European Review for Medical and Pharmacological Sciences 2015; 19: 4835-4840

Evaluation of serum levels of zinc, copper, and *Helicobacter pylori* IgG and IgA in iron deficiency anemia cases

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Abstract. – OBJECTIVE: Iron deficiency anemia (IDA) is the most common form of anemia. Impaired intake absorption and blood loss are the main factors in the etiology. Impaired absorption can be caused by a decrease in trace elements such as copper and zinc, which are found in the structure of enzymes that coordinate iron metabolism or act as a catalyst for them, and the existence of *Helicobacter pylori* (*H. pylori*), which inhibits iron absorption in the stomach. Serum levels of zinc, copper, and *H. pylori* antibodies were measured in IDA cases, and correlations with IDA were evaluated.

PATIENTS AND METHODS: The study group was composed of 115 IDA cases who were followed at hematology outpatient clinics, and the control group was composed of 92 gender- and age-matched healthy individuals. Patients were diagnosed with iron deficiency anemia according to hemoglobin, serum ferritin, and iron levels and total iron-binding capacity. Serum zinc, copper, *H. pylori* immunoglobulin A (HpIgA) and immunoglobulin G (HpIgG), vitamin B12, and folic acid levels were examined in the blood specimens collected.

RESULTS: No statistically significant difference in zinc and copper serum levels between the study and control groups was observed ($p > 0.05$ for both groups). Although no difference was observed between the HpIgG levels of the two groups, patients with IDA had a statistically significant increase in HpIgA levels ($p < 0.05$). Pearson's correlation analysis showed that the zinc levels of the IDA group did not have a correlation with any parameters ($p < 0.05$ for all). Copper levels had a positive correlation with only the HpIgA level in the IDA group ($r = 0.222$, $p = 0.017$).

CONCLUSIONS: Trace elements and *H. pylori* infection did not have a correlation with IDA. Elevated levels of HpIgA and positive correlation of HpIgA with copper levels were observed. The literature review clearly suggests that several points require further explanation, and extensive research with larger samples is required.

Key Words:
Copper, Zinc, Iron deficiency anemia, *Helicobacter pylori*.

Introduction

Iron deficiency anemia (IDA) is the most common type of anemia globally and poses a serious risk to public health particularly in developing countries. Although iron, B_{12}, and folic acid deficiencies have been clearly shown to be the most common causes of anemia today, the mechanisms of rare deficiencies of trace elements such as copper, zinc and vitamins as well as chronic infections such as *Helicobacter pylori* (*H. pylori*) in the development of anemia remain controversial. Copper is found in the structure of many primary enzymes that act on iron metabolism and in the structure of ceruloplasmin, which ensures intestinal absorption of iron and its mobilization from tissues to plasma with ferroxidase activity. Another element, zinc, is found in the structure of metalloproteins and more than 300 types of enzymes. Zinc functions as the catalyst in iron metabolism in the activity of alpha-aminolevulinic acid dehydratase enzyme, which has a role in heme synthesis. Zinc is found in the structure of the Gfi-1B zinc finger protein, which acts as a major regulator in erythroid cell growth by modulating gene expression specific to erythroid series, performs transcriptional regulation during erythropoiesis, supports proliferation of immature erythroblasts and provides normal erythro-

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poiesis by taking a potential role in the serial development of hematopoietic stem cells and megakaryocytes[1-6]. However, the exact role of *H. pylori* in iron deficiency, currently a common topic in research, has not been determined. The *cag* (cytotoxin-associated gene) pathogenicity island (*cag* PAI) is an important determinant of pathogenicity expressed by approximately 60-70% of *H. pylori* strains present in Western countries and virtually 100% of strains in East Asian countries. *H. pylori* strains that express the *cag* PAI (*cag* PAI$^+$) significantly increase the risk of severe gastritis, atrophy, dysplasia, and gastric adenocarcinoma compared to strains that lack the *cag* PAI (*cag* PAI$^-$)[7,8]. *Helicobacter pylori* increases gastric pH by reducing gastric acid secretion, which results in impaired iron absorption. In another hypothesis *H. pylori* contributes to iron deficiency anemia by competing with the host for iron and directly using iron for its own development in addition to reducing levels of vitamin C, another factor in improving iron absorption. Several studies[9-12] showed a correlation between *H. pylori* and iron deficiency anemia and researchers reported that the serum hemoglobin and ferritin levels increased as a result of eradication treatment. These studies did not investigate the difference between iron and *H. pylori* immunoglobulin A (HpIgA) and immunoglobulin G (HpIgG); therefore, their roles in iron deficiency anemia have not been clearly revealed.

