Fetuin-A: a possible link between *Helicobacter pylori* infection and insulin resistance

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Introduction *Helicobacter pylori* infection has been associated with many extragastrointestinal diseases such as cardiovascular and neurological diseases. Recently, several studies have reported a relationship between nonalcoholic fatty liver disease and *H. pylori* infection. Indeed, *H. pylori* infection is involved in the pathogenesis of insulin resistance (IR), which is closely linked with nonalcoholic fatty liver disease. Furthermore, fetuin-A has been linked with impaired insulin sensitivity.

Aim Therefore, we aimed to evaluate fetuin-A as a possible link between *H. pylori* infection and IR.

Patients and methods A total of 160 patients included in our study were divided into two groups according to the presence or absence of *H. pylori* infection. We determined serum fetuin-A, serum insulin, homeostasis model assessment-IR, and liver and kidney functions. Upper gastrointestinal tract endoscopy and antral biopsy was taken for *H. pylori* examination.

Results We found that fetuin-A was significantly elevated in *H. pylori*-positive group when compared with *H. pylori*-negative group, and a significant positive correlation of fetuin-A was observed with fasting insulin levels and homeostasis model assessment-IR.

Conclusion We conclude that fetuin-A was implicated as a possible link between *H. pylori* infection and the development of nonalcoholic steatohepatitis through its effect on IR.

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Introduction

Helicobacter pylori is a gram-negative, noninvasive bacterium. It is one of the most common cause of bacterial infections worldwide, particularly in developing countries. It is accompanied by various gastrointestinal diseases such as chronic gastritis, peptic ulcer, and gastric malignancies [1]. Several studies reported that *H. pylori* infection is implicated in many extragastrointestinal diseases, like ischemic heart disease, type 2 diabetes mellitus, and nonalcoholic fatty liver disease (NAFLD) [2–4].

NAFLD refers to a spectrum of disorders ranging from the simple nonalcoholic fatty deposition of the liver and nonalcoholic steatohepatitis to fibrosis, cirrhosis, and eventually hepatocellular carcinoma [5]. Several studies reported an association between NAFLD and *H. pylori* infection; however, the definitive pathophysiological mechanism is still unclear [6–8]. Indeed, insulin resistance (IR) is a key mechanism in the pathogenesis of NAFLD. On the contrary, *H. pylori* infection can play a pivotal role in the development in IR, which may contribute to NAFLD development [9,10].

Fetuin-A is a hormone mainly secreted by the liver and delivered into circulation. Fetuin-A has an important role in the regulation of metabolism, mainly in the modulation of insulin sensitivity. So, it serves as one of the biomarkers of IR [11]. Some studies have found the circulating level of fetuin-A was elevated in atherosclerosis, metabolic syndrome, type 2 diabetes mellitus, and accumulation of fat in the liver [12,13]. Therefore, we aimed to evaluate fetuin-A as a possible link between *H. pylori* infection and IR.

Patients and methods

This cross-sectional study enrolled 160 Egyptian patients who had dyspeptic complaints such as distension, postprandial fullness, early satiety, flatulence, epigastric pain, heartburn, nausea, and vomiting. Their ages were above 18 years. All patients were selected from the outpatient's clinic and Hepatology Department at El Galaa Military Hospital from March 2017 to August 2018. All patients were informed about the study protocol, and a written consent was obtained from them. The study was approved according to the ethical committee of Al-Azhar University and was conducted in accordance with the Declaration of Helsinki.

Inclusion criteria

(1) Patients who complained of dyspeptic symptoms with age above 18 years were included.

Exclusion criteria

The following were the exclusion criteria:

(1) Diabetic patients.
(2) Chronic liver cell failure.
(3) Chronic renal cell failure.
(4) Intake of proton pump inhibitors, histamine type 2 receptor antagonist, and *H. pylori* eradication therapy in the past month.
(5) Patients receiving lipid-lowering therapy.
(6) Systemic or local infection.
(7) Malignancy.
(8) Patients who had undergone gastric surgery.
(9) Pregnancy.

A total of 160 patients were divided into two groups according the presence or absence of *H. pylori* infection, and each group included 80 patients.

Group A: patients who were *H. pylori* infection positive.
Group B: patients who were *H. pylori* infection negative.

H. pylori infection was diagnosed by upper gastrointestinal endoscopy and taking of gastric biopsy.

NAFLD was diagnosed by HAIR score (systemic hypertension, alanine transaminase, and IR). The grading of steatosis was done by abdominal ultrasonography [14].

All patients were subjected to the following:

(1) Full medical history taking and full medical examination were done.
(2) Laboratory investigations were:
    (a) Complete blood count.
    (b) Fasting serum glucose level and glycated hemoglobin (HbA1c).
    (c) Alanine aminotransferase and aspartate aminotransferase.
    (d) Prothrombin time and international normalized ratio.
    (e) Serum creatinine, blood urea, and serum uric acid.
    (f) Serum lipid profile [total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein].
    (g) Quantitative C-reactive protein.
    (h) Serum fetuin-A level.
    (i) Fasting serum insulin level.
(3) IR, using the homeostasis model assessment of insulin resistance (HOMA-IR) index, was calculated using the formula: fasting insulin (μu/ml)×fasting glucose (mg /dl)/450. The patients were considered to have IR when HOMA-IR more than 2.5 [15,16].
(4) Radiological investigations:
    Pelvi-abdominal ultrasonography was done using GE LOGIQ S8 with a convex probe (3.75 mHz). MedCorp LLC is a full-service ultrasound company (5735 Benjamin Center Dr. Tampa, FL 33634)
(5) Upper gastrointestinal endoscopy:

This was done using sterile upper gastrointestinal video scope (Fujinon Fujifilm EG – 590 WR and Olympus GIF type XQ 260; Olympus, Shinjuku Monolith, 2-3-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 163-0914, Japan) after good preparation of the patient. Two antral biopsies were taken and preserved in a sterile container using diluted formalin solution.

Methodology

(1) The venous blood samples were taken and immediately centrifuged and then preserved at –20°C.
(2) Serum insulin was estimated using Insulin Enzyme-linked Immunoassay Kit which is a solid-phase enzyme-linked immunosorbent assay. The kit was provided by Calbiotech Catalog Number IS 130D, which is intended for the quantitative measurement of insulin in human serum or plasma.
(3) Serum level of fetuin-A was measured by enzyme-linked immunosorbent assay technique. The kit was provided by Glory Science Co. Ltd has extended the products to more than 20 countries world wide, and have one in USA (Catalog Number #: 95469; 4/F, Hehuayuan, Lianhua Street, Hangzhou, Zhejiang, China). Detection range of the kit is 200–4000 ng/ml.

