum. Microplate were subjected to coating with the HCV recombinant antigens. Throughout the testing process, the specimen's diluent and specimens were added to the antigen coat micro well plate and subjected to incubation.

In the case specimens contain certain antibodies to the HCV; it will bind to the antigen coated on a microplate for forming immobilized antigen-HCV antibody complexes. With regard to the conditions when specimens contain no antibodies to the HCV, the complexes will not be created. After the initial incubation, the micro well plate was washed to remove un-bound materials.

The enzyme conjugated anti-human IgG antibodies were added to the micro well plate and subjected to incubation. The enzyme conjugate anti-human IgG antibodies were bound to the immobilized antigen-HCV antibody complexes. Then, micro well plate were washed to remove the unbound materials. The substrates A and B were added and subjected to incubation to produce a blue color to specify the HCV antibodies present in the specimen. Also, adding the solution of the sulfuric acid was added into the micro well plate to stop the reaction generating change in color from blue to yellow. Colour's intensity, corresponding to HCV antibodies present in the specimen, was evaluated with the micro-plate reader at 450/630-700 nm or 450 nm.

Molecular Diagnosis of *H. pylori*

Stool DNA Extraction

Stool DNA from stool samples was extracted by using (Presto™ Stool g DNA Extraction Kit Geneaid Taiwan and done according to company instructions as the following steps.

Sample Lysis step

- A180–220 mg of stool sample was transferred to a bead beating tube containing ceramic beads. and then 800 µL of ST1 Buffer and vortex briefly then incubated at 70ºC for 5 minutes. Vortex at maximum speed for 10 minutes at room temperature. Then, centrifuge at 8,000 x g for 2 minutes at room temperature.
- 500 µL of supernatant was transferred to a new 1.5 mL microcentrifuge tube.
- PCR inhibitor removal (150 µL of ST2 buffer was added then vortexed for 5 seconds. Incubate at 0–4ºC for 5 minutes. Then, centrifuge at 16,000 x g for 3 minutes at room temperature to precipitate insoluble particles and PCR inhibitors. Place an inhibitor removal column (purple ring) in a 2 mL centrifuge tube.
- 500 µL of clear supernatant was transferred to the inhibitor removal column. Then, centrifuge at 16,000 x g for 1 minute at room temperature then discard the column. Save the flow-through in the 2 mL.

DNA Binding step

- A 800 µL of ST3 buffer were added into the flow-through then mixed immediately by shaking vigorously for 5 seconds. Then place a GD column (green ring) in a 2 mL collection tube.
- A700 µL sample mixture was transferred to the GD Column. Then, centrifuge at 16,000 x g for 1 minute at room temperature then discard the flow-through.
- The GD column back in the 2 mL collection tube. Then, transferred the remaining sample mixtures to the GD column. Then, centrifuge at 16,000 x g for 1 minute at room temperature.
- The flow-through was discarded then placed in the GD column back in the 2 mL collection tube.

Wash step

- 400 µL of ST3 buffer was added to the GD column. Then, centrifuge at 16,000 x g for 30 seconds at room temperature.
- The flow-through was discarded, then placed in the GD column back in the 2 mL collection Tube.
- 600 µL of wash buffer was added to the GD column. Then, centrifuge at 16,000 x g for 30 seconds at room temperature.
- The flow-through was discarded, then placed in the GD column back in the 2 mL collection tube.
- 600 µL of wash buffer was added to the GD column again. Then, centrifuge at 16,000 x g for 30 seconds at room temperature.
- The flow-through was discarded, then placed in the GD column back in the 2 mL collection tube. Then, centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix

Elution step

- The dry GD Column was placed into a new 1.5 mL microcentrifuge tube. Then 30–100 µL of preheated Elution Buffer was added into the center of the column matrix.
- The GD Column was Let stand for at least 2 minutes to allow the Elution Buffer to be completely absorbed. Then, Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified DNA.

If a higher DNA concentration is required, use 30 µL of elution buffer (10 mM Tris-HCl, pH8.5) then repeat the elution step by adding the same 30 µL of elution buffer (which now contains the eluted DNA) to the center of the column matrix again.

