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Estimation of some trace elements level in children with Entrobiasis in Babylon province, Iraq

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Abstract---Background: Enterobiasis or pinworm infection is a common, contagious, parasitic infestation found mainly in children, and is caused by *Enterobius vermicularis* one of the most common human parasitic helminthes . Objective : The present study aimed to check the influence of enterobiasis infections on some trace elements (serum zinc, iron, and copper) in children of Babylon province in Iraq. Methods : A total of ninety children participated in this study and their ages ranged between (2-12)years old , and included both sexes , All samples were collected cellophane tape technique then examined under light microscope. blood sample also collected to estimate the level of serum zinc, copper and iron by ELISA test in infected children. Results: the results show mean value of serum zinc of patients and control group were (79.34 ± 25.09), (86.40 ± 25.54), ($t = 1.251$, $p\text{-value} = 0.214$) respectively , the mean value of serum copper in patients was (99.16 ± 36.23) and in control group were (103.59 ± 36.52), ($t = - 0.544$, $p\text{-value} = 0.587$), and the mean value of serum iron in patients with enterobiasis and control group were (104.80 ± 125.12), ($t = - 1.774$, $p\text{-value} = 0.08$) respectively. The positive correlation between copper and iron were (-0.253), ($p = 0.051$). Conclusion : From the recent study, we found that the elements were effected when infected with entrobiasis and there is a significant correlation between copper and iron($p = 0,051$).

Keywords---Enterobius vermicularis, trace elements, serum zinc.

Introduction

Enterobius vermicularis (pinworms) is one of the most common human parasitic helminthes that may cause enterobiasis, which is common among primary school children in many countries (Moosazadeh *et al.*, 2017) . Pinworm infection can be facilitated by certain factors such as poor personal or group hygiene, and overcrowding in preschools, schools, orphanages, and family groupings (Otu-Bassey *et al.*, 2011).

Human infection was directly associated with the ingestion of infective eggs through oral routes or from contaminated clothes and bed linens. Additionally, transmission through the respiratory tract has also been speculated by inhaling dust contaminated with eggs, Approximately 40% of affected individuals are oligo- or asymptomatic (Kubiak *et al.*, 2017).

Pinworm infection may be symptomless in most patients, some of them may suffer perianal pruritus, insomnia, restlessness, and irritability, particularly children .It should be stressed that pinworms may cause serious morbidity such as appendicitis and eosinophilic enterocolitis, and sometimes ectopic infections can result in pelvic inflammatory disease or urinary tract infections in females(Tsai *et al.*,2018).

A trace element , also called minor element, is a chemical element whose concentration (or other measure of amount) is very low (a "trace amount") . They are classified into two groups: essential and non-essential. Essential trace elements are needed for many physiological and biochemical processes in both plants and animals. Not only do trace elements play a role in biological processes but they also serve as catalysts to engage in oxidation and reduction mechanisms(Bhattachary *et al.*, 2016).

Zinc is an essential trace elements for all forms of life. Clinical zinc deficiency in humans was first described in 1961, Nutritionists have long been concerned that zinc deficiency affects large numbers of women and world wild. Zinc deficiency was indicated as a risk factor for immune deficiency. Zinc play important roles in growth and development, immune function , neurotransmission, vision, reproduction, and intestinal ion transport (Terrin *et al.*,2015).

Copper (Cu) is an essential trace element for humans and animals. In the human organism,copper exists in two forms – the first and second oxidation form, as most of the copper in the human organism is in the second form.The ability of copper to easily attach and accept electrons explains its importance in oxidative reduction processes and in disposing and removing free radicals from the organis. Although scientists identified copper compounds to treat diseases in 400 BC (during Hippocrates) researchers still discover new information regarding the biochemistry,physiology ,toxicology, many clinical, laboratory and other indicators of the impact copper in the organism(Angelova *et al.*, 2011).

Iron is the most abundant essential trace element in the human body. The total content of iron in the body is about 3–5 g with most of it in the blood and the rest in the liver, bone marrow, and muscles in the form of heme. Iron is absorbed in

the gut from diet in case of depletion and transported in the form of ferritin. It's a key element in the metabolism of all living organism . Iron is an essential component of hundreds of proteins and enzymes supporting essential biological function , such as oxygen transport, energy production , DNA synthesis, and cell growth and replication (Bhattacharya *et al.*,2016). The targets of this research were to check the influence of enterobiasis infections on some trace elements (serum zinc, iron, and copper) in children in the Babylon province .

