

## Nutritional status of children infected with *Giardia lamblia* and *Entamoeba histolytica* infections in Kalar town, Iraq

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### Abstract

*Giardia lamblia* is a flagellate protozoon which causes giardiasis. The infection is limited to the small intestine and/ or the biliary tract. It may produce a wide range of gastrointestinal symptoms especially in children; which include vomiting, flatulence, diarrhea and malabsorption syndrome. *Entamoeba histolytica* causes amoebiasis in human beings which is an infection of the large intestine; it may invade the host tissues. It may produce acute amoebic dysentery which is accompanied with blood, mucus and tenesmus; chronic and asymptomatic amoebiasis or extra-intestinal amoebiasis. This study was carried out from second of May to thirties of November 2008 to show the relationship between *Giardia lamblia* and *Entamoeba histolytica* infections with some biochemical parameters and anthropometric measurements among 348 children aged 2-13 years old in Kalar General Hospital. Stool examination was done by direct method using normal saline and lugols iodine solution. Biochemical parameters (total protein, albumin, globulin, zinc, copper, and iron were estimated by spectrometry. The nutritional status were determined by measuring weight for age (WFA), weight for height (WFH) and height for age (HFA) according to National Center for Health Statistics (NCHS). The overall prevalence of infections was 48%. The infection rate of *E. histolytica* 31.6% was higher than *G. lamblia* 16.37%. Total serum protein and albumin concentrations were decreased significantly in *G. lamblia* (7.15 mg/dl, 4.91 mg/dl) and *E. histolytica* (6.48mg/dl, 4.76 mg/dl) infected children compared to non-infected (7.49 mg/dl, 5.02mg/dl), while total globulin concentration increased significantly in *G. lamblia* (3.41 mg/dl) and decreased slightly in *E. histolytica* (2.22mg/dl) infected children compared to non-infected (2.56 gm/dl) ones. Serum zinc and iron element concentrations decreased significantly in *G. lamblia* (79µg/dl, 54µg/dl) and *E. histolytica* (78 µg/dl, 68 µg/dl) infections compared to non-infected (96µg/dl, 87µg/dl) respectively, while copper level decreased slightly in *G. lamblia* (110µg/dl) and significantly in *E. histolytica* (65 µg/dl) infections in comparison to non-infected group (125µg/dl). *G. lamblia* and *E. histolytica* infections were more prevalent in underweight children (low WFA), and stunted children (low HFA), whereas in wasted children (WFH) had no relation with both infections.

الحالة التغذوية للاطفال المصابين بطفيلي جيارديا لامبليا والمتحولة النسيجية في قضاء كلالر / سلیمانیاة/ العراق

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المستخلص

جيارديا لامبليا هي من السوطيات الاوالي التي تسبب مرض داء الجيارديا ،الإصابة محدودة في الأمعاء الدقيقة والقنوات الصفراوية . ربما يسبب طيفا واسعا من الاعراض المعوية المعدية خاصة عند الاطفال والتي تتضمن