Histopathological examination of the antral biopsies was done at El Galaa Military Hospital histopathology laboratory.

Statistical analysis

The data were collected and statistically analyzed using 17.1.0.0 for Windows. Descriptive data were represented as mean and SD, whereas nonnumerical data as number and percentage (%). Independent *t*

test was used to compare between two independent groups. Pearson χ^2 test was used to test the association between categorical variables. Pearson correlation coefficient test was used to test the correlation between variables of numerical pattern. Receiver operating characteristic (ROC) curve was used to identify optimal cutoff values of serum fetuin-A, with maximum sensitivity and specificity for prediction of *H. pylori*. Area under the curve (AUC) was also calculated, where more than 0.60–0.70 was considered acceptable. *P* value was considered significant if less than 0.05.

Results

The characteristics data of groups

Table 1 shows the comparative study of the demographic, clinical, and laboratory data between the two study groups. The current study enrolled 160 patients who were divided into 80 patients having *H. pylori* infection-positive result as group A and 80 patients having *H. pylori* infection-negative result as group B. Both groups were matched regarding the age, sex, and smoking habits. Considering the smoking habits, 13.75% of group A and 7.5% in group B were smokers, but there was no significant difference in smoking habits between the groups (*P*=0.3).

There was no statistically significant difference between group A and group B with respect to hypertension [group A: 10 (12.5%) vs. group B: 8 (10%); *P*=0.7] and abdominal distension [group A: 27 (33.75%) vs. group B: 34 (42%); *P*=0.3]. However, there was a significant difference of postprandial fullness between group A [21 (26.35%)] and group B [46 (57.50%)], with *P* value=0.001; early satiety [group A: 14 (17.50%) vs. group B: 30 (37.50%); *P*=0.001], and flatulence [group A: nine (11.25%) vs. group B: 34 (42.50%); *P*=0.001), as shown in Table 1.

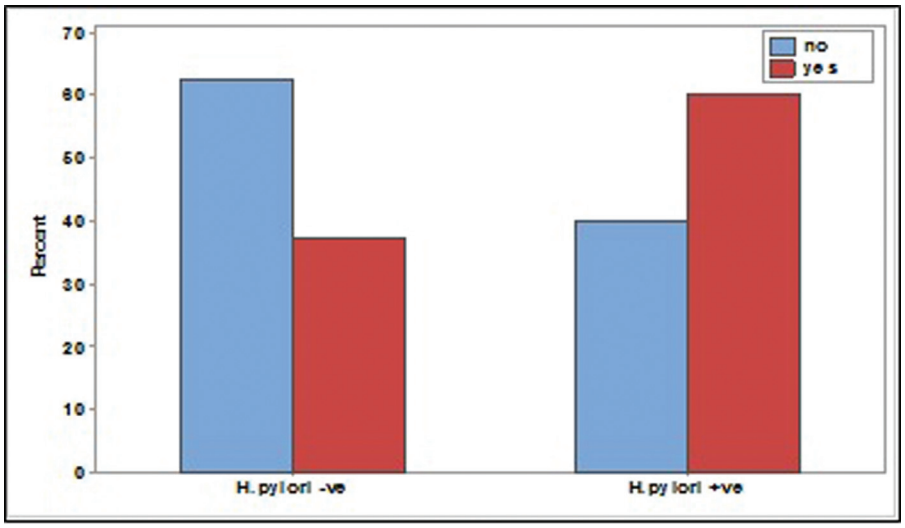
Regarding the heartburn, nausea, and vomiting, we found a significantly associated with *H. pylori*-positive cases (*P*=0.02, 0.005, and 0.001, respectively), whereas

Table 1 Demography and clinical data of the studied groups

Variables	Group A [*n* (%)] *N*=80	Group B [*n* (%)] *N*=80	*P* value	Significance
Age (mean±SD)	40.8±12.1	44.7±12.9	0.12	NS
Sex (female)	47 (58.75)	50 (62.5)	0.6	NS
Smoking status (smoker)	11 (13.75)	6 (7.5)	0.3	NS
BMI (kg/m^2) (mean±SD)	23.88±2.50	24.38±2.20	0.2	NS
NSAI use	16 (20.00)	12 (15)	0.6	NS
Hypertension	10 (12.50)	8 (10)	0.7	NS
Abdominal distention	27 (33.75)	34 (42.5)	0.3	NS
Postprandial fullness	21 (26.25)	46 (57.50)	0.001	S
Early satiety	14 (17.50)	30 (37.50)	0.001	S
Flatulence	9 (11.25)	34 (42.50)	0.001	S
Epigastric pain	49 (61.25)	42 (52.50)	0.3	NS
Heartburn	48 (60.00)	30 (37.50)	0.02	S
Nausea	34 (42.50)	14 (17.50)	0.005	S
Vomiting	27 (33.75)	6 (7.50)	0.001	S
Total leukocytic count (μl) (mean±SD)	7214.00±1858.00	6974.00±1902.00	0.50	NS
Hb (g/dl) (mean±SD)	12.51±1.42	12.14±1.42	0.10	NS
PLT (10^9/l) (mean±SD)	223 013±58 627	230 564±53 367	0.40	NS
ALT (IU/l) (mean±SD)	27.20±10.70	23.50±11.70	0.10	NS
AST (IU/l) (mean±SD)	19.97±8.66	27.10±12.90	0.001	HS
Serum total bilirubin (mg/dl) (mean±SD)	0.87±0.25	0.76±0.31	0.04	S
Serum total protein (g/dl) (mean±SD)	6.98±6.83	6.26±0.65	0.3	NS
Prothrombin time (s) (mean±SD)	13.00±11.30	11.91±0.73	0.3	NS
INR (mean±SD)	1.01±0.04	1.01±0.04	0.7	NS
Total cholesterol (mg/dl) (mean±SD)	178.5±39.9	176.5±24.6	0.7	NS
Triglycerides (mg/dl) (mean±SD)	124.4±63.8	103.1±13.4	0.005	S
LDL (mg/dl) (mean±SD)	87.40±31.40	61.30±16.90	0.001	HS
HDL (mg/dl) (mean±SD)	46.40±4.42	45.69±2.51	0.2	NS
Serum creatinine (mg/dl) (mean±SD)	0.86±0.19	0.91±0.17	0.11	NS
Blood urea (mg/dl) (mean±SD)	27.74±8.16	26.33±7.69	0.3	NS