- If maximum DNA yield is required, use 100 µL of elution buffer (DNA concentration was diluted). Ensure that the elution buffer was added into the center of the GD column matrix and is completely absorbed.
- Using TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed.
- If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added

into the center of the GD Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Nano-drop Spectrophotometer

This is the second step after DNA extraction. It is an easy and fast method. It reveals the possible error rate in the sample where the measurement read for DNA 1.8. As for the readings that are less than these % indicate the % of contamination in the sample, where the sample still contains protein or some other substances. The reading is done at the wavelength (260–280).

PCR and Electrophoresis

- Thermocycler, where the idea of this technique is based on rapid temperature control.
- DNA polymerase: Ex Taq polymerase is a single chain of polypeptide extracted from thermous aquitics bacteria that live in a hot spring and its optimum temperature is 72ºC
- Primer: The primers Hp23S 1835F (5′-GGTCTCAGCAAAG AGTCCCT-3′) and Hp23S 2327R (5′-CCCACCAAGCATT GTCCT-3′) are used for first PCR, and the primers Hp23S 1942F AGGATGCGTCAGTCGCAAGAT and Hp23S 2308R CCTGTGGATAACACAGGCCAGT are used for second PCR.
- dNTP Mixture[11]
- Buffer
- Template from sample by DNA extraction
- Deionized distilled water
- Electrophoresis buffer (50× TAE buffer)
- DNA ladder marker 100 kbp.

Nested-PCR Targeting the 23 S rRNA Gene of *H. pylori*

- Prepare the first PCR reaction mixture on ice by adding the following quantities of reagents: 25 µL in the order listed: 7.5 mL of sterile deionized water, 12.5 µL Master mix, 3 µL DNA from sample(template), 1.0 µL of primer Hp23S 1835 F (10 pmol/mL), 1.0 µL of primer Hp23S 2327R (10 pmol/mL).
- Aliquot 20 µL of the PCR reaction mixture to each PCR tube. And add 5 µL from DNA sample (template) mix well.
- Use a thermal cycler, perform PCR: initial denaturation at 95°C for 2 minute,followed by 5 cycles: 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds; then 30 cycles: 94°C for 15 seconds, 57°C for 15 seconds, and 72°C for 20 seconds.
- Prepare the second PCR mixture on ice by adding: 25 µL in the order listed: 7.5 µLof sterile ultra-pure water, 12.5 µL of master mix, 1.0 µL of primer Hp23S 1942 F (10 pmol/µL), and 1.0 µL of primer Hp23S 2308R (10 pmol/µL).
- Aliquot 22 µL of the PCR reaction mixture to each PCR tube and add 3 µL of the first PCR product. Mix well.
- Perform PCR as follows; initial denaturation at 95°C for 2 min, followed by 25 cycles: 94°C for 10 seconds and 63°C for 20 seconds.
- Final PCR products are confirmed using electrophoresis in 2% agarose gels. The PCR product should be 367 bp[11]

Statistical Analysis

The statistical analysis system - program was used to detect the effect of different factors on study parameters. The least significant difference –LSD test (analysis of variation-ANOVA) was used to compare between means significantly. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability).[12]

RESULTS AND DISCUSSION

Prevalence of *H. pylori* in β-thalassemia

Prevalence of *H. pylori* among β-thalassemia in Wasit province/ Iraq who have been admitted in Thalassemia Hematology Center in Al Kut Women and Children Hospital, 13 patients of thalassemia with the percentage 43.33% (according molecular diagnosis) out of 90 patients of total β-thalassemia. So there is a high prevalence of *H. pylori* in β-thalassemia. This result agrees with[7] who reported that the overall *H. pylori* infection rate was 43%.

The infection was significantly more prevalent in thalassemia with HCV patients than thalassemia without HCV. The prevalence of *H. pylori* among β-thalassemia with HCV about 9 patients (28.1%) while in β-thalassemia without HCV was about 4(6.9%) so theirs higher significantly (*p-value*=0.0073 **). This study shows *H. pylori* infected β-Thalassemia with HCV more than β-thalassemia without HCV. The results as shown in Table 1.

Table 1: The prevalence of *H. pylori* among β-Thalassemia with HCV and β-thalassemia without HCV.

H. pylori	*β-thalassemia patients with HCV No (%)*	*β-thalassemia patients without HCV No (%)*	*p.value*
Negative	23 (71.9)	54 (93.1)	0.0073 **
Positive	9 (28.1)	4 (6.9)	
**($p \leq 0.01$).			

Table 2: Relationship of *H. pylori* to β-thalassemia level factor in Thalassemia with and without HCV infected patients.