Most studies investigating the levels of trace elements and the presence of *H. pylori* in the development of iron deficiency anemia have been performed in children and information in adults is lacking. In our study, we aimed to evaluate the association of these factors with IDA in an adult population to shed light on their role in etiology.

Patients and Methods

Patients

This cross-sectional study was conducted with 115 patients with IDA who were followed at hematology outpatient clinics and 92 age- and gender-matched healthy controls. Serum levels of zinc, copper, and *H. pylori* IgG and IgA were compared.

Patients were diagnosed with iron deficiency depending on the hemoglobin, serum ferritin and iron levels and total iron-binding capacity. Folic acid and vitamin B_{12} levels were analyzed in all patients to exclude co-existing deficiencies of vitamin B_{12} and folic acid. Etiologic investigation such as gynecologic examination, urologic examination, gastroscopy and/or colonoscopy were performed in certain patients whenever necessary. Patients with malignancy, chronic disease, dimorphic anemia, or acute infection were excluded from the study. Venous blood specimens of 5 cc from each individual were taken in blood collection tubes. Complete blood count was performed with the Roche Sysmex Xt-2000i analyzer. Ferritin was measured with the Elecsys 2010 (Roche Diagnostics, Istanbul, Turkey) using a Roche Diagnostics kit through the electrochemiluminescence immunoassay (ECLIA) method. Serum levels of iron were measured with the colorimetric method with a Roche Modular Analyzer. Total iron binding capacity was measured with the Roche Modular Analyzer. Standard solutions of trichloroacetic acid, copper and zinc used for serum copper and zinc analyses were obtained from Merck Co (Readington Township, NJ, USA). Serum specimens were analyzed based on supernatant using trichloroacetic acid (TCA) precipitation without diluting. The Philips PO 9100 X Flame Atomic Absorption Spectrophotometer was used for the analysis. HpIgA and HpIgG analyses were conducted with the ELISA method (Phoenix Pharmaceuticals, Burlingame, CA, USA).

Statistical Analysis

SPSS 17 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Compliance of data with normal distribution was evaluated with the one-sample Kolmogorov-Smirnov test. Results were presented as mean ± standard deviation in parametric data and as the minimum, maximum, and median in non-parametric data. The chi-square test for categorical data and independent sample *t*- and Mann-Whitney U tests for parametric and non-parametric data comparison between groups were used. Pearson correlation analysis was carried out for correlative parameters in patients with iron deficiency. Values below $p < 0.05$ were considered statistically significant.

Results

The demographic characteristics of and biochemical findings for the patients are given in Table I. There was no significant difference between the groups in terms of age or gender ($p >$

Table I. Demographic characteristics of and biochemical findings for the participants.

	Patients with IDA (n = 115)	Healthy controls (n = 92)	*p* value
Age (years)	36.30 ± 13.27	39.05 ± 12.44	0.127
Gender (F/M)	109/6	83/9	0.282
Hemoglobin (g/dl)	10.30 (6.10-11.90)	13.50 (12-17.20)	< 0.001
MCV (fL)	72 (49-92.50)	86.70 (59-818)	< 0.001
WBC (/μl)	6.33 ± 2.00	7.01 ± 2.47	0.034
PLT (/μl)	288 (24-760)	241 (49-722)	< 0.001
Ferritin (μg/l)	4.40 (1.04-37)	38.4 (12.5-190)	< 0.001
B_{12} (pg/ml)	299 (93-862)	371.5 (154-1704)	< 0.001
Folic acid (ng/dl)	9.60 (4.30-20)	9.15 (4.20-126)	0.759
Zinc (mg/dl)	83.0 ± 14.76	83.97 ± 18.97	0.685
Copper (mg/dl)	111.6 ± 28.16	109.75 ± 22.90	0.598
HpIgA (U/ml)	35 (2.09-219)	23.1 (0.6-231)	0.003
HpIgG (U/ml)	67.40 (0.8-3386)	56.5 (0.6-5863)	0.578
Iron (μg/dl)	25 (6-284)	72.5 (13-189)	< 0.001
TIBC (μg/dl)	477.12 ± 60.96	386.30 ± 67.00	< 0.001