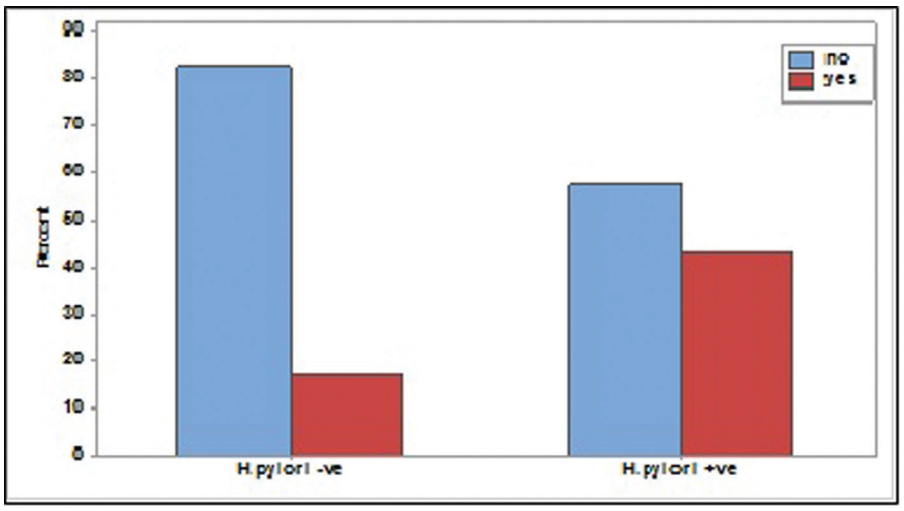
ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; HDL, high-density lipoprotein; HS, highly significant; INR, international normalized ratio; LDL, low-density lipoprotein; NSAI, non-steroidal anti inflammatory; PLT, platelet; S, significant.

Figure 1



Heartburn symptoms in both groups.

Figure 2



Nausea symptom in both groups.

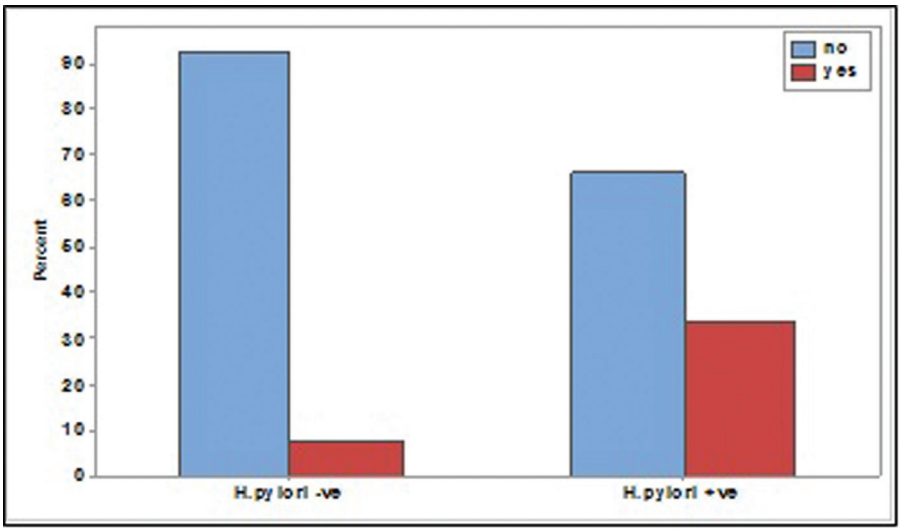
postprandial fullness, early satiety, and flatulence were significantly associated with patients with negative *H. pylori* (P=0.001, 0.01, and <0.001, respectively) (Table 1) (Fig. 1 for heartburn, Fig. 2 for nausea, and Fig. 3 for vomiting).

Both liver and kidney functions which were recorded in both groups showed nonsignificant statistical difference between groups except serum total bilirubin (mg/dl) and AST (IU/l) which showed significant statistical elevation in group A (patients with positive *H. pylori* infection) (P=0.04 and 0.001, respectively). Moreover, the lipid profile [LDL (mg/dl) and triglyceride (mg/dl)] showed significant elevation in patients with positive *H. pylori* infection group (P=0.001 and 0.005, respectively), as shown in Table 1 (Fig. 4 for serum bilirubin, Fig. 5 for aspartate aminotransferase, and Fig. 6 for LDL and triglyceride).

The fasting blood sugar, serum insulin, glycated hemoglobin, and homeostasis model assessment of insulin resistance

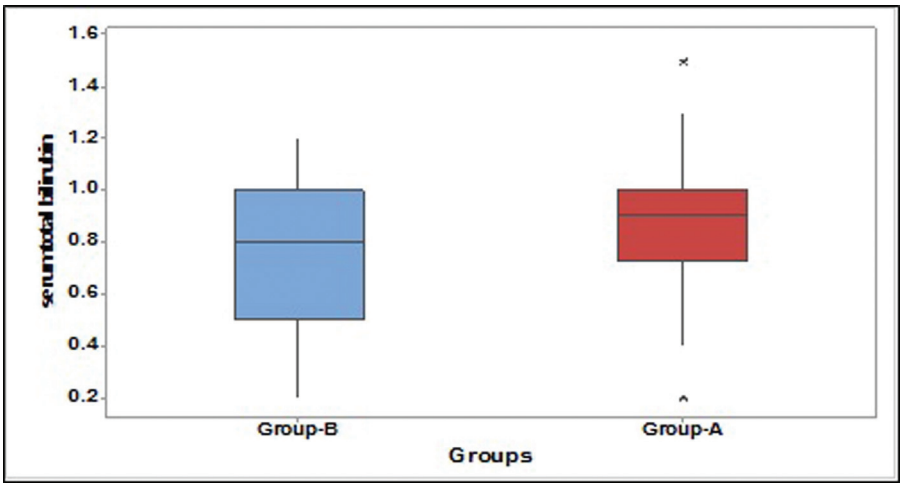
The mean serum fasting glucose level, serum insulin level, HbA1c, and HOMA-IR were significantly higher in patients who had positive *H. pylori* infection (98±10.6, 5.41±4.58, 5.475±0.858, and 1.25±1.11, respectively) than the group who had negative *H. pylori* infection (88.38±8.94, 2.13±1.31, 4.995±0.632, and 0.463±0.303, respectively) (P=0.001, 0.001, 0.001, and 0.001, respectively) (Table 2) (Fig. 7 for serum insulin level).