2. Materials and method

2.1. Study duration and samples

During the period of time from September 2021 to November 2022 , a total of ninety children participated in this study and their ages ranged between (2-12)years old , and included both sexes (42 males and 48 females), the chosen areas for sampling included AL noor hospital for children, Emergency Babylon hospital for maternity and children , the second AL Hilla sector , and villages and countryside in Hilla city, Iraq. A special questionnaire form was prepared for each participant child in the study and filled out by interviewing their mothers.

2.1.1 Cellophane tape sample collection

The samples were collected by pressing the sticky side of the tape several times on the anal and perianal region of the children and then sticking the tape on the labeled glass slide and putting it in a sterile clean nylon envelope and then enclosing it tightly . This method was carried out with the help of the children's mothers at night or in the early morning before defecation , using the toilet or taking bath. The collected samples were examined under light microscope .

2.1.2. Blood sample collection .

Five milliliter blood was collected from each child infected with *E. vermicularis* and healthy (control group) by using disposable syringe (5 ml) .The blood sample was placing in EDTA tube and in gel tube, the gel tube was left standing for 15-20 minutes at room temperature to clot, then the tubes were centrifuged at 3000 rpm for 10 minutes to collect the serum.The serum obtained was added in eppendorf tubes (200µl) into many portions for different tests to avoid repeated freezing and thawing of the samples which is not recommended because this may affect the quality of the results. All sera were stored at -20°C until being analyzed for trace elements. After completing the sampling , all samples were simultaneously extracted from the freezer and tested.

2.2. Serological test

Determination of the level of trace elements

2.2.1. Iron direct method (Ferne)

After dissociation of iron –transferrin bound in acid medium, ascorbic acid reduces Fe^{+3} iron into Fe^{+2} iron then form a coloured complex with 3-(2-pyridyl)-5,-6-difuryl-1,-2,-4-triazine-disulfonate (Ferene) . The absorbance thus measured at 600 nm (580-620) is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent tom prevent the copper interference. The reagents and specimens was stored at room temperature. the determination zinc by atomic absorption spectrophotometer according to manufactures instructions.

2.2.2. Copper

The chromogen 3,5-Di-Br-PAESA react with cupric ions and forming a blue – violet compound, which intensity is proportional to the copper concentration in the sample. The method does not require de- proteinization of the serum nor the blank sample. Atomic absorption spectrophotometry Systems (Milano, Italia) was used to (IgG Ab), Zinc react with the chromogen present in the reagent, forming a colored compound which color intensity is proportional to the zinc concentration present in the sample. determine Zinc level in serum in recurrent spontaneous aborted women with chronic infection.

2.2.3.4. Zinc colorimetric

Zinc forms with 2-(5- Brom-2-pyridylazo)-5-(N-propyl-N-sulfo-propylamino)-phenol a red chelate complex. The increase of absorbance can be measured and is proportional to the concentration of total zinc in the sample. Atomic absorption spectrophotometry Systems (Milano, Italia) was used to (IgG Ab), Zinc reacts with the chromogen present in the reagent, forming a colored compound which color intensity is proportional to the zinc concentration present in the sample. determine Zinc level in serum in recurrent spontaneous aborted women with chronic infection Mixed and incubated for 8 minutes at 25 °C or 5 minutes at 37 °C. The absorbance of the sample A(sample) and of the standard a(standard) were measured against the reagent blank A(RBL).

2.3. Statistical analysis

Statistical analysis was carried out using SPSS version 27. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means $\pm$ SD). Student t-test was used to compare means between two groups. Pearson correlation coefficient was used to find the relationship between two continuous variables. A p-value of ≤ 0.05 was considered as significant.

3. Results and discussion

3.1 The mean differences of Zinc according to study group

The results in table (1) show the mean differences of Zinc according to study group including (patients with *Enterobius vermicularis* and control group). There were no significant differences between Zinc according to study group (figure 1).

