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Biochemical assays for children infected with Giardia lamblia

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Original Research article

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Abstract: The objective of this study was to evaluate the possible alteration of serum copper, caeruloplasmin, and iron in children infected with Giardia lamblia in various age groups. In Kirkuk, North Iraq, 50 patients in a paediatric hospital infected with G. lamblia were included in this study. Age- and sex-matched patients and the control group were categorised into five age groups. The serum copper and iron levels were measured photometrically using 6305, JENWAY Productes, Spectrophotometer, Man. in EU by Barloworld Scientific Ltd, Dunmow, Essex, OM6 3LB. Caeruloplasmin was measured with the single radial immunodiffusion test using KINT Laboratory, INC, Vorgensen place, Bellinghgam, WA, 98226, USA. The mean copper serum level shows a significant elevation in the patients as compared with the control ($p < 0.05$), and a high significant elevation ($p < 0.01$) is seen in the infant group. Copper shows a weak negative correlation with age in infected children. The mean caeruloplasmin serum level significantly ($p < 0.01$) in patients as compared with the control and the significance is present in all age groups. Copper and caeruloplasmin show a weak positive correlation in patients with giardiasis. The mean iron. Serum level shows a high significant decrease ($p < 0.01$) in patients with giardiasis, with a weak negative correlation with age in patients and a weak positive correlation in the control. Conclusion: Copper and caeruloplasmin can be good markers of infection with G. lamblia, and children infected with this parasite may be at an increased risk of iron deficiency anaemia.
Keywords: Giardia lamblia, serum copper, caeruloplasmin, anaemia.

INTRODUCTION

Giardia lamblia is arguably the most widespread protozoan parasite causing diarrhoea in 200 million symptomatic individuals worldwide [1]. Giardiasis is a parasitic disease caused by the flagellated protozoan G. lamblia (also sometimes called Giardia intestinalis or Giardia duodenalis) [2]. Giardia species are parasites of mammals and other animals, including reptiles and birds. It has distinct morphological characteristics in the vegetative trophozoite and cystic stages [3]. Giardiasis is transmitted via the cystic stage to humans through contaminated water or food [4, 5]. Although infection by G. duodenalis is diagnosed by the presence of gastrointestinal symptoms, such as acute or chronic diarrhoea, bloating, and stomach cramps, asymptomatic infections may occur, particularly in endemic areas [6].

The World Health Organization stated that infections by intestinal protozoan parasites are age dependent and are found to occur in greater severity in children. Parasitic infections are thought to contribute to child malnutrition and micronutrient deficiency through subtle reduction in digestion and absorption, chronic inflammation, and loss of nutrients [7]. Copper is an essential trace element, an important catalyst for haem synthesis and iron absorption. Following zinc and iron, copper is the third most abundant trace element in the

Copper stimulates the immune system to fight infections, and copper deficiency reduces cytokine response [8]. At this juncture, there is a need to find out whether a clear-cut association exists between serum copper levels and the presence of different clinical disorders, and if this relationship is also seen in children infected with G. lamblia [9, 10]. Caeruloplasmin (CP) is a ferroxidase enzyme synthesised in the liver; it is the major copper-carrying protein in the blood and, in addition, plays a role in iron metabolism. It contains eight atoms of copper in its structure. CP carries about 70% of the total copper in human plasma. It is worth to mention as well that the copper atoms are interacting with iron in CP. For this reason, the CP will be considered as an essential parameter for measuring the malnutrition severity for the people are infected with *Giardia lambilia*. [11]. Iron is a trace element essential for almost all forms of life. Its biological role is attributable to its properties as a transition metal. Iron is

a critical component of haem in haem globin and myoglobin, where it serves in oxygen binding and transport, which is essential for respiration [12]. Anaemia in children can be caused by iron deficiency and by health factors such as parasites [13].

This study aimed to evaluate the possible alteration of serum copper, CP, and iron in children infected with G. lamblia in various age groups, and also the detection of possible correlations between those parameters with age and the correlation between each other's.

Patients and methods

Fifty G. lamblia-infected children were included in this study, and 50 other age- and sex-matched children were chosen as a control group. Both patients and the control were categorized into five groups as follows: infants <1 year, toddlers (1–3 years), preschoolers (3–5 years), middle childhood (6–8 years), and 9–11-year-olds.