Figure 3



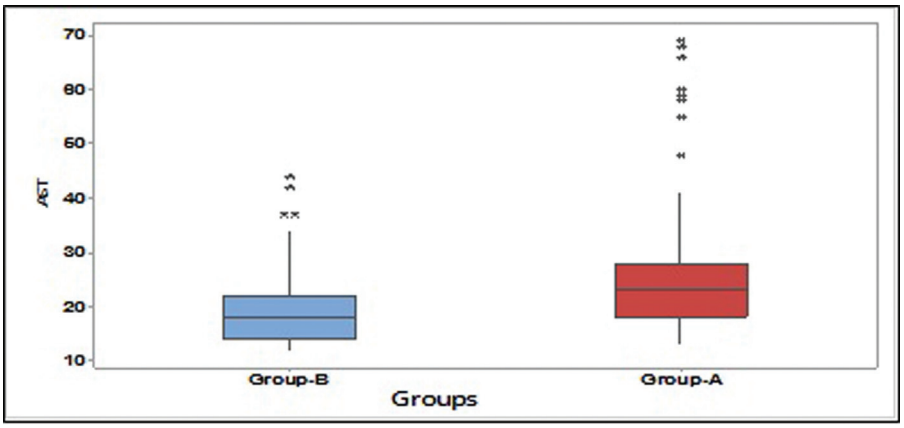
Vomiting in both groups.

Figure 4



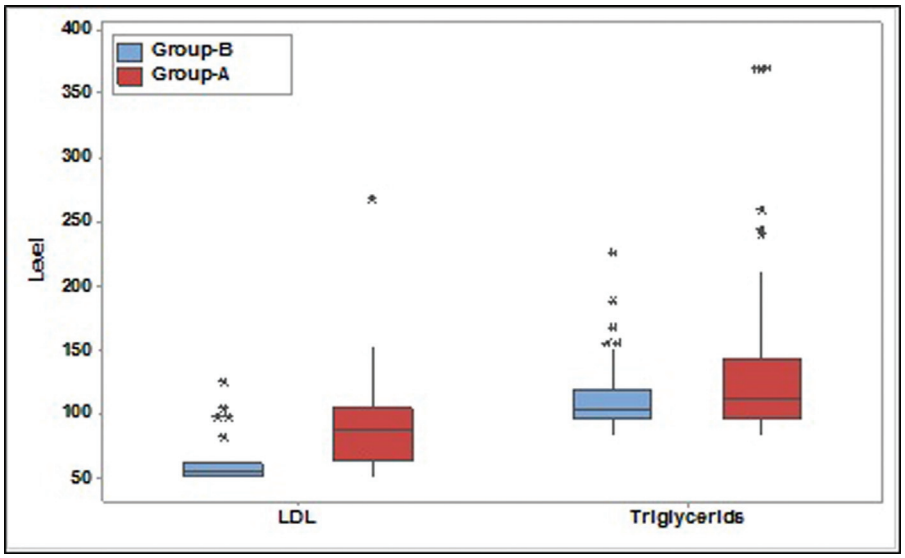
Serum total bilirubin in both groups.

Figure 5



AST in both groups. AST, aspartate aminotransferase.

Figure 6



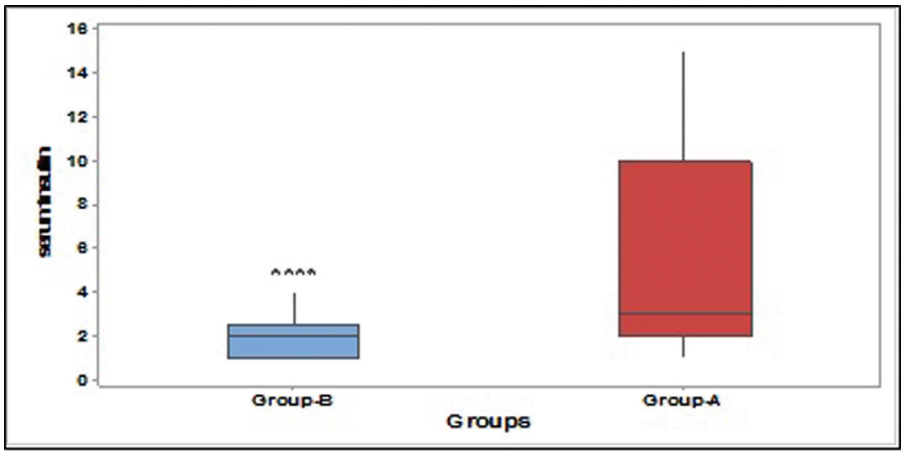
LDL and triglycerides in both groups. LDL, low-density lipoprotein.

Table 2 Insulin resistance parameters and serum fetuin-A level in both groups

Variables	Group A (mean±SD) *N*=80	Group B (mean±SD) *N*=80	*P* value	Significance
Serum fasting glucose (mg/dl)	98±10.6	88.38±8.94	0.001	HS
Serum insulin (μU/l)	5.41±4.58	2.13±1.31	0.001	HS
HbA1c %	5.475±0.858	4.995±0.632	0.001	HS
HOMA-IR	1.25±1.11	0.463±0.303	0.001	HS
Serum fetuin-A (ng/ml)	297±27.0	39.7±4.40	0.001	HS

HbA1c, glycated hemoglobin; HOMA, homeostasis model assessment; HS, highly significant; IR, insulin resistance.

Figure 7



Serum insulin level in both groups.

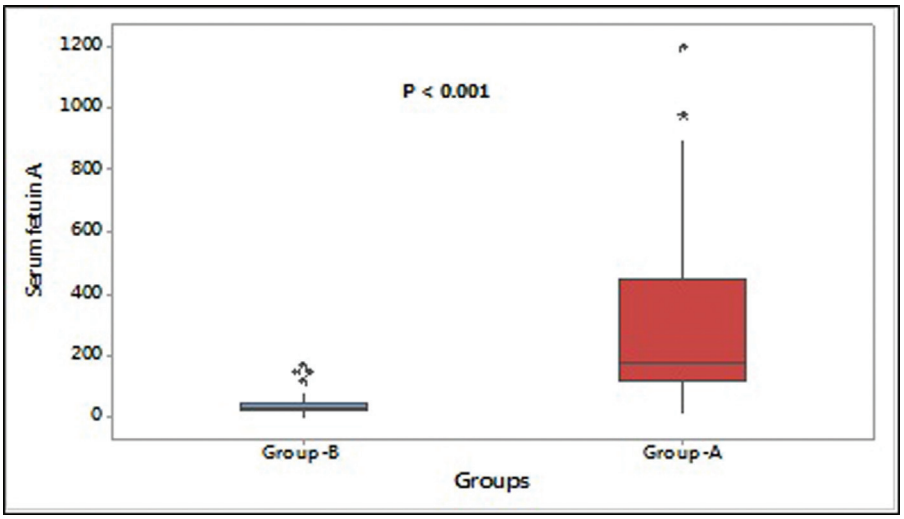
The serum fetuin-A

Table 2 shows a statistically significant elevation of the mean serum fetuin-A level in group A (297±27.0) in comparison with group B (39.7±4.40) ($P<0.001$, Fig. 8).