Table (1) : The mean differences of zinc according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Zinc($\mu\text{g}/\text{dl}$)	Patients	60	79.34	25.09	1.252	0.214
	control	30	86.40	25.54		

P value ≤ 0.05 was considered as significant

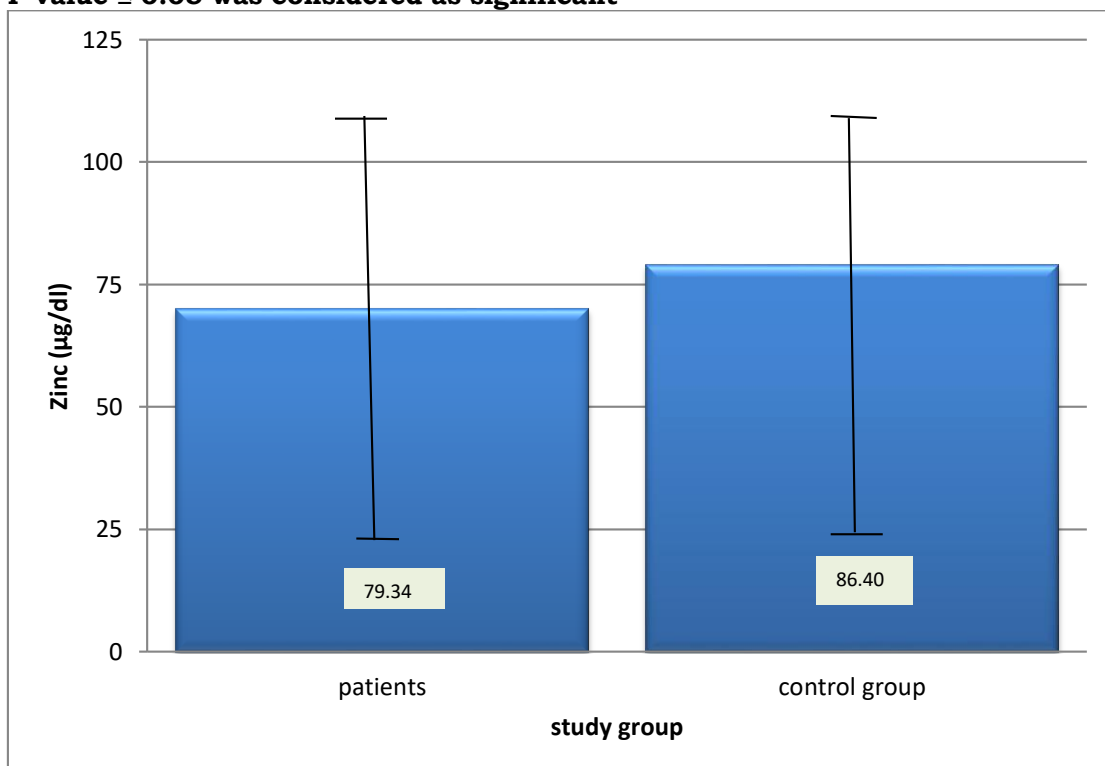


Figure 1: The mean differences of Zinc ($\mu\text{g}/\text{dl}$) according to study group (P= 0.214)

The present result show that the mean value of serum zinc between patients and control group were (79.34 ± 25.09), (86.40 ± 25.54), ($t = 1.251$, $p\text{-value} = 0.214$) respectively. The present study was accept with the study by Arbabi *et al.*, (2015) he found that the levels of zinc in children with *Enterobius vermicularis* was (72.7 ± 17.92) and in control group (80.66 ± 23.58), ($p\text{-value} = 0.05$). also study of Al-Masoudi *et.al.* (2020) confirm the Mean serum Zn level in seropositive toxoplasmosis abortive women were significantly ($P < 0.05$) lower than in control group (67.7 ± 7.778).

Zinc from animal sources has higher bioavailability compared to zinc sourced from plant products. People who abstain from eating red meats, vegetarians, vegans, and people living in developing country who rely mainly on plant-based foods are at higher risk of developing zinc deficiency due to inadequate zinc intake (King *et al.*, 2015).

Zinc is not stored in the body in large amounts, serum zinc levels could easily decline during infections in children with low zinc intake. However elevation of serum zinc levels could cause acute infections due to the immediate emptying of body stores (Ertan *et al.*, 2002). Insufficient intake of nutrients which the body needs, in particular meats, vegetables, fruits, and other foods, has an adverse effect on levels of essential elements (Koltas *et al.*, 1997).

3.2. The mean differences of copper according to study group

The present study show the mean differences of copper according to study group including (patients with *Enterobius vermicularis* and control group). There were no significant differences in copper according to study group (Table 2, figure 2).

Table(2): The mean differences of copper according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Copper($\mu\text{g}/\text{dl}$)	Patients	60	99.16	36.23	-0.544	0.587
	Control group	30	103.59	36.52		

P value ≤ 0.05 was considered as significant.