0.05). There was a statistically significant difference between the groups in terms of Hb, mean corpuscular volume (MCV), serum iron, total iron binding capacity, and ferritin. However, there was no statistically significant difference between the two groups in the zinc and copper serum levels ($p > 0.05$ for both groups; Figures 1 and 2). Although no difference was observed between the groups in terms of HpIgG levels, the patients with iron deficiency anemia had statistically significant increased HpIgA levels ($p < 0.05$). The Pearson's correlation analysis showed that zinc levels of the patients with IDA did not have a correlation with any parameters ($p < 0.05$ for all). Copper levels had a positive correlation with only IgA levels in the patients with IDA ($r = 0.222$, $p = 0.017$; Figure 3).

Discussion

Iron deficiency is a nutritional deficiency that affects approximately 2 billion people worldwide. Anemia, which is caused by this nutritional iron deficiency, is mainly observed in children during the developmental period and pregnant women. Iron deficiency anemia can co-exist with deficiencies of other trace elements such as copper and zinc, which is more frequently encountered in developing countries. Iron and zinc have

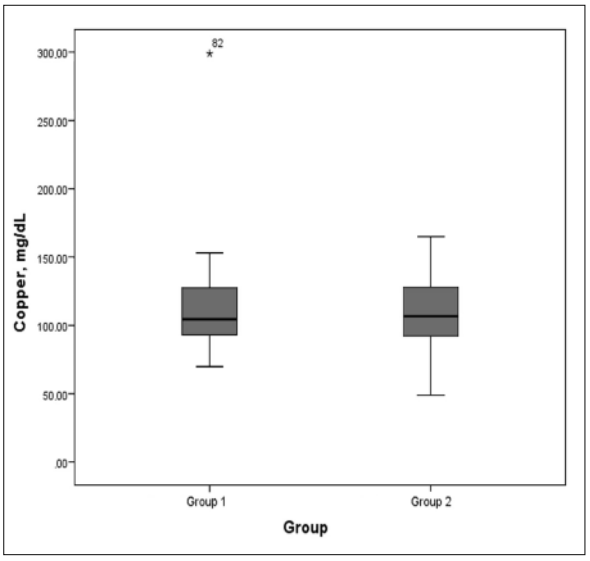

Figure 1. Serum copper levels in IDA and control groups.

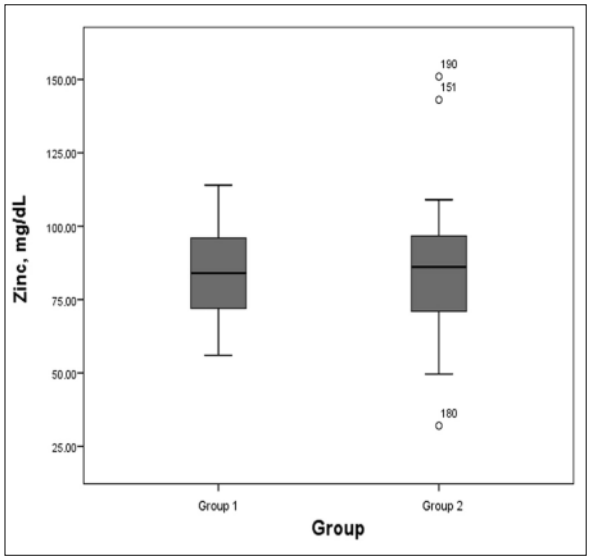

Figure 2. Serum zinc levels in IDA and control groups.

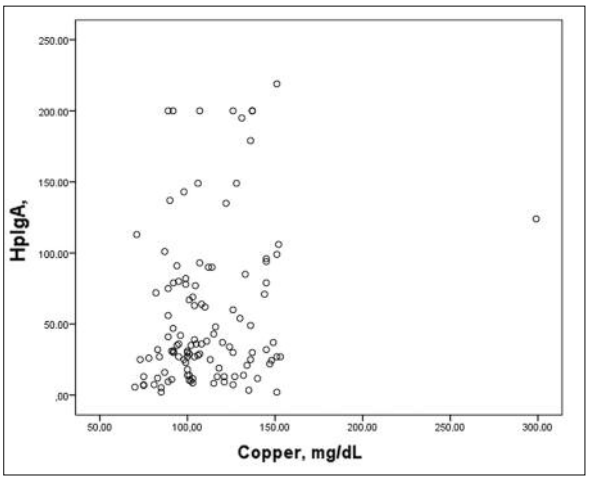

Figure 3. Correlation of serum copper and HpIgA levels in IDA group (r = 0.222, p = 0.017).