From each child, 5 ml of venous blood sample was collected in a vaccutainer tube, and all samples were analyzed for serum copper, CP, and iron level. Serum copper and serum iron were measured spectrophotometrically using Jenway (Denmark) according to the manual instructions of the serum copper and iron kits LAT S.R.I Via, Milano 15/F, Bussero (Milan) , ITALY.BIOLABO SAS, Les Hantes RIVES, Maizy, FRANCE respectively. The serum CP level was measured by the single radial immunodiffusion (SRID) test using KINT Laboratories INC, Vorgensen Place, Bellingham, WA 98226, USA. The results were sorted depending on the children's age and analyzed.

Statistical Analysis

Statistical evaluations were carried out by samples t-test equal variances. Power analyses were performed by Excel 2013 data analysis for the tests. The significant level was determined to be 5%; p values < 0.05 were considered statistically significant and p < 0.01 considered highly significant. The R-value was used to find the correlation coefficients between the biochemical parameters in patients and the control; r > 0.75 was considered to be a strong correlation, r = 0.5–0.75 considered a moderate correlation, r = 0.25–0.5 considered a weak correlation, and <0.25 considered no correlation.

RESULTS

The mean copper serum level has a high significant increase (p = 0.003), in patients as compared to the control. In addition, the mean CP serum level has a very high significant increase (p = 0.00001), in patients as compared to the control. Furthermore, Table 1 shows a very high significant decrease in serum iron level (p = 0.000027) in patients as compared to the control.

The mean serum levels of copper, CP, and iron in patients and the control according to age group are shown in Table 2. In the age group <1 year, the serum copper level has a high significant increase in patients as compared with the control, p = 0.004. In other age groups, no statistically significant increase was observed.

The mean CP serum level has a significant increase in all age groups. The mean iron serum level decreased significantly in patients in comparison with the control in all age groups. The correlation between the child's age and the mean serum level of biochemical parameters are shown in Table 3. There was a weak negative correlation between the age and copper level in the Giardia-infected group, and the correlation was significant (p = 0.01), while there was no correlation in the control. There was no correlation between the age and CP level in the Giardia infected group, while it was weak negative in the control the correlation is considered significant (p = 0.01). There was a weak negative correlation between the age and the level of iron in the Giardia-infected group and a weak positive correlation in the control. The correlation between the age and serum iron level was significant in the patients and the control group (p = 0.01). Table 4 shows the correlation between copper, CP, and iron in patients infected with G. lamblia and the control. There was a weak positive correlation between copper and CP in the Giardia-infected group (r = 0.32), and the correlation was significant (p = 0.01), while it was not in the control.

Table−1: Biochemical finding in children infected with Giardia lamblia

Variables	Patient		Control		p-value
	mean	SD	mean	SD	
Copper(µg/dl)	57.06	32.77	42.42	9.07	0.003
Ceruloplasmin(mg/dl)	55.2	21.22	28.4	12.37	0.00001
Iron(µg/dl)	43.46	20.12	85	18.44	0.000027

Table-2: Biochemical findings in children infected with Giardia lamblia according to age group

Age group (year)	Copper			Caeruloplasmin			Iron		
	Mean ± SD patient	Mean ± SD control	PV	Mean ± SD patient	Mean ± SD control	PV	Mean ± SD patient	Mean ± SD control	PV
<1[3]	122.7 ±78.01	40.1±9.12	0.004	73±6.93	41±14.92	0.005	59.67±27.0	89.4±22.88	0.03
1–3[6]	70.17±42.74	43.7±9.25	0.07	57.33±35.14	28.8±9.15	0.02	45.83±20.23	77.4±11.61	0.001
3–5[19]	54.63±26.05	44±9.48	0.23	54.63±19.59	25.9±8.44	0.0001	43.68±22.85	75.7±21.76	0.001
6–8[14]	49.14±10.82	43.3±8.99	0.18	54.29±20.41	29.3±9.99	0.002	39.57±15.71	90.8±16.36	$1.0* 10^{-7}$
9–11[8]	42.25±11.25	41±9.74	0.80	49.88±17.16	17±5.16	0.00004	41.88±19.53	91.7±13.55	$8*10^{-6}$