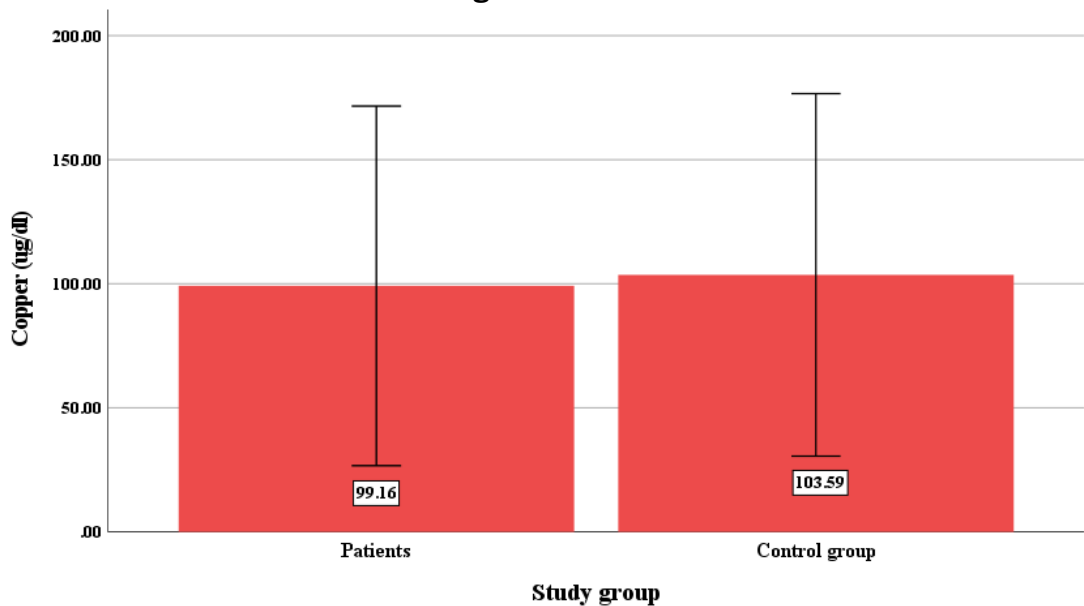


Figure 2: The mean differences of Copper ($\mu\text{g}/\text{dl}$) according to study group (P = 0.587)

The mean value of serum copper in patients was (99.16 $\pm$ 36.23) and in control group were (103.59 $\pm$ 36.52), (t= - 0.544, p-value = 0.587). study by Çulha and Sangün (2007) in Turkey found that the levels of serum copper in patients were decrease than that in control group as he reported in his result (9.35 $\pm$ 0.71),(12.45 $\pm$ 2.46) ,(p= 0.112) respectively .

Sadraei *et al.* (2007) show conformed with the recent study in the level of serum copper, decreased in patients group than that in control group (94.75 ± 12), (127.75 ± 12.02). Copper deficiency usually presents as anemia and neutropenia. If zinc is ingested in high enough amounts, copper malabsorption may ensue since copper binds avidly to metallothionein. Over an extended time period of months or years, this may lead to copper deficiency. One of the causes of copper deficiency are consuming too much zinc, as zinc competes with copper to be absorbed (Duncan *et al.*, 2015). The path physiology is not clearly understood; however, micronutrients deficiencies may be linked to malabsorption due to mucous affection (Shalaby *et al.*, 2017).

3.3. The mean differences of Iron according to study group

The mean differences of iron according to study group including (patients with *Enterobius vermicularis* and control group) was shown in table (3).

Table (3) : The mean differences of Iron according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Iron($\mu\text{g}/\text{dl}$)	Patients	60	104.80	50.26	-1.774	0.08
	Control group	30	125.12	53.14		

