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Evaluation of Iron Status and Helicobacter Pylori in Iraqi Patients with Type 2 Diabetes Mellitus

Biomedical

KEYWORDS : Helicobacter pylori infection; type 2 diabetes; iron statu

Dr. Fatin Fadil AL-Kazazz

Assistant Professor in clinical biochemistry, Chemistry Department, College of Science, Al-Mustansirya University, Iraq

ABSTRACT

Objective: The aim of the current study was to assess the iron status and the prevalence rate of H. pylori infection in Iraqi Patients with type 2 diabetes.

Methods: The author enrolled 60 type 2 diabetic patients T2DM age (35-75) years, and 30 healthy volunteers served as a control group. Groups were matched for gender and age, and had similar geographical origin and socioeconomic status

The status of iron was evaluated through determination iron levels (Fe) and total iron binding capacity (TIBC) was measured spectrophotometrically, while serum ferritin was measured using ELISA technique. Unsaturated iron-binding capacity (UIBC), transferrrin saturation percentage (TS %), transferrin concentration and estimated total iron body stores (ETIBS) were calculated mathematically.

Results: It was found that (51.42%) of T2DM patients were infected with H. pylori with a highly significant increase in FBG or HbA1c levels (P<0.001). Results of serum (Fe) and ferritin levels were decreased and the differences in TIBC, UIBC, Transferrin and ETIBS values were non-significant (p > 0.05) in both infected and non-infected T2DM patients .

Conclusion: The presence of H. pylori infection is one of the risk factor that must be considered in evaluation of iron status in diabetic patients.

INTRODUCTION

Iron is an essential micronutrient, as it is required for satisfactory erythropoietin function, oxidative metabolism and cellular immune response (1). Iron is a component of several metalloproteinase and plays a crucial role in vital biochemical activities, such as oxygen sensing and transport, electron transfer, and catalysis, thus indispensable for life. Iron deficiency, defined as decreased total body iron content variety of causes such as inadequate iron intake, chronic blood loss, chronic disease, malabsorption, hemolysis or a combination of these, can induce IDA (2, 3, 4), and is among the most common nutritional deficiencies in the world. It develops through three stages: first stage (pre-latent iron deficiency) may be "diagnostically silent" as laboratory parameters still remain within normal limits. Second stage (latent iron deficiency) decreased serum ferritin, low serum iron, high serum transferrin (and consequent low iron saturation of transferrin), and increased plasma levels of soluble transferrin receptor. In the third stage (iron deficiency anemia), hemoglobin concentration (Hb) decreased (5). Continuous shortage of iron may profoundly affect production of Hb while the impact on muscular myoglobin is less pronounced (6, 1). Iron-deficiency anemia (IDA), generally attributed to abnormal blood loss, is often caused by gastrointestinal bleeding due to peptic ulcer and chronic gastritis (7, 8).

Iron deficiency anemia is a common tropical disease. Iron plays a very important role in the human body. Serum iron and a total iron-binding capacity (TIBC), or sometimes an unsaturated iron-binding capacity (UIBC) or transferrin test are ordered together, and transferrin saturation is calculated to determine how much iron is being carried in the blood. A ferritin test may also be ordered to evaluate a person's current iron stores. Plasma ferritin is an acute phase protein (9).

Transferrin is the iron transport protein in plasma. It is responsible for shuttling iron between sites of absorption, storage and utilization for the biosynthesis of iron-containing macromolecules (10).

Several seroepidemiological studies have suggested a link between IDA and H. pylori infection. Barabino hypothesized that gastritis increased levels of neutrophil-derived lactoferrin, and since H. pylori has a lactoferrin-binding protein receptor, the infection would result in increased iron losses related to bacterial turnover (11).

Some patients with refractory IDA have no gastrointestinal symptoms but H. pylori gastritis, as the only cause of their anemia (12).

Most dietary iron is in the non-hemic ferric form, and an acidic intra gastric pH is needed to reduce it to the ferrous form for absorption (13, 14).

This reaction is promoted by gastric acidity and ascorbic acid, which is thus considered the most potent regulator of iron absorption patients with H. pylori gastritis showed an increase in intra gastric pH with a median of > 3, a pH that is known to be critical in the process of iron absorption (15, 16, 17).

The blood loss in chronic gastritis and bleeding from duodenal or gastric ulcers related to H. pylori infection (18) plays an important role in the development of iron deficiency. In response to H. pylori chronic gastric inflammation, the epithelial cells in the mucosa are damaged, leading to detachment and apoptosis. In the absence of bleeding lesions, the possible mechanisms by which H. pylori is involved in the development of IDA remain unclear (19).

The aim of this study was to diminish the iron status and prevalence of H. pylori infection among Iraqi T2DM patients.

