Detection Zinc and Copper Serum in Helicobacter Pylori-Infected Patients

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Abstract

Background and Objectives: Helicobacter pylori (Hp) colonizes the human gastric and duodenal mucosa and the infection may cause peptic ulcers and gastric adenocarcinoma. Most study appear that (HP) infection effect of different micronutration zinc and copper. The purpose of the study was to compare serum concentrations of trace elements (zinc [Zn], copper [Cu]) of Helicobacter Pylori-Infected patients with those of healthy subjects and to determine the relationships between trace elements.

Materials and Methods: The study was conducted in 45 Helicobacter pylori (Hp) infection patients and 20 age- and gender-matched healthy volunteers. Flame atomic absorption spectroscopy method was employed to analyze serum trace element concentrations zinc and copper.

Results: Compared to the control subjects, 45 Helicobacter pylori (Hp) infection patients had significantly (P < 0.05) independent sample t-test depicted that serum concentrations of Zn were decreased while serum concentration of Cu increased significantly in the Helicobacter pylori (Hp) patient group. The concentrations of Zn, Cu, and were 92.8 mg/L and 132.2 µg/L, respectively, in Helicobacter pylori (Hp) infection patients, and 142.3 mg/L and 84.2 mg/L respectively, in the healthy controls.

Conclusion: Helicobacter pylori (Hp) infection associated with metabolism zinc and copper. Serum concentrations of Zn were decreased, but serum concentration of Cu increased significantly in Gastritis Helicobacter pylori patient groups.

Keywords: Gastritis Helicobacter pylori, serum zinc (Zn), serum copper (Cu).

INTRODUCTION

Infection with Helicobacter pylori (H. pylori) is one of the most common bacterial infections. This organism causes chronic gastritis, gastric and duodenal ulcers and could be the cause of some neoplasm (gastric cancer and MALT lymphoma).1,2

Trace minerals and vitamins are essential for life. They act as essential cofactors of enzymes and as organizers of the molecular structures of the cell. Deficiencies of micronutrients influence immunohomeostasis and thus affect infection-related morbidity and mortality.3 Micronutrients copper and others are powerful antioxidants and have a significant impact on infection-related morbidity in humans. Subclinical deficiencies are known to impair biological and immune functions in the host.4 Helicobacter pylori can change the secretion and acidification functions of stomach, because it penetrates especially into the stomach. This situation can affect digestion and absorption of some components of the nutrients and micronutrients. Although nutrient absorption does not take place in the stomach, this organ contributes to the process by means of secretion of hydrochloric acid and several enzymes. These substances help partly release the micronutrients from the food matrix, but also, in the case of the essential minerals, render them soluble during the digestive process. In the last few years, a number of studies have suggested that HP infection may affect the homeostasis of different micronutrients.

Materials and Methods

Patients

We studied 45 volunteer patients of both genders with gastric disease. Mean patient age was 59.2 ± 11.36 years (range: 19-87 years). Exclusion criteria were as follows: previous gastroduodenal surgery, prior treatment with antimicrobial therapy to eradicate the microorganism, use of NSAIDs and/or proton pump inhibitors in the last three months, and being a smoker, user of therapeutic drugs in the last 30 days.

Control

A total of 40 apparently healthy subjects were involved as controls group. The age range of controls was matched to the patients (30 – 60) years.

Blood samples

A 5-mL venous blood sample was collected from the antecubital vein of each of the lung cancer patients and healthy volunteers in a metal-free sterile tube, between 8 to 9 AM, and after an overnight fasting. Standard precautions for trace element determination were taken; samples with signs of hemolysis were discarded. The blood was then allowed to clot and centrifuged for 15 minutes at 3000 rpm to extract the serum. The serum was aliquoted into eppendorf tubes and stored at -80°C for analysis of trace elements.
Determination of Serum Zinc by Using Zinc kit.
This study was part of a larger study that evaluated the impact of maternal trace element (copper and zinc). The level of serum zinc was determined according to the instructions manual of (LTA):

**Principle**
Serum zinc reacts with chromogen present in the reagent forming a colored compound, which color intensity that is proportional to the zinc concentration present in the sample.

**Reagents**
- **Reagent A:** borate buffer 0.37 M, pH 8.2; salicylaldoxime 12.5 mM; dimetilgioxime 1.25 mM; surfactants and preservatives.
- **Reagent B:** NITRO-PAPS; 0.4 mM, preservatives.
- **Standard:** Zinc ion 200 µg/dl (30.6 µmol/l); stabilizers and preservatives.

**Assay Procedure for Detection of Serum Zinc**
Three sets of tubes were prepared as follows:
Mix and read the absorbance against blank at 578nm. Color is stable for 30 minutes.

**Calculation:**

\[
\text{Zn µg/dl} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 200
\]

Standard absorbance was (0.152) (5).

2- Determination of Serum Copper level by commercial kit.
The level of serum zinc was determined according to the instructions manual of (LTA): **Principle** The cupric ions react with the chromogen Di-Br-PAESA forming a blue complex; its intensity is proportional to the copper concentration present in the sample.