P value ≤ 0.05 was considered as significant

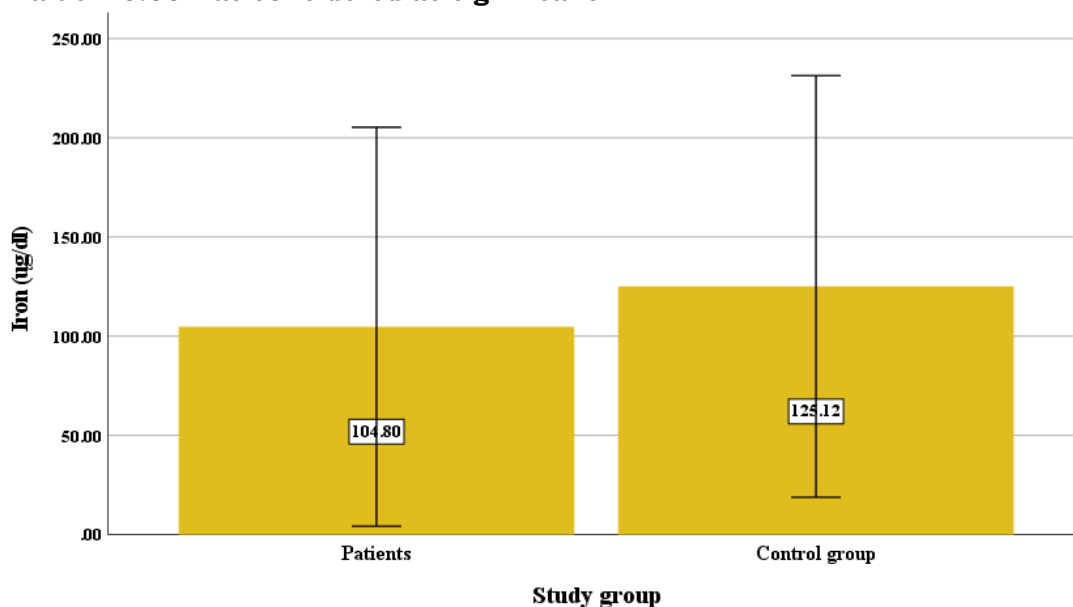


Figure 3: The mean differences of Iron ($\mu\text{g}/\text{dl}$) according to study group (P= 0.08)

There were no significant differences in iron levels according to study group. The result show that the mean value of serum iron between patients with enterobiasis and control group were (104.80 ± 125.12), ($t = -1.774$, $p\text{-value} = 0.08$) respectively. The amount of iron that body need each day depends on age, sex, and whether a consume a mostly plant-based diet. Vegetarians who do not eat

meat, poultry, or seafood need almost double amount of iron because the body doesn't absorb non heme iron in plant foods as well as heme iron in animal foods . Humans require iron for energy production, oxygen transport and utilization, cellular proliferation, and pathogen destruction. (Lynch *et al* .,2018).

Study by Çulha and Sangün (2007) in Turkey revealed that the levels of the serum iron in patients were (128.9±17.9), and in control group were (293.2±62.57), ($p = 7.7 \times 10^{-6}$; $p < 0.01$) and that was in with the recent study . Also the result of (Al-Daoudy and Al-Bazzaz, 2020) be through with the recent study about the level of serum iron in patient and control group were he reported as (68.74 ± 32.01), (86.40 ± 42.59), ($p\text{-value} = 0.036$) respectively . Iron levels decreased due to the malabsorption. There were negative effectors reduce iron absorption or compete for/inhibit absorption, such as manganese, zinc, lead, and calcium. Conversely, positive effectors are fructose, copper, vitamin A enhance absorption of iron (Briguglio *et al*.,2020).

3.4. The correlation between Zinc, copper and iron among patients with *Enterobius vermicularis*

There were significant correlation between copper and iron($p = 0,051$) table 4. Copper, like iron, is required for normal erythropoiesis; copper deficiency causes an iron-deficiency-like anemia. Iron-copper interactions in biological systems may be attributed to their positive charges, similar atomic radii, and common metabolic fates. For example, dietary iron and copper are both absorbed in the proximal small intestine (Gulec and Collins, 2014).

Table (4): The correlation between Zinc, copper and iron among patients with *Enterobius vermicularis* (N=60).

Study variables	Number	Mean	SD	r	P-value
Zinc (ug/dl)	60	86.40	25.09	0.089	0.5
Copper (ug/dl)	60	99.16	36.23		
Zinc (ug/dl)	60	86.40	25.09	0.224	0.086
Iron (ug/dl)	60	104.79	50.26		
Copper (ug/dl)	60	99.16	36.23	-0.253	0.0 51
Iron (ug/dl)	60	104.79	50.26		

P value ≤ 0.05 was considered as significant.

Also, iron and copper must be reduced before uptake into enterocytes and further, both metals are oxidized after (or concurrent with) export into the interstitial fluids (enzymatic iron oxidation may occur while copper oxidation is likely spontaneous). Moreover, both metals are involved in redox chemistry in which they function as enzyme cofactors, and both can be toxic when in excess. Furthermore, a reciprocal relationship between iron and copper has been established in some tissues. For example, copper accumulates in the liver during iron deficiency, and iron accumulates during copper deficiency .Copper levels also

increase in the intestinal mucosa and blood during iron deprivation (Ha *et al.*, 2016).

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