β-thalassemia level	*β-thalassemia without HCV*			*β-thalassemia with HCV*		
	+H. pylori No. (%)	*+H. pylori No (%)*	*p-value*	*+H. pylori No. (%)*	*+H. pylori No (%)*	*p-value*
Major	4 (100)	53 (98.1)	0.0001**	9 (100)	20 (87)	0.0001**
Intermediate	0	1 (1.9)		0	3 (13%)	
** ($p \leq 0.01$).						

Table 3: Relationship of *H. pylori* to gender factor in the β-thalassemia with and without HCV infected patients.

Gender	*β-Thalassemia without HCV*			*β-Thalassemia with HCV*		
	+H. pylori No. (%)	*-H. pylori No. (%)*	*p. vale*	*+H. pylori No.(%)*	*-H. pylori No. (%)*	*p-value*
Male	2 (50)	33 (61)	0.0001**	4 (44.4)	13 (56.5)	0.00047**
Female	2 (50)	21 (39)		5 (55.6)	10 (43.5)	
** (P ≤0.01).						

Table 4: Relationship of *H. pylori* to Age factor in the β-thalassemia with and without HCV infected patients

Age	*β-thalassemia without HCV*			*β-thalassemia with HCV*		
	+H. pylori No. (%)	*-H. pylori No (%)*	*p-value*	*+H. pylori No. (%)*	*-H. pylori No (%)*	*p-value*
< 26	2 (50)	50 (92.6)	0.0001**	6 (66.7)	15 (65.2)	0.0031**
> 26	2 (50)	4 (7.4)		3 (33.3)	8 (34.8)	
** (P ≤0.01).						

Table 5: Relationship of *H. pylori* to ferritin level factor in the β-thalassemia with and without HCV infected patients.

Ferritin level	*β-thalassemia without HCV*			*β-thalassemia with HCV*		
	+H. pylori No. (%)	*-H. pylori No. (%)*	*p-value*	*+H. pylori No. (%)*	*-H. pylori No. (%)*	*p-value*
less than 3000 ng/dL	3(75)	28(51.9)	0.0001**	2(22.2)	10(43.5)	0.0007**
more than 3000ng/dL	1 (25)	26(48.1)		7(77.8)	13(56.5)	
** (p ≤0.01).						

This result agrees with,[6] who reported a statistically significantly higher rate of *H. pylori* among adult β-thalassemia patients (35%).

Relationship of *H. pylori* to Different Characteristics

The first factor analyzed in the current study is the correlation of the β-thalassemia level (major or intermediate) and β-thalassemia within two groups (β- thalassemia with HCV and β-thalassemia without HCV). The results are shown in (Table 2)

In β-thalassemia with HCV group and *H. pylori* positive about 9(100%) patients β-thalassemia major and 0 (0%) β-thalassemia intermediate while *H. pylori* negative about 20(87%) β-thalassemia major with higher significant *p-value* =0.0001** So in this study *H. pylori* positive 100% in β-thalassemia major also in β-thalassemia without HCV and *H. pylori* positive 4 (100%) β- thalassemia major and 0 (0%) in β- thalassemia intermediate while *H. pylori* negative about 53(98.1%) β-thalassemia major and 1(1.7%) there's high significant difference *p-value* 0.0001**. So *H. pylori* positive in both groups increased in β-thalassemia major more than β-thalassemia intermedia.

The second factor analyzed in the current study is the correlation of the gender and β-thalassemia within two groups (β- thalassemia with HCV and β-thalassemia without HCV). The results are shown in Table 3. In β-thalassemia with HCV about 5 females (55.6%) and 4 males (44.4%) were recorded with higher significant *p-value* =0.0047**

This study agrees with other studies[13] who reported that female gender was also another factor associated with a higher rate of *H. pylori*. And agrees with other studies[14] who recorded *H. pylori* seropositive infected females about 59.2% while infected male about 40.8%. So *H. pylori* infected females more than males in β-thalassemia with HCV.

While gender factor in the β-thalassemia without HCV and *H. pylori* was positive for two patients male gender with percentage of 50% but *H. pylori* was negative in male gender about 33(61%) and 2 patients female gender with percentage of 50% *H. pylori* positive and 21 (39%) *H. pylori* negative and *p-value* are 0.0001** with a highly significant difference. This result agrees with [15] who reported that the distribution of *H. pylori* seropositivity was about 38 (51.4%) of the cases with *H. pylori* in girls.