التقيء وتنفخ البطن والاسهال ومتلازمة سوء الامتصاص . المتحولة الحالة للنسج بسبب داء الاميبيا في الإنسان والذي هو يعتبر من امراض الامعاء الغليظة وربما يغزو انسجة المضيف قد ينتج إصابة الاميبيا الحادة والذي يصاحبه الدم والمخاط واستط لاق البطن وحالات مزمنة وبدون اعراض او داء الاميبيا خارج الأمعاء . أجريت هذه الدراسة خلال الفترة الزمنية التي تراوحت من اليوم الثاني من شهر مايس حتى الثلاثين من شهر كانون الاول لسنة 2008، للكشف عن علاقة الإصابة بجارديا لامبليا والاميبيا المتحولة الحالة للنسج ببعض المتغيرات الكيميائية والجسمانية في دم 348 طفلا من المراجعين للمستشفى العام في كلار، والذين تراوحت اعمارهم ما بين 2-13 سنة. فحصت عينات البراز بالطريقة المباشرة باستعمال المحلول الفسيولوجي ومحلول الايودين وتم اجراء قياس مستوى بروتين الكلى والالبومين وبع ض معادن (زنك، نحاس وحديد) بطريقة المطياف الضوئي Spectrophotometry. تم تحديد الحالة التغذوية بواسطة قياس الوزن الى العمر (WFA) الوزن الى الطول (WFH) وأخيرا الطول إلى العمر (HFA) وتم الحساب وفق دليل المركز القومي للإحصاء الصحي (NCHS). تم استخدام طريقة اختبار (T) لبيان الاختلاف المعنوي بين المجموعتين . كان المعدل الكلي لنسبة الإصابة 48% ، كانت نسبة الإصابة بالأميبيا المتحولة الحالة للنسج 31,6% حيث كانت اكثر من نسبة الإصابة بجارديا لامبليا 16,37. المعدل الكلي للبروتين و البومين الدم لدى الأطفال المصابين ب جارديا لامبليا (7,15 ملغرام\100مل ، 4,91 ملغرام\100مل) على التوالي و المتحولة الحالة للنسج (6,48 ملغرام\100مل ، 4,76 ملغرام\100مل ) على التوالي ب المقارنة مع الأطفال الاصحاء (7,49 ملغرام\100مل، 5,02 ملغرام\100مل) على التوالي بينما كانت نسبة الكلوبولين اعلى في المصابين بجارديا (3,41 ملغرام\100مل) و اقل في الاميبيا المتحولة للنسج (2,22 ملغرام\100مل) في المصابين مقارنة بالاصحاء ( 2,56 ملغرام\100مل ). لوحظ نقصان في عنصر الخارصين و الحديد في مصل الدم لدى الأطفال المصابين بجارديا لامبليا (79 مايكروغرام\100مل، 54 مايكروغرام\100مل) على التوالي و ب المتحولة الحالة للنسج (78 مايكروغرام\100مل، 68 مايكروغرام\100مل) على التوالي بالمقارنة مع الأطفال الاصحاء (96 مايكروغرام\100مل ، 87 مايكروغرام\100مل) على التوالي أما عنصر النحاس فقد كان اوطأ قليلا عند الإصابة بجارديا لامبليا (110 مايكروغرام\100مل) وقل معنويا عند الإصابة ب المتحولة الحالة للنسج (65 مايكروغرام\100مل) عند المقارنة مع الاصحاء (125 مايكروغرام\100مل). وجدت علاقة قوية بين الإصابة بجارديا لامبليا و بالمتحولة الحالة للنسج وحالات سوء التغذية طبقا للوزن الى العمر (WFA) و الطول الى العمر (HFA)، ولكن لم نجد اية علاقة بين إصابة جارديا لامبليا و ب المتحولة الحالة للنسج و حالات سوء التغذية طبقا للطول الى الوزن (HFW).

## Introduction

Parasitic infections can affect the nutritional status of infected people, by modifying the key stages of food intake, digestion and absorption. Indirect morbidity is particularly important in children with parasitic infections, ranging from malnutrition, anemia, growth retardation, irritability and cognitive impairment to increase susceptibility to other infections and acute complications (1), protein energy malnutrition is another complication of parasitic infection which is common problem in populations with low socioeconomic status and low level of public health sanitation (2). Attachment of a large number of *G. lamblia* to intestinal mucosa may exert an irritative, mechanical, or possibly a toxic action on the intestinal mucosa may lead to shortening and blunting of the villi, thus interfering with intestinal

absorption of nutrients and producing malabsorption syndrome (3). *E. histolytica* have numerous virulence factors such as proteinases, lectin and amoebapore for digestion and penetration of intestinal mucosa leading to diarrhea as well as malabsorption of nutrients and minerals (4). It has been reported that blood levels of iron, zinc, and copper might decrease in children infected with amoebiasis (5). Epidemiological studies indicate an association between relatively low zinc concentration and increased diarrheal morbidity. Moreover, zinc supplementation reduces duration and treatment failure of diarrhea. It is suggested that zinc alters the function of *E. histolytica* in vitro reflected by decreasing in replication and adhesion, and in vivo as manifested by inhibition of amoebic pathogenicity (2), serological level of zinc is also decreased during giardiasis

due to malabsorption (6). Although a relation of copper deficiency and diarrheal morbidity has not been shown, *E. histolytica* acid phosphatase activity is significantly inhibited by copper suggesting a possible role in amoebic intestinal disease (5), while studies revealed that giardiasis increased serological level of copper like other infections (6 &7).