The abdominal ultrasound finding

The number of patients who had normal abdominal ultrasound finding was 30 (37.5%) in group B and 20 (25%) in group A. The number of fatty liver in group A was 35 (43.75%) patients, but 18 (22.5%)

Figure 8



Serum fetuin-A in both groups.

Table 3 Abdominal ultrasound picture in both groups

Variables	Group A [*n* (%)] *N*=80	Group B [*n* (%)] *N*=80
Normal US finding	20 (25)	30 (37.5)
Splenomegaly	2 (2.5)	4 (5)
Fatty liver	35 (43.75)	18 (22.5)
Calcular gall bladder	8 (10)	8 (10)
Fatty liver and calcular gall bladder	2 (2.5)	0
Gaseous distension	13 (16.25)	20 (25)

US, ultrasound.

Table 4 Fatty liver feature in the studied patients

Fatty liver	Group A [*n* (%)] *N*=80	Group B [*n* (%)] *N*=80	*P* value	Significance
Yes	37 (46.25)	18 (22.5)	0.01	S
No	43 (53.75)	62 (77.5)		

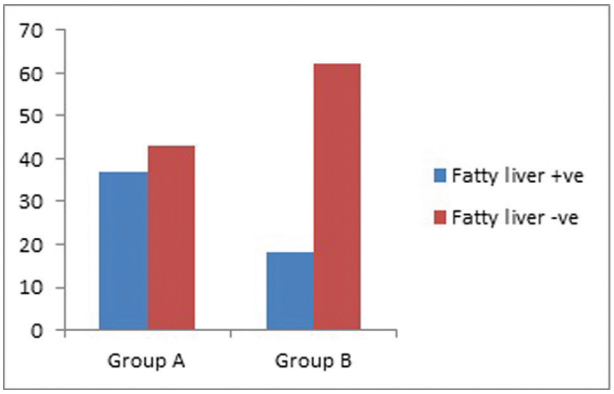
S, significance.

in group B. There were 13 (16.25%) patients who had gaseous distension in group A, whereas 20 (25%) in group B, as shown in Tables 3 and 4 [Fig. 9 for fatty liver (+ve) and (–ve) in both groups].

The correlation study and the cutoff point

Table 5 shows a significantly positive association between serum fetuin-A and serum insulin level (r=0.713, P=0.001) in group A. Moreover, there was a significant positive correlation between the serum fetuin-A and HbA1c (r=0.342, P=0.001) and HOMA-IR (r=0.726, P=0.001) in group A (Fig. 10).

Figure 9



Fatty liver in both groups.

The best cutoff point of fetuin-A in prediction of positive *H. pylori* infection was 40, and above this point, the sensitivity and specificity were 95 and 75%, respectively; the AUC was 91%, with significant performance of this biomarker, P value less than 0.001 (Fig. 11).

Discussion

Our study aimed to evaluate fetuin-A as a possible link between *H. pylori* infection and IR. This study was conducted on 160 patients at age above 18 years with dyspeptic complaints. They were divided into two groups according to the presence of *H. pylori* infection: group A had *H. pylori*-positive patients (80 patients), and group B had *H. pylori* infection-negative patients (80 patients).

Both groups were matched regarding age, sex, and smoking habits. Table 1 shows that the mean age of

patients with positive *H. pylori* infection was 40 years old and that of the other group (negative *H. pylori*) was 44 years, with insignificant statistical difference (P=0.12). Moreover, most patients of both groups were female, with insignificant association of specific sex with any group (P=0.6). Considering the smoking habits, 13.75% of group A and 7.5% of group B patients were smokers, with insignificant association of smoking habits with any group (P=0.3). This result is in agreement with several studies [17–20].

Regarding BMI, there was no statistically significant difference between *H. pylori*-positive and *H. pylori*-negative cases, which is in agreement with Aydemir *et al.* [17] and Ozdem *et al.* [21], who found no statistically significant difference between the two groups regarding the BMI of the patients (P>0.05). Moreover, this means that the increase in HOMA-IR in *H. pylori*-positive patients and its decrease in *H. pylori*-negative patients were not related to BMI difference.

Table 5 Correlation between fetuin-A and parameters of insulin resistance

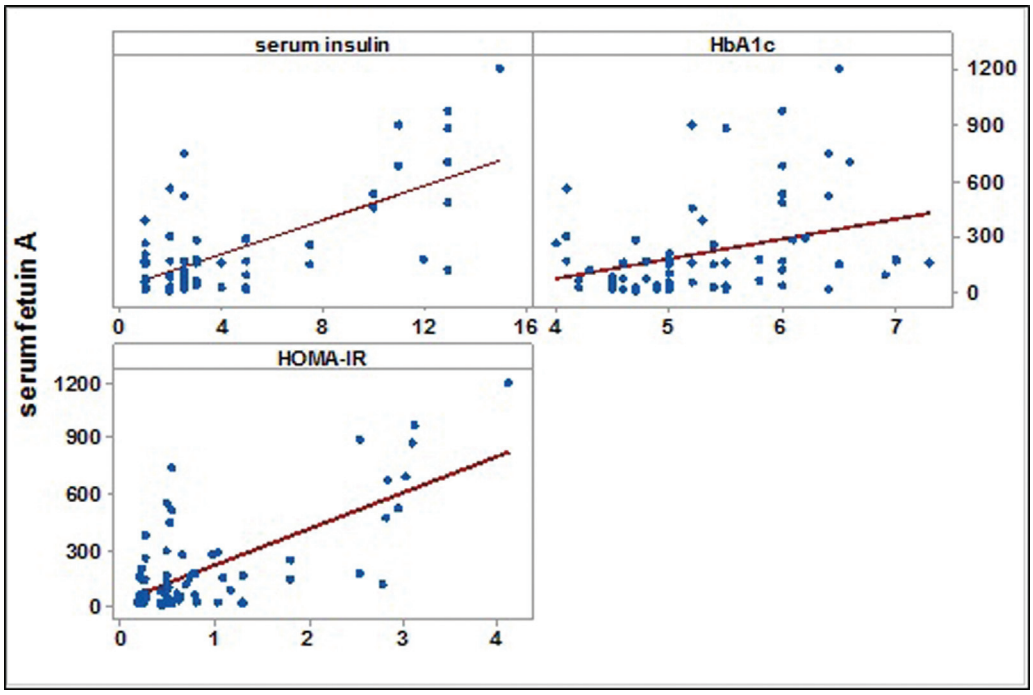
Variables	Serum fetuin-A	
	r	*P*
Serum insulin (μU/l)	0.713	0.001
HbA1c %	0.342	0.001
HOMA-IR	0.726	0.001

HbA1c, glycated hemoglobin; HOMA, homeostasis model assessment; IR, insulin resistance.

