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Detection Zinc and Copper Serum in *HelicobacterPylori*-Infected Patients

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Abstract
Background and Objectives: Helicobacter pylori (Hp) colonizes the human gastric and duodenal mucosa and the infection maycause peptic ulcers and gastric adenocarcinoma..Most study appear thate (HP) infection effect of defferent micronutretion zinc and copper The purpose of the study wasto compare serum concentrations of trace elements (zinc [Zn], copper [Cu] of *HelicobacterPylori*-Infected patients with those of healthy subjectsand to determine the relationshipsbetween trace elements**Materials and Methods:** The study was conducted in 45Helicobacter pylori (Hp) infection patients and 20 age- andgender-matched healthy volunteers.Flame atomic absorption spectroscopymethod was employed to analyze serum trace element concentrations zinc and copper. **Results:** Compared to the control subjects,45Helicobacter pylori (Hp) infection patients had significantly($P < 0.05$) Independent sample *t*-testdepicted that serum concentrations ofZn 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 and132.2µg /L, respectively,in Helicobacter pylori (Hp) infection patients, and 142.3 mg/L and84.2 mg/L respectively, in the healthy controls..**Conclusion:** Helicobacter pylori (Hp) infection assosated with metabolism zinc and copper, Serum concentrations of Zn were decreased, but serum concentration of Cu increased significantlyin Gastritis *Helicobacter pylori* patient groups.
Keywords- Gastritis Helicobacter pylori , serum zinc (Zn) , serum copper(Cu).

INTRUDUCTION

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

Trace minerals and vitamins are essential forlife. They act as essential cofactors of enzymes andas organizers of the molecular structures of the cell.Deficiencies of micronutrients influence immunehomeostasis and thus affect infection-related morbidity and mortality . (3)Micronutrients copper and othersare powerful antioxidants and have a significantimpact on infection related morbidity in humans.Subclinical deficiencies are known to impair biological and immune functions in the host(4). *H.pylori* can change the secretion and acidification functions of stomach, because it penetratesespecially into the stomach. This situation canaffect digestion and absorption of some componentsof the nutrients and micronutrients Although nutrient absorption does not takeplace in the stomach, this organ contributes to theprocess by means of secretion of hydrochloric acid and several enzymes. These substances help notonly release the micronutrients from the foodmatrix, but also, in the case of the essentialminerals, render them soluble during the digestiveprocess. In the last few years, a number of studieshave suggested that HP infection may affect thehomeostasis 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

Atotal 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 collectedfrom the antecubital vein of each of the lung cancer patients and healthyvolunteers in a metal-free sterile tube, between 8 to 9 AM, and after anovernight fasting. Standard precautions for trace element determination weretaken; samples with signs of hemolysis were discarded.The blood was thenallowed to clot and centrifuged for 15 minutes at 3000 rpm to extract theserum.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 impactof 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; saliciladoxime 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.table1

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

Calculation:

$$\text{Zn µg /dl} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \text{ X } 200$$

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:tabal2.
Mix and wait for 10 minutes then read the absorbance against the blank at 580nm. The color is stable for 30 minutes.

Calculation

$$\text{Cu µg /dl} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \text{ X } 200$$

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 pathologicconditions has been extensivelyinvestigated 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 notadequately researched. A protein that strongly bindsto zinc has been identified on the membrane and inthe 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 thisbacterium causes serious or moderate

micronutrient deficiencies(9).

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

Copper Concentration

The mean level of serum copper was 132.2µg /dl for *H. pylori*-infected patients and 84.2µg /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 theexperimentally induction of gastric ulceration followed up byhighly significant increase of serum copper level oncomparison with the mean value in the control group that dueto copper ions are essential for: the viability and maintenanceof connective tissue metabolism, vascular and immunologicalfunctions, protection against oxygen radical-induced tissue destruction, and regulations of lysosomal induced tissueautolysis [10]

In this aspect the increased copper level associated withulcer to endocrine disturbance, where growth hormone andinsulin-like growth factor-1 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 followedgastric ulceration [13].

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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

Reagents	Sample	Standard	Blank
work reagent	1ml	1ml	1ml
distilled water	-	-	66µl
standard	-	66µl	-
serum	66µl	-	-

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

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

***there is significance defferances of p>0.05**

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

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

***there is significance defferances of p>0.05**

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