MATERIALS AND METHODS

Sample collections

Five milliliters (5 ml) venous blood was obtained after a 12 hour fasting period. 2 ml of this blood was transferred to EDTA tube for estimation of HA1C (A1C), the rest of the blood transferred to polyethylene plain tube. Serum sample was obtained by centrifugation at 3000 rpm for 5 min. Hemolysed samples were discarded and the sera were stored and frozen at about -8°C until analysis.

Measurements

Fasting serum glucose FBG concentrations had been measured by enzymatic oxidation in the presence of glucose oxidase by (GOD-PAP) method (20), patients with fasting blood sugar more than 126 mg/dl were selected and considered diabetic according to the American Diabetes Association criteria. Percentage of glycol hemoglobin (HA1C) test was done by fast ion-exchange Resin separation methods (21).

The presence of H. pylori were diagnosed by using a rapid, one step test, a simple test that utilizes a combination of H. pylori antigen coated particles and anti-human IgG to qualitatively and selectivity detect H. pylori antibodies in serum (22).

Concentration of H. pylori levels (anti-H. pylori IgG antibody) were determined through the use ELISA kit (Demeditec Diagnostics GmbH Germany Kit) (23).

The status of iron was evaluated through determination iron levels (Fe), and total iron binding capacity (TIBC) was measured spectrophotometrically in the sera of patients and control groups, while serum ferritin was measured using ELISA technique. Unsaturated iron-binding capacity (UIBC), transferrrin saturation percentage (TS%), transferrin concentration and estimated total iron body stores (ETIBS) were calculated mathematically.

Statistical analysis

The data collected were analyzed using statistical package for social science (SPSS). Percentage prevalence rates were calculated with their respective 95% confidence intervals. Differences between proportions were evaluated using the T- tests. Statistical significance were achieved at ($p < 0.05$).

RESULTS AND CONCLUSION

Levels for each H. pylori, F.B.G, HbA1C% were illustrated as mean ± SD in Table 1 with their P–values between control and T2DM patients groups.

Table 1: Mean ± SD for each H. pylori, FBG, HbA1C% in control and patients groups.

		H. pylori (U/mol) Mean ± SD	FBG µmol/l Mean ± SD	HbA1C % Mean ± SD
Control	H. pylori (+ve)	64.46 ± 38.9	5.36 ± 1.33	5.15 ± 0.5
	H. pylori (-ve)	2.61 ± 0.81	3.8 ± 0.54	4.79 ± 0.50
Patient	H. pylori (+ve)	63.33 ± 35.4	13.84 ± 6.0	9.75 ± 2.23
	H. pylori (-ve)	3.18 ± 1.4	14.39 ± 5.65	9.01 ± 2.0
P-value patients &control HP(+ve		0 .00I	0.001	0.001

There was a highly statistically significance in overall prevalence of H. pylori in patients and controls ($p < 0.001$) Recent reports suggested that Helicobacter pylori (H. pylori) might have high prevalence among patients with diabetes. Test of the equality of means between H. pylori-positive type 2 DM patients and H. pylori negative according to FBG and HbA1C, shows highly statistically significant in the means of both groups ($p < 0.001$) In this study presence of H. pylori bacteria is associated with elevated levels of glycosylated hemoglobin (HbA1c)an important biomarker for blood glucose levels in diabetes. Study reported highly statistically significance between T2DM patients and non-diabetics (control) regarding to HA1C and FBG that infected H. pylori ($P < 0.001$). Table 3 shows mean ± SD for each S.Fe, Ferritin and Transferrin saturation (TS%).

Table 2: Mean ± SD and P-values for iron status (S.Fe, Ferritin and Transferrin saturation).

Groups	H. pylori	S.Fe (µmol/l)		Ferritin (ng/ml)		Transferrin saturation (TS%)	
		Mean ± SD	SE	Mean ± SD	SE	Mean ± SD	SE
Control	H. pylori (+ve)	17.06 ± 7.7	1.94	84.54 ± 37.13	9.28	21.82 ± 11.0	2.75
	H. pylori (-ve)	14.05 ± 5.42	1.44	72.40 ± 28.55	7.9	20.74 ± 11.0	0.29
T2DM	H. pylori (+ve)	15.57 ± 5.6	0.9	74.40 ± 59.80	10.1	22.84 ± 12.18	2.1
	H. pylori (-ve)	13.13 ± 4.8	1.3	66.78 ± 36.72	7.2	13.13 ± 4.8	1.3
P-value : patientsHP(+ve) &control HP(+ve)		0.31		0.31		0.71	

It is important to mention here that there are other four parameters (TIBC, UIBC, Transferrin and ETIBS) that should be calculated to cover the study on Iron status. These parameters are listed in Tables 2&3,they also show the multiple comparisons significant which have been done by T-test analysis between the different groups.