Considering the risk factors of dyspepsia and need for upper gastrointestinal tract endoscopy exploration, non-steroidal anti inflammatory (NSAI) use was considered as an important factor, which appeared to be matched in both groups (P=0.6). In the same line, hypertension as a comorbidity factor was found to insignificantly associate with any group (P=0.7). Moreover, abdominal distention and epigastric pain were insignificantly associated with complaint in any group (P=0.3 for both). Some symptoms were found to be significantly associated with *H. pylori*-positive cases such as heartburn, nausea, and vomiting (P=0.02, 0.005, and 0.001, respectively), whereas postprandial fullness, early satiety, and flatulence were significantly associated with patients with negative *H. pylori* (P=0.001, 0.01, and <0.001, respectively). This result was in agreement with that reported by Aslan *et al.* [22].

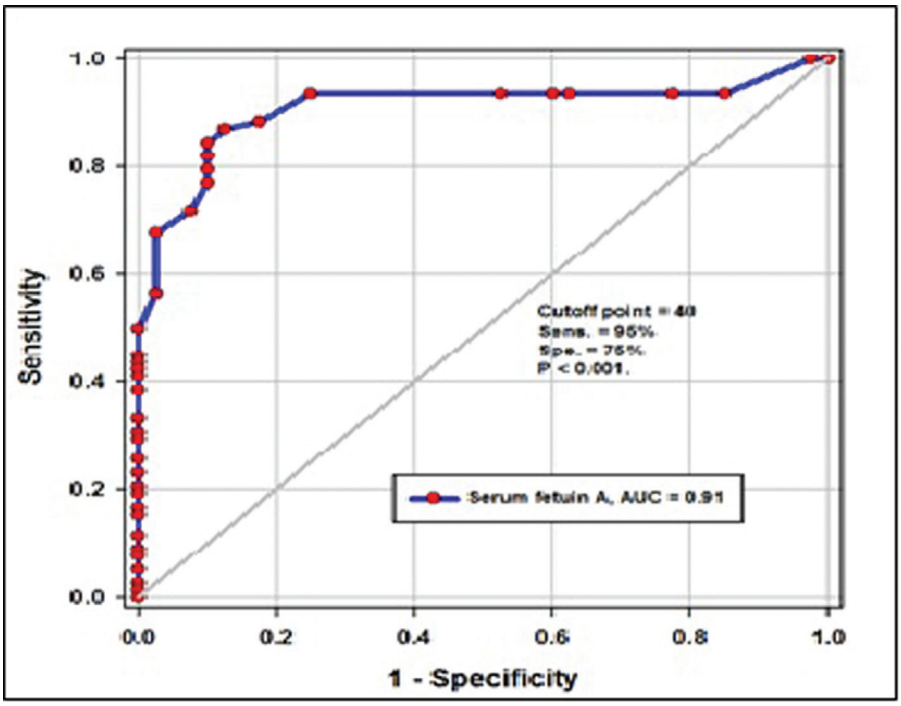
In the present study, both liver and kidney functions which were recorded in both groups showed insignificant statistical difference in between, except serum total bilirubin and AST, which showed significant statistical elevation in patients with positive *H. pylori* infection, (P=0.04 and 0.001, respectively). Furthermore, the lipid profile, including LDL and triglyceride, showed significant elevation in patients with positive *H. pylori* (P=0.001 and 0.005, respectively). These results were close to those of Haala *et al.* [19] who stated

Figure 10



Correlation between fetuin-A and parameters of insulin resistance within group A.

Figure 11



ROC curve of fetuin-A in prediction of *Helicobacter pylori* infection. ROC, receiver operating characteristic.

no statistical significant difference between the two groups regarding cholesterol level (P=0.69), and disagreed with significance elevation of triglycerides in positive group, and that may be owing to fatty liver association with positive group of *H. pylori*.

Regarding the ultrasound features of patients in the current work, most patients with negative *H. pylori* had normal ultrasound picture and some gaseous distension, whereas in group A, fatty liver was the most prominent feature. Further solo analysis of fatty liver presence revealed that it was significantly associated with group A patients (*H. pylori* positive) (P=0.01). It came in consistence with Cheng *et al.* [23] and Abdel-Razik *et al.* [24], who found that *H. pylori* infection was related to an increased risk of NAFLD development, through increased markers of IR, inflammatory mediators, and lipid metabolism. NAFLD is a complex disease that is affected by genetic and environmental factors. The incident rate of NAFLD is high, and most of the NAFLD patients have a good prognosis; however, up to 25% of NAFLD patients have H. pylori infection and probably can progress into cirrhosis and liver cancer. Thus, effective treatment regimen for prevention of this progress is extremely urgent. IR is considered to be a crucial part of NAFLD development, and many studies have confirmed that *H. pylori* infection may be a causal factor for IR. The mechanisms of *H. pylori* infection that contribute to NAFLD may include the following: *H. pylori* infection may cause chronic low-grade systemic inflammation, increasing the levels of inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α and leading to IR; *H. pylori* infection may also inhibit white adipose tissue to release leptin, and then promote the accelerating very LDL-cholesterol and fatty deposits in the liver tissue; And owing to the interaction of the stomach and the intestines, *H. pylori* infection may lead to gastrointestinal flora dysbiosis, increasing serum lipopolysaccharides, stimulating systemic inflammation, and causing a decrease in lipoprotein activity followed by dyslipidemia. If we understand the pathogenic role of *H. pylori* infection in NAFLD, it will provide a new direction for NAFLD treatment strategies.

In the present study, all parameters that evaluated the IR such as serum insulin, fasting blood sugar, HbA1c, HOMA-IR, and serum fetuin-A were significantly higher in patients with positive *H. pylori* infection (P<0.001, <0.001, <0.001, <0.001, and <0.001, respectively). This result was in agreement with that reported by Işıktaş Sayılar *et al.* [15] who stated that there was a statistically significant difference between the two groups regarding serum insulin and serum fasting glucose (P<0.01 and <0.05, respectively). Analysis of the results of this study revealed that there was an association between *H. pylori* infection and IR, as HOMA-IR in *H. pylori*-positive cases

ranged between 0.46 and 2.54 with median value of 0.74, whereas it was ranged between 0.2 and 0.55, with median value of 0.44 in *H. pylori*-negative cases, with a high statistically significant difference between the two groups (P=0.001). This result was in agreement with that reported by several studies [15,17,19,21].