The third factor analyzed in the current study is the correlation of the age factor and β- thalassemia within two groups (β- thalassemia with HCV and β- thalassemia without HCV). The results are shown in (Table 4). In β- thalassemia with HCV about 6 patients were less than 26 years with a percentage of (66.7%) and 3 patients were more or equal to 26 years with a percentage of (33.3%) and the *p-value* was 0.0031** highly significant difference.

This result agrees with researchers[16] who recorded there was a statistically significantly higher seroprevalence of *H. pylori* among the total number of β-TM children than among the total number of control children. So *H. pylori* increased in the younger age group (less than 26 years) more than the older age. While in β- thalassemia without HCV in this study was *H. pylori* positive in 2 patients less than 26 years with a percentage of (50%) but in negative *H. pylori* and beta thalassemia without HCV it was about 50 (92.6%) less than 26 years and 2 patients with *H. pylori* positive were with age more or equal to 26 years with a percentage of (50%) and 4 patients *H. pylori* negative (7.4%) were more than 26 years. *p-value* =0.0001** was a

Table 6: Relationship of *H. pylori* to splenoctomized factor in Thalassemia with and without HCV infected patients.

Factor	*β-thalassemia with HCV and positive H. pylori*	*β-thalassemia without HCV and positive H. pylori*
Splenoctomized	6 (66.66%)	1 (25%)
Non splenoctomized	3 (33.33%)	3 (75%)

Table 7: ELISA test for *H. pylori* IgG in serum patients of β-thalassemia patients with and without HCV.

ELISA test for H. pylori IgG in β-thalassemia patients with HCV		*p-value*
Positive	18 (56.2)	0.0372 *
Negative	14 (43.8)	
ELISA test for H. pylori IgG in β- thalassemia patients without HCV		*p-value*
Positive	12 (20.7)	0.0001**
Negative	46 (79.3)	

Table 8: Molecular detection using Nested PCR for *H. pylori*

β- thalassemia patients with HCV		*p-value*
	Number (%)	
Positive	9 (50)	1.00 NS
Negative	9 (50)	
β- thalassemia patients without HCV		*p-value*
	Number (%)	
Positive	4 (33.3)	0.0057**
Negative	8 (66.7)	

highly significant difference. Therefore, it could be concluded that there was a relationship between *H. pylori* and age less than 26 years.

The fourth factor analyzed in the current study is the correlation of the ferritin level factor and β- thalassemia within two groups (β- thalassemia with HCV and β- thalassemia without HCV). The results are shown in (Table 5).

In β-thalassemia with HCV and *H. pylori* positive about 2 patients less than 3000 ng/dL with a percentage of (22.2%) and 7 patients more or equal to 3000ng/dL with a percentage of (77.8%) and *P-value* 0.0007** recorded high significant difference but inβ- thalassemia with HCV and *H. pylori* negative about 10 patients less than 3000 ng/dL with a percentage of (43.5%) and 13 patients more or equal to 3000 ng/dL with a percentage of (56.5%) and *P-value* 0.0007** was highly significant difference.

This result agrees withc studies[17] who reported that Serum ferritin level was significantly higher in participants with *H. pylori* infection (536.82 ± 117.0ng/dL vs. 391.31 ± 101.54 ng/dL; *p-value:* <0.0001). While in β- thalassemia without HCV and *H. pylori* positive about 3 patients were less than 3000 ng/dL with percentage (75%) and 1 patient more or equal to 3000 ng/dL with a percentage of (25%)and *p-value* 0.0001** was highly significantly difference but in β-thalassemia without HCV and *H. pylori* negative about 28 patients were less than 3000 ng/dL with percentage (51.9%) and 26 patients more or equal to 3000 ng/dL with a percentage of (48.1%)and *p-value* 0.0001** highly significant difference.

Therefore, it could be concluded that serum ferritin increased in patients of β-thalassemia with HCV and *H. pylori* positive more than β-thalassemia without HCV.

Splenoctomized Factor

The relationship of the splenoctomized factor and β-thalassemia within two groups (β- thalassemia with HCV and β-thalassemia without HCV) and *H. pylori* positive The results are shown in (Table 6). The comparisons between the patients of beta thalassemia with HCV and *H. pylori* positive and beta thalassemia without HCV and *H. pylori* positive are shown in Table 6.