active roles in absorption, transport, and heme structure and exhibit competitive inhibition in transport and bio-efficiency via important interactions with each other[14]. Several studies[15,16] conducted mainly in children have been reported in the literature. Turgut et al[15] analyzed serum levels of iron, copper and zinc in a total of 256 children with a mean age of 6, and found that the IDA group had indifferent zinc levels (IDA 1.37 ± 0.16 mg/l, control 1.68 ± 0.08 mg/l) while copper was significantly elevated in the IDA group. Ece et al[16] compared the zinc and copper serum levels in 60 children with IDA and 64 healthy children, ages 1 to 14. The researchers observed that zinc was significantly decreased while copper was significantly increased in the IDA group (zinc 109 ± 59 mg/dl, 135 ± 56 mg/dl; copper 189 ± 49 mg/dl, 163 ± 37 mg/dl). Other studies[13,17,18] conducted in children found decreased levels of zinc and copper in IDA cases. The number of studies conducted in adults is limited compared to children. The study performed by Van Nhien et al[19] with 123 adult cases found IDA in 30% of the patients, yet no significant difference in zinc and copper levels between two groups was observed. We also did not find a significant difference in zinc and copper levels between the IDA group and the healthy controls group. Since iron and microelements are consumed faster in higher amounts during the rapid growth process of childhood, this status observed during the developmental period seems normal. However, in adults, this status can more commonly manifest due to nutrition deficiencies.

Similar to IDA, *H. pylori* infection is another condition commonly observed in the Turkish population. The effects of *H. pylori* infection on several extra-gastric systems have been shown in several studies[20]. In particular, the correlation between *H. pylori* with iron deficiency anemia and immune thrombocytopenic purpura has been analyzed. One proposed hypothesis suggests that *H. pylori* decreases the levels of gastric acid and ascorbic acid in the stomach, leading to reduced iron absorption, directly binds iron to iron-binding protein and uses it for its own development, resulting in IDA[21]. The literature includes studies with controversial results regarding the relationship between *H. pylori* and IDA. In Turkey similar studies were performed mainly among children. Kürekci et al[22] performed *H. pylori* screening with the urea breath test and antibody testing in stool in 140 children aged between 6 and 16, which showed that hemoglobin and ferritin increased only with *H. pylori* eradication even without iron replacement therapy. The studies that supported a positive correlation between *H. pylori* and IDA indicated *H. pylori* was an etiologic factor in patients with IDA who were refractory to iron therapy or had an undetermined cause. However, studies that do not suggest such a correlation also exist[23-29]. In the current study, we did not detect a correlation between IDA and *H. pylori*. According to the latest Maastricht IV report[30], *H. pylori* eradication therapy is recommended in patients who are positive for *H. pylori* with IDA after other anemia-causing factors have been excluded.

For a long time, the urea breath test has been the first recommended test for diagnosis of *H. pylori* infection, followed by antibody testing in stool. Serological tests are third. American and European guidelines recommend investigating IgG type antibodies in serology. In the literature, the role of serum IgG in diagnosis has been well described. However, fewer data have been found regarding IgA type antibodies[31-36]. Studies have shown that serum IgA levels are elevated in *H. pylori* infections with severe and advanced destruction such as gastric cancer and duodenal ulcer, and even though the sensitivity is high similar to IgG, the specificity is lower. In this study, the HpIgA levels were significantly elevated. HpIgA can possibly affect iron absorption as a mucosal immunoglobulin. In addition, the increase in copper levels accompanied by HpIgA in the IDA group suggests that copper might be elevated in response to the infection and inflam-

mation process of *H. pylori*, which agrees with the literature. In accordance with our study, Janjetic et al[37] conducted a study in children and found no correlation between IDA and zinc but observed elevated levels of copper in *H. pylori*-positive patients.

Conclusions

No correlation between IDA and trace elements and *H. pylori* was found in the current study. *Helicobacter pylori* IgA levels were significantly higher in the IDA group, and the copper levels were also positively correlated with the HpIgA levels. Compared to studies that involved children, a limited number of studies have been performed regarding the association of IDA and *H. pylori* in adults. Extensive research through studies conducted with larger patient groups is required.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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