Işıktaş Sayılar *et al.* [15] found a high statistically significant difference regarding HOMA-IR between *H. pylori*-positive patients and *H. pylori*-negative patients (P<0.01), as the mean of HOMA-IR in *H. pylori*-positive patients was 2.72±1.39 and in *H. pylori*-negative patients was 2.02±1.17. Haala *et al*. [19] found a statistically significant difference regarding HOMA-IR between *H. pylori*-positive patients and *H. pylori*-negative patients (P<0.05), as the mean of HOMA-IR in *H. pylori*-positive patients was 3.21±1.26 and in *H. pylori*-negative patients was 2.05±1.2. Gen *et al.* [25] also reported that there was a relationship between *H. pylori* infection and IR, as the mean value of HOMA-IR was 3.89 in *H. pylori*-positive cases whereas it was 2.2 in *H. pylori*-negative cases. In addition, HOMA-IR decreased from 3.79 to 2.21 after successful *H. pylori* eradication, whereas it remained unchanged in patients for whom *H. pylori* eradication had failed.

On the contrary, Kachuei *et al*. [20] reported that *H. pylori* eradication by the 2-week antibiotic medication did not decrease IR and even increased fasting plasma insulin and IR indices. The mean of HOMA-IR in *H. pylori*-positive cases was 2.94±1.84 and the mean of HOMA-IR after eradication of *H. pylori* was 2.52 ±1.11. Gao *et al*. [18] stated that the mean value of HOMA-IR was 1.6 in *H. pylori*-positive cases and it was 1.7 in *H. pylori*-negative cases. This may be owing to selection of apparently healthy participants for the study. The primary end point was not the association of *H. pylori* infection with HOMA-IR but with ghrelin and obestatin, so a selection bias incurred given that 100 of 257 participants were finally selected for the study.

This can be explained through there is an association between *H. pylori* infection and IR. The difference between this study and other studies that discovered no association of IR with *H. pylori* infection may be owing to the difference in host factors, ethnicity, and lifestyle. Moreover, it may be owing to selection of diabetic and dyslipidemic patients in the studies. In addition, the diagnosis of *H. pylori* infection was done by serum *H. pylori* immunoglobulin G antibody concentration and not by histopathological examination of antral biopsies, which is considered the actual diagnostic gold standard.

Analysis of the results of this study revealed that there was a highly statistical significant difference between *H. pylori*-positive and *H. pylori*-negative cases regarding fetuin-A, as fetuin-A in *H. pylori*-positive cases ranged between 90 and 383 ng/ml, with median value of 160 ng/ml, whereas it was ranged between 15 and 50 ng/ml, with median value of 30 ng/ml in *H. pylori*-negative cases, with a high statistically significant difference between the two groups (P<0.001). This results was in agreement with that reported by Manolakis *et al.* [26] which revealed that *H. pylori* +ve group showed higher mean±SD levels of fetuin-A than *H. pylori* –ve group, as the mean of fetuin-A in *H. pylori*-positive patients was 0.77±0.03 g/l, whereas in *H. pylori*-negative patients was 0.58 ±0.02 g/l (P<0.01). These results agree with our study owing to using the same methodology (same type of study with same inclusion and exclusion criteria) [27].

On the contrary, Jasim [28] revealed that fetuin-A level was very highly significant lower (P<0.0001) in patients group when compared with healthy participants group (29.53±5.25 vs. 53.45±8.37 ng/ml). This result is opposite to our results, which may be owing to selection of apparently healthy participants for this study and owing to using different methodology. Moreover, Kebapcilar *et al.* [29] revealed that *H. pylori*-infected patients had significantly lower fetuin-A levels (28.7±7.7 vs. 50.1 ±20.9 ng/mL), with P value=0.001, and *H. pylori* eradication was associated with a progressive increase in serum fetuin-A levels (28.7±7.7 vs. 36.8±16.9 ng/m), with P value=0.007. The difference between our study and this study which discovered opposite results may be owing to the difference in host factors, ethnicity, lifestyle, and the time that was used for eradication of *H. pylori*, or evaluation of serum fetuin-A level after the eradication may be not enough for giving accurate results.In the present study, serum fetuin-A had significant positive correlation with serum insulin, HbA1c, and HOMA-IR. This result was in agreement with that reported by Manolakis *et al.* [26] who revealed that there was a positive correlation between fetuin-A and HOMA-IR (r=0.2, P=0.01). So, serum fetuin-A acts as a possible mediator in the biological process linking *H. pylori* infection to the onset of IR. This results was in agreement with that reported by Khalid *et al.* [30]. Regarding the correlations between serum fetuin-A level and other parameters, there was a high statistically significant positive correlation between serum fetuin-A level and HOMA (r=+0.39; P<0.01). ROC curve showed the diagnostic

performance of fetuin-A as a marker of *H. pylori*, as it was found that the best cutoff point of fetuin-A in prediction of positive *H. pylori* infection was 40, and above this point, the sensitivity and specificity were 95 and 75%, respectively; the AUC was 91% with significant performance of this biomarker ($P<0.001$). So serum fetuin-A may represent a promising index for assessing the association between *H. pylori* and IR. There is no previous data about using ROC curve analysis to show the diagnostic performance of fetuin-A as a marker of *H. pylori* infection.

Conclusion

The data from the present study are consistent with that *H. pylori* infection may induce IR. Moreover, the increased IR was accompanied by high serum fetuin-A levels in our population. The coexistence of increased fetuin-A and IR, and also the recorded modulation of the association between *H. pylori* infection and IR by serum fetuin-A, seem to be suggestive for *H. pylori*–IR–fetuin-A interactions which give the ability of fetuin-A to increase IR. So, fetuin-A could, at least in part, be responsible for the altered IR in *H. pylori*-infected individuals.

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Conflicts of interest

There are no conflicts of interest.

References

1 Fock KM, Graham DY, Malfertheiner P. *Helicobacter pylori research: historical insights and future directions*. *Nat Rev Gastroenterol Hepatol* 2013; **10**:495–500.

2 Wong F, Rayner-Hartley E, Byrne MF. Extra intestinal manifestations of *Helicobacter pylori*: a concise review. *World J Gastroenterol* 2014; **20**:11950–11961.