Table 3: Mean ± SD and P-values for each TIBC, UIBC, Transferrin and ETIBS.

Groups	H. pylori	TIBC (µmol/l)		UIBC (µmol/l)		Transferrin (g/l)		ETIBS (µmol/l)	
		Mean ± SD	SE	Mean ± SD	SE	Mean ± SD	SE	Mean ± SD	SE
Control	H. pylori (+ve)	84 ± 13.7	5.6	67.3 ± 14.4	7.7	3.3 ± 0.9	0.3	11.9 ± 5.3	1.3
	H. pylori (-ve)	84.0 ± 22.1	11.1	70.0 ± 20.2	6.1	3.3 ± 1.6	0.4	10.0 ± 4.4	1.1
T2DM	H. pylori (+ve)	72.3 ± 14.0	4.1	57.8 ± 16.1	4.5	2.8 ± 0.9	0.1	10.6 ± 8.5	1.3
	H. pylori (-ve)	64.2 ± 19	3.8	51.1 ± 19	3.9	2.5 ± 0.7	0.2	10.0 ± 4.8	1.1
P-value : patientsHP(+ve) &control HP(+ve)		0.30		0.41		0.73		0.31	

Results of serum iron levels in Tables 2 and 3 decreased non-significantly ($p > 0.05$) in both infected and non-infected T2DM patients as compared with control group. The results are consistent with M. R. KERAMATİ et al who found no significant difference among patients referred with the upper and lower gastrointestinal tract infected H. pylori (24). Thankachan P et al (2010) reported that the presence of H. pylori infection in 543 Indian children, aged 6-10 years, did not affect the efficacy of long-term iron and vitamin B12 for the infection (25). Other studies observed that iron levels were significantly lower than the normal ranges in H. pylori infected patients than in anti-H. pylori-IgG seronegative control group (26). Baysoy et al. had investigated H. pylori related-changes in gastric physiology and histology in children. So, it is associated with low serum iron levels and with a decrease in gastric juice ascorbic acid concentration (27, 28).

Table 2 appears clearly that serum ferritin decreased non-significantly ($p > 0.05$) for T2DM patient compared to control groups for both infected and non- infected with H. pylori. Similar results have been reported previously by Henry C et al who observed that active *H. pylori* infection by UBT was independently associated with child's age ≥10 years and iron deficiency anemia among children is defined solely on the basis of ferritin levels (29, 30). Furthermore and in comparison with other studies by Xin-Hua Qu et al (31) and ZHANG Zhi-feng et al (32). They suggested that H. pylori infection may play some roles in the development of ID. They concluded that H. pylori infection may not be the only cause of iron deficiency in their subjects; chronic blood loss and poor intake iron rich food in their subjects could have played a role in the causation of iron deficiency anemia.

Results in Table 3 shows that the differences in TIBC, UIBC, Transferrin and ETIBS values between infected and non-infected T2DM patients and control groups were non-significant ($p > 0.05$). The presence of H. pylori infection would cause iron defiance as a beginning for anemia in T2DM patients under this study.

Iron deficiency anemia is the most common cause of nutritional anemia throughout the world. The prevalence of iron defi-

ciency anemia in developing countries has been reported to be in more than 50% of the population (33, 34), which is mainly due to poor nutrition. Numerous studies have shown that iron deficiency anemia is associated with cognitive disorders, poor learning ability and lack of concentration, poor memory, educational failure, and behavioral or physical problems. One of the factors affecting iron deficiency anemia is H. pylori infection, which has a high prevalence in developing countries and some believe that resistant iron deficiency anemia.

In the current study, we did not find any association between H. pylori infection and S.Fe, serum ferritin TIBC, UIBC, Transferrin and ETIBS levels. Indeed, there was no significant correlation between H. pylori infection and patients with iron deficiency among T2DM patient and control group. The biologic mechanism by which H. pylori induces the alteration in the iron stores is not fully understood, but it seems to involve several pathways, including gastrointestinal blood loss, decrease in the absorption of dietary iron, and enhanced uptake of the iron by the bacterium. Iron deficiency anemia (IDA) is often caused by gastrointestinal bleeding due to peptic ulcer and chronic gastritis. H. pylori infection, which has been proved to play the main role in peptic ulcer and chronic gastritis, has been implicated as a cause of IDA refractory to oral iron treatment. In the absence of bleeding lesions, the possible mechanisms by which H. pylori is involved in the development of IDA remain unclear (31). In fact, the observed contrasts between different studies are expected since similar condition have been applied between different studies, and could also be attributed to the fact that our subject had any gastrointestinal bleeding.

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