Table-3: Age correlation with copper, caeruloplasmin, and iron in children infected with Giardia lamblia

Variable	Patient		Control	
	r value	p value	r value	p value
Copper	0.35	0.01	0.12	NS
Caeruloplasmin	0.09	NS	0.37	0.01
Iron	0.35	0.01	0.35	0.01

Table-4: Correlation among copper, caeruloplasmin, and iron in children with Giardia lamblia

Variable	Patient		Control	
	r value	p value	r value	p value
Copper with Caeruloplasmin	0.32	0.01	0.19	NS
Copper with iron	0.21	NS	0.02	NS
Iron with caeruloplasmin	0.045	NS	0.19	NS

DISCUSSION

In the present study, the serum copper level was significantly elevated in the patient group, which was in agreement with the findings of a previous study performed in Turkey [14]. However, in a few reports, there was no significant difference in the serum copper level between the patients and the control group [15, 16]. Generally, elevated copper levels are reported in response to infections; this may be attributed to the changes in the concentration of specific tissue proteins controlled by cytokines [17]. The mean CP serum level was measured in this study for the first time in Iraqi patients with G. lamblia infection. The level of

CP increased significantly among infected patients compared with the control. This may be due to the fact that CP is an acute phase reactant and it increasingly acts as a part of the innate immune system through its role in oxidized iron and inhibiting microbe iron uptake [18]. The serum iron level decreased significantly in

Giardia-infected children compared with the control. Micronutrient deficiency was previously reported in Iraq, Turkey, and Egypt [14, 19, and 20], where iron decreases significantly in patients infected with Giardia protozoan infections [19]. In addition, elevations of the serum copper level are observed in most of the acute and chronic parasitic infections [22]. Whenever zinc becomes deficient, copper tends to accumulate [23]. This can explain the significant negative correlation between the age and copper level seen in Giardia-infected children.

The mean serum level of CP increased significantly in patients with Giardia infection in comparison with the control in all age groups. Thus, this led to an increase in serum copper in children infected with G. lamblia, as CP represents the main copper carrier. The higher-than-normal CP level may be due to acute and chronic infections [24]. In addition, there is a weak negative correlation between the age and CP level in the control but not in the patients. Infection with G. lamblia leads to a disturbance of the metabolic process. In the present study, a significant decrease in serumiron is present in all patient groups in compared with non-infected children. The exact mechanism of micronutrient deficiency in giardiasis is not clear. A number of interacting factors are thought to be response capacity [21].The mean copper serum level increased in children infected with Giardia compared with the control, but only infants (<1 year) have a statistically significant increase; this result may be due to the fact that some infants are born with excessive tissue copper that passes through the placenta from the high copper content in the mother's blood. Another fact

may explain this result: many researchers reported a decreased zinc level during Giardia infection that may be due to nutrient malabsorption, whereas others reported that the zinc concentration in the serum decreases during comparison with the control. In addition, a weak negative correlation between the age and the level of serum iron in the Giardia-infected group and a weak posi tive correlation in the control were demonstrated in this study. This might be due to repeated bowel infections during the child's life leading to malabsorption. This result is in agreement with Abou-Shady [20] and Cheek *et al*. [25] who showed that the concentration of iron is lower in children with a high prevalence of giardiasis compared with normal children [25]. Other studies showed that there was no significant difference in the serum iron level between the patients and the control [21]. In Iraq, a previous study reported a significant decrease in serum iron levels in children with giardiasis [19]. The positive significant correlation between the level of serum copper and serum CP in patients infected with G. lamblia is based Principally on the fact that variations in copper and CP concentrations are usually in step with one another. It is thus conceivable that this enzyme plays an important role in copper metabolism. However, this finding needs to be evaluated in a large-scale research to prove this hypothesis. It was revealed in this study that serum copper, CP, and iron levels changed in parasitic infection with G. lamblia. These bio Chemical parameters may be explored in future as a marker of this protozoan parasite disease and a large-scale study is needed to confirm such findings.

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