3 Dogan Z, Sarikaya M, Ergul B, Filik L. The effect of *Helicobacter pylori* eradication on insulin resistance and HbA1c level in people with normal glucose levels: a prospective study. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2015; **159**:242–245.

4 Nasif WA, Mukhtar MH, Nour Eldein MM, Ashgar SS. Oxidative DNA damage and oxidized low density lipoprotein in type II diabetes mellitus among patients with *Helicobacter pylori* infection. *Diabetol Metab Syndr* 2016; **8**:34.

5 Huang Y, Huang X, Ding L, Wang P, Peng K, Chen Y, *et al.* Serum fetuin-A associated with fatty liver index, early indicator of nonalcoholic fatty liver disease: a strobe-compliant article. *Medicine (Baltimore)* 2015; **94**:e1517.

6 Tang DM, Kumar S. The association between *Helicobacter pylori* infection and nonalcoholic fatty liver disease. *Curr Gastroenterol Rep* 2017; **19**:5.

7 Cai O, Huang Z, Li M, Zhang C, Xi F, Tan S. Association between *Helicobacter pylori* infection and nonalcoholic fatty liver disease: a single-center clinical study. *Gastroenterol Res Pract* 2018; **2018**:6.

8 Jiang T, Chen X, Xia C, Liu H, Yan H, Wang G, *et al.* Association between *Helicobacter pylori* infection and non-alcoholic fatty liver disease in North Chinese: a cross-sectional study. *Sci Rep* 2019; **9**:4874.

9 Assal AH, Gad MA, El Badawy RM, Emara NM, Soliman MS. The Association between *Helicobacter pylori* infection and insulin resistance. *Int Med J Malay* 2013; **12**:49–52.

10 Deibert P, Lazaro A, Schaffner D, Berg A, Koenig D, Kreisel W, *et al.* Comprehensive lifestyle intervention vs soy protein-based meal regimen in non-alcoholic steatohepatitis. *World J Gastroenterol* 2019; **25**:1116–1131.

11 Park SE, Park CY, Sweeney G. Biomarkers of insulin sensitivity and insulin resistance: past, present and future. *Crit Rev Clin Lab Sci* 2015; **52**:180–190.

12 Haukeland JW, Dahl TB, Yndestad A, Gladhaug IP, Løberg EM, Haaland T, *et al.* Fetuin A in nonalcoholic fatty liver disease: in vivo and in vitro studies. *Eur J Endocrinol* 2012; **166**:503–510.

13 Sato M, Kamada Y, Takeda Y, Kida S, Ohara Y, Fujii H, *et al.* Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects. *Liver Int* 2015; **35**:925–935.

14 Oana S, Mariana F, Petru D, Victorita S, Laurentiu S. Non-alcoholic fatty liver disease.*From the cardiologist perspective*. *Anatol J Cardiol* 2016; **16**:534–541.

15 Işıktaş Sayılar E, Çelik B, Dumlu . Relationship between *Helicobacter pylori* infection and metabolic syndrome. *Turk J Gastroenterol* 2015; **26**:468–473.

16 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 1985; **28**:412–419.

17 Aydemir S, Bayraktaroglu T, Sert M, Sokmen C, Atmaca H, Mungan G, *et al.* The effect of *Helicobacter pylori* on insulin resistance. *Dig Dis Sci* 2005; **50**:2090–2093.

18 Gao XY, Kuang HY, Liu XM, Duan P, Yang Y, Ma ZB. Circulating ghrelin? obestatin ratio in subjects with Helicobacter pylori infection. *Nutrition* 2009 **25**:506–511.

19 Haala M, Mohammed E, Elmutuz H, Hani K, Nour M, Sharif A. Effect of *Helicobacter pylori* infection on insulin resistance in asymptomatic Sudanese patients. *Pharma Innov J* 2015; **3**:68–71.

20 Kachuei A, Amini M, Sebghatollahi V, Feizi A, Hamedani P, Iraj B. Effect of *Helicobacter pylori* eradication on insulin resistance among prediabetic patients: a pilot study and single-blind randomized controlled clinical trial. *J Res Med Sci* 2016; **21**:8.

21 Ozdem S, Akcam M, Yilmaz A, Artan R. Insulin resistance in children with *Helicobacter pylori* infection. *J Endocrinol Invest* 2007; **30**:236–240.

22 Aslan M, Horoz M, Nazligul Y, Bolukbas C, Bolukbas FF, Selek S, *et al.* Insulin resistance in *H. pylori* infection and its association with oxidative stress. *World J Gastroenterol* 2006; **12**:6865–6868.

23 Cheng DD, He C, Ai HH, Huang Y, Lu NH. The possible role of Helicobacter pylori infection in non-alcoholic fatty liver disease. *Front Microbiol* 2017; **8**:743.

24 Abdel-Razik A, Mousa N, Shabana W, Refaey M, Elhelaly R, Elzehery R, *et al. Helicobacter pylori and non-alcoholic fatty liver disease: a new enigma? Helicobacter* 2018; **23**:e12537.

25 Gen R, Demir M, Ataseven H. Effect of *Helicobacter pylori* eradication on insulin resistance, serum lipids and low-grade inflammation. *South Med J* 2010; **103**:190–196.

26 Manolakis AC, Tiaka EK, Kapsoritakis AN, Georgoulias P, Tsiopoulos F, Valotassiou V, *et al.* Increased fetuin A levels in *Helicobacter pylori* infection: a missing link between *H. pylori* and insulin resistance? *Diabetologia* 2011; **54**:472–474.

27 Zhou BG, Yang HJ, Xu W, Wang K, Guo P, Ai YW. Association between Helicobacter pylori infection and nonalcoholic fatty liver disease: a systematic review and meta-analysis of observational studies. *Helicobacter* 2019; **24**:e12576.

28 Jasim TM. Evaluation of novel immunological mediator in patients with *Helicobacter pylori* in Baghdad City, Iraq. *Int J Curr Microbiol App Sci* 2016; **5**:1–9.

29 Kebapcilar L, Bilgir O, Cetinkaya E, Akyol M, Bilgir F, Bozkaya G. The effect of *Helicobacter pylori* eradication on macrophage migration inhibitory factor,*C-reactive protein and fetuin-a levels*. *Clinics (Sao Paulo)* 2010; **65**:799–802.

30 Khalid M, Hussein G, Ghanem AI, Omar GA. Association of serum fetuin-A with insulin resistance in type 2 diabetic patients. *Med J Cairo Univ* 2013; **81**:1067–1071.