**Reagents**
- **Reagent A:** acetate buffer 0.1M, pH 4.9; reducing agents and preservatives.
- **Reagent B:** 3,5Di Br-PAESA
- **Standard:** ion copper 200 µg/dl; preservatives.

**Assay Procedure for Detection of Serum Copper**
Three sets of tubes were prepared as follows:
Mix and wait for 10 minutes then read the absorbance against the blank at 580nm. The color is stable for 30 minutes.

**Calculation**

\[
\text{Cu µg/dl} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 200
\]

Standard absorbance was (0.296)

**Statistical Analysis**
T-test (p <0.05) were carried out according to (6).

**Results and Discussion**
The biological role of trace elements, especially copper (Cu) and zinc (Zn), in different physiologic and pathologic conditions has been extensively investigated in many diseases.7

**Zinc Concentration**
The mean level of serum zinc was 81.6µg/dl for patients and 114.1µg/dl for control subjects. This study reveals a significant difference between the serum zinc level for patients and control subjects (p<0.05), table (3). It showed that zinc level was lower in *H. pylori*-infected patients than controls.

Relation between HP infection and zinc is not adequately researched. A protein that strongly binds to zinc has been identified on the membrane and in the cytosol of HP(8). Because zinc is absorbed mainly in the small intestine, by binding dietary zinc in the stomach, HP may possibly contribute to serum zinc deficiency. HP infection might cause zinc deficiency because it is possibility that this bacterium causes serious or moderate
micronutrient deficiencies (9).

It has been reported that the amount of zinc ingested per day may be insufficient relative to the daily requirement in some groups of individuals (children, elderly people, young women on weight-reducing diets, and some other groups). These individuals may develop quasi-deficiency or true deficiency of zinc (10).

**Copper Concentration**

The mean level of serum copper was 132.2 µg/dl for *H. pylori*-infected patients and 84.2 µg/dl for control subjects. The study shows significantly difference between *H. pylori*-infected patients and control subjects (p<0.05), table (4). The study shows that the copper level higher in *H. pylori*-infected patients than controls.

The present data in Table (2) revealed that the experimentally induction of gastric ulceration followed up by highly significant increase of serum copper level on comparison with the mean value in the control group that dueto copper ions are essential for: the viability and maintenaneof connective tissue metabolism, vascular and immunological functions, protection against oxygen radical-induced tissue destruction, and regulations of lysosomal induced tissue autolysis [10]

In this aspect the increased copper level associated with ulcer to endocrine disturbance, where growth hormone andinsulin-like growth factor-I decline with ulcer where [11,12] found that when the main serum binding protein-3 (IGFBP-3) that carries insulin-like growth factor-I increases, the freehormone decreases and results in body weight loss followed gastric ulceration [13].

**REFERENCES**


| Table (1) Volumes of Reagents Used in Determination of Zinc level |
|---------------------|---------------------|---------------------|---------------------|
| **Reagents** | **Sample** | **Standard** | **Blank** |
| work reagent | 1ml | 1ml | 1ml |
| distilled water | - | - | 50µl |
| standard | - | 50µl | - |
| serum | 50µl | - | - |
Table (2) Volumes of reagents used in determination of copper level

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Sample</th>
<th>Standard</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>work reagent</td>
<td>1ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>distilled water</td>
<td>-</td>
<td>-</td>
<td>66µl</td>
</tr>
<tr>
<td>standard</td>
<td>-</td>
<td>66µl</td>
<td>-</td>
</tr>
<tr>
<td>serum</td>
<td>66µl</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (3) Concentration of Zinc Mgldl for *H. pylori*-infected patients and Controls

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Groups</th>
<th>Zinc Mgldl (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31-40</td>
<td>Patient</td>
<td>*89.1±6.347</td>
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<tr>
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<td>Control</td>
<td>120.8±11.694</td>
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<tr>
<td>41-50</td>
<td>Patient</td>
<td>*81.6±5.102</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>114.1±4.112</td>
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<tr>
<td>51-60</td>
<td>Patient</td>
<td>86.8±12.292</td>
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<tr>
<td></td>
<td>Control</td>
<td>198.9±13.419</td>
</tr>
<tr>
<td>60-70</td>
<td>Patient</td>
<td>92.8±7.972</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>142.3±15.334</td>
</tr>
</tbody>
</table>

*there is significance differences of p>0.05

Table (4) Concentration of copper Mgldl for *H. pylori*-infected patients and Controls

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Groups</th>
<th>copper Mgldl (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31-40</td>
<td>Patient</td>
<td>*131.55±22.578</td>
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<tr>
<td></td>
<td>Control</td>
<td>98.1±13.58</td>
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<tr>
<td>41-50</td>
<td>Patient</td>
<td>132.2±5.055</td>
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<tr>
<td></td>
<td>Control</td>
<td>84.2±6.22</td>
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<tr>
<td>51-60</td>
<td>Patient</td>
<td>*127.0±8.27</td>
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<tr>
<td></td>
<td>Control</td>
<td>94.1±10.241</td>
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<tr>
<td>60-70</td>
<td>Patient</td>
<td>*118.1±15.616</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>84.3±9.0338</td>
</tr>
</tbody>
</table>

*there is significance differences of p>0.05
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