H. pylori was detected more frequently in thalassemia patients with hepatitis C virus a longer duration of splenectomy of about 6 (66.66%) while non splenoctomized patients about 3 (33.33%) and about 1 (25%) of β-TM without HCV and *H. pylori* positive were splenoctomized patient but 3(75%) β-TM with non HCV and *H. pylori* positive are non splenoctomized patient.

Moreover, the mean duration of splenectomy was higher in β-TM with HCV positive and *H. pylori* positive than β-TM negative HCV and *H. pylori* positive. This means that splenectomy weakens the immunity against *H. pylori*, establishing the role of the immune system in protection against *H. pylori* infection

This study agrees with[16,6] who reported *H. pylori* was more frequent in Splenoctomized than non Splenoctomized patients in β-thalassemia than non β-thalassemia patients as shown in Figure 1.

ELISA test for *H. pylori* IgG in serum patients of β-thalassemia showed 30 (33.3%) positive for *H. pylori* out from 90 while the negative results for *H. pylori* were 60 (66.7%) as in Table 7.

This agrees with a study[18] recorded the seropositive patients. 26 patients were ELISA positive. This finding agrees with

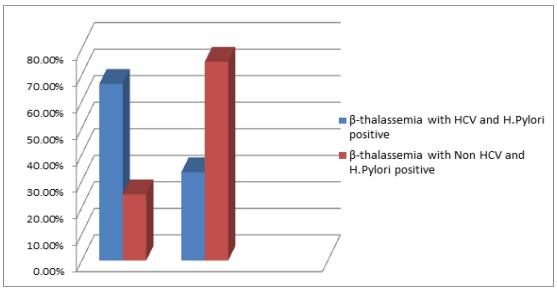

Figure 1: Comparison between β-thalassemia with HCV and β-thalassemia without HCV according to splenloctomy.

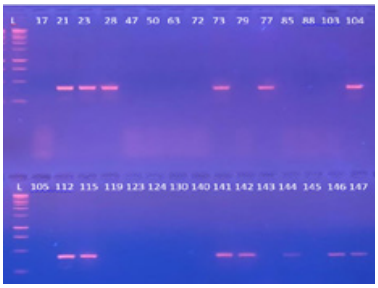

Figure 2: Gel electrophoresis of amplified 23S rRNA gene from H. pylori using Nested PCR. Agarose 2%, 70V/cm for 120 min, stained with ethidium bromide dye and visualized on a UV transilluminator. DNA ladder 100 kbp.

a study[6] recorded that there was a statistically significant high seroprevalence of *H. pylori* among adult β-thalassemia patients than among the controls (35%). In this study patients of β-thalassemia with HCV positive and *H. pylori* positive were 18 with a percentage of (56.2%) and 14 were negative with a percentage of (43.%) (p.value = 0.0372). While in β-thalassemia patients without HCV 12 patients were positive with a percentage of (20.7%) and 46 were negative with a percentage of (79.3%) (*p-value* = 0.0001).

Molecular Detection

The results of molecular detection using PCR diagnosis by amplifying the conserved gene 23S rRNA had the highest performance than other PCR assays. Therefore, 23S rRNA PCR was used to identify *H. pylori* infection in this study. In addition, it was efficient to rule out the other neighboring species close to the phylogenetic cluster of *Helicobacter* bacteria and increasing the diagnostic sensitivity and specificity.[1] The result of molecular detection using nested PCR for β-thalassemia patients was 13 patients (43.3%). This result agrees with the study achieved by[7] who recorded that the prevalence of *H. pylori* infection rate was 43%. The infection was significantly more prevalent in thalassemia patients. This study was carried out on 30 of *H. pylori* seropositive patients of the 2 group involved as: (18) β-thalassemia patients with HCV about 9(50%) positive and 9 negative (50%) there was no significant relationship (*p-value* = 0.286). While in β-Thalassemia patients without HCV with *H. pylori* positive 4 with percentage (33.3%) and 8 patients were negative with a percentage of (66.7%) and the *p-value* was 0.0057. There was a high significant difference as shown in Table 8.

This study agrees with [(19)]who reported that 50% of β-thalassemia with HCV infected by *H. pylori* and agrees with[1] who reported that sensitivity of PCR positive was 53%, while (12) seropositive of β-thalassemia patients without HCV. The molecular positive *H. pylori* in patients of beta thalassemia without HCV was 4 (33.3%), while in *H. pylori* negative was 8 (66.7%) with a highly significant difference *p-value* was 0.0057 as shown in Figure 2.

DISCUSSION

According to the result, the prevalence of *H. pylori* among β-thalassemia with HCV patients was about 9(28.1%), while in β-thalassemia without HCV about 4(6.9%) with high significant (*p-value*=0.0073) referring to *H. pylori* infected β-thalassemia with HCV is higher than β-thalassemia without HCV. In this study, different factors were analyzed within two groups (β- thalassemia with HCV and β- thalassemia without HCV). The first factor is the level of the β-thalassemia, which is major or intermediate. In β-thalassemia with HCV group and *H. pylori* positive about 9(100%) patient β-thalassemia major and 0(0%) β-thalassemia intermediate while *H. pylori* negative about 20 (87%) β-thalassemia major with high significant (*p-value* =0.0001). Thus, *H. pylori* positive100% in β- thalassemia major also in β-thalassemia without HCV and *H. pylori* positive 4(100%) β-thalassemia major and 0 (0%) in β- thalassemia intermediate while *H. pylori* negative about 53 (98.1%) β-thalassemia major and 1 (1.7%) there's high significant difference (*p-value* 0.0001). Thus, *H. pylori* positive in both groups increased in β-thalassemia major more than β-thalassemia intermediate. The second factor is age, and the ages ranged from (2 to 52 years) in β-thalassemia with HCV about 6 patients less than 26 years with percentage (66.7%) and 3 patients more or equal to 26 years with percentage (33.3%) with (*p-value*= 0.0031) high significant deference. In β- thalassemia without HCV, *H. pylori* positive in 2 patients less than 26 years with percentage (50%), so *H. pylori* increased in β- thalassemia with HCV in age less than 26 years in β- thalassemia with HCV. Gender factor in β- thalassemia with HCV about 5 females (55.6%) and 4 males (44.4%) with highly significant (*p-value* =0.0047), while gender factor in the β-thalassemia without HCV and *H. pylori* positive 2 patients male gender with percentage 50% and 50% female so *H. pylori* more infected female more than male in β-thalassemia with HCV and *H. pylori* positive. forth factor is Serum Ferritin were, which analyzed in two groups of patients. In β-thalassemia with HCV and *H. pylori* positive about 2 patients less than 3000ng/dL with percentage (22.2%) and 7 patients more or equal to 3000ng/dL with percentage (77.8%) with high significant deference (*p-value*= 0.0007) but in β- thalassemia with HCV and *H. pylori* negative about 10 patients less than 3000ng/dL with percentage (43.5%) and 13 patients more or equal to 3000ng/dL with percentage (56.5%) with high significant difference (*p-value*=0.0007) so there's relation between *H. pylori* and increase serum ferritin level in β- thalassemia with

HCV more than in β- thalassemia without HCV group. *H. pylori* was detected more frequently in thalassemic patients with HCV a longer duration of splenectomy about 6(66.66%). In comparison, non splenectomized patient about 3(33.33%) and about 1(25%) of β-thalassemia without HCV and H. pylori positive are splenectomized patient but 3(75%) β-thalassemia without HCV and *H. pylori* positive are non splenectomized. High significant difference with splenectomized more than non splenectomized patients were investigated for the presence of *H. pylori* IgG in serum sample for 90 patients of β-thalassemia (32 β-thalassemia with HCV and 58β-thalassemia without HCV, about 30 patients are positive with *H. pylori* IgG (18 β-thalassemia with HCV and 12β-Thalassemia without HCV). These seropositive patients are tested by nested PCR for stool samples and the result showed only 13(43.3%) are positive out of 30 seropositive (9 β-thalassemia with HCV and 4 β-thalassemia without HCV). There was positive correlation between hepatitis C status and high *H. pylori* rate when compared with beta thalassemia without HCV. Ferritin levels have a significant correlation with *H. pylori* in β-thalassemia with HCV group.

CONCLUSIONS

The predominant of *H. pylori* in Wasit patients in β-thalassemia was 43.3%. Levels of ferritin have significant correlation with *H. pylori* in HCV patients. There was positive correlation between hepatitis C status and high *H. pylori* rate when compared with beta β-thalassemia without HCV. Splenectomy was a positive correlation with *H. pylori* in β-thalassemia with HCV.

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