### **Materials and Methods**

This study was carried out from second of May to thirties of November 2008 to investigate the relation of *E. histolytica* and G infection with some biochemical parameters and anthropometric measurements among 348 children aged 2-13 years old visited Kalar General Hospital. General stool examination was carried out for each child using direct wet mount technique.

### **Biochemical parameters**

Total protein determination was performed by spectrophotometer wave length 540 nm, following the procedure of Tietz (8). Albumin determination was performed using spectrophotometer at 630 nm following the procedure of Daumas *et al.*, (9). Globulin calculation was calculated by subtracting the value of albumin from total protein. Determination of Zinc (Zn) was carried out following the procedure of Makino (10) using Spectrophotometer at 580 nm. Determination of Copper (Cu) was carried out according to Pasquinelli, (11) using spectrophotometer at 578 nm. Determination of iron (Fe) was

performed according to procedure of Hennesy *et. al.*, (12), using spectrophotometer at 600 nm.

### **Nutritional status**

Nutritional status was determined as weight for age (WFA), weight for height (WFH), and height for age (HFA) according to National Center for Health Statistics (13).

### **Statistical analysis**

Statistical analysis was carried out using statistically available software (SPSS version 17). Comparisons between control and patient groups were made using t-test at (0.05 and 0.01) levels (14).

### **Results**

#### **Biochemical parameters:**

Total serum protein concentration in gm/dl±SE of *G. lamblia* 7.15±0.47, and *E. histolytica* 6.48±0.31 infected children were significantly lower at (p<0.05) than non-infected 7.49±0.26, while albumin concentration of *G. lamblia* 4.91±0.16 and *E. histolytica* 4.76±0.08 infected children were significantly lower at (p<0.01) than non-infected group 5.02±0.02 (1). The total serum globulin concentration in gm/dl±SE of *G. lamblia* were 3.41±0.53 and total 2.68±0.28 infected children were significantly higher than non-infected group 2.56±0.24 at (p<0.05), but the results of *E. histolytica* infected group 2.22±0.29 recorded no significant difference at (p>0.05).

**Table (1):- Concentrations of total serum protein, albumin and globulin in *G. lamblia* and *E. histolytica* infections.**

Proteins Infections	Total protein (gm/dl±SE+--)	Albumin (gm/dl±SE)	Globulins (gm/dl±SE)
Non-infected	7.49±0.26	5.02±0.02	2.56±0.24
<i>G. lamblia</i>	7.15±0.47*	4.91±0.16**	3.41±0.53*↑
<i>E. histolytica</i>	6.48±0.31*	4.76±0.08**	2.22±0.29

\*significant decrease at (p<0.05).

\*\*significant decrease at (p<0.01).

\*↑significant increase at (p<0.05).

**Serum concentration of trace elements in relation to *G. lamblia* and *E. histolytica* infections:**

In this study 69 infected children with *G. lamblia* 32, *E. histolytica* 37 and 37 non-infected children were examined for serum zinc, copper and iron concentrations. As illustrated in table (2), serum zinc concentration mean±SE µg/dl of *G. lamblia* 79±15 and *E. histolytica* 78±13 and infected children were significantly lower than non-infected 96±7 at (p<0.05). See figure (3-6). Serum copper concentration (mean±ES) µg/dl of *G.*

*lamblia* infected group 110±19 µg/dl was not significantly different from non-infected 125±11 µg/dl, while *E. histolytica* infected children 65±7 µg/dl were significantly lower than non-infected at (p<0.01). The serum iron levels decreased significantly (p<0.05) in children with giardiasis 54±6 µg/dl and amoebiasis 68±11 compared to the non-infected group 87±10 µg/dl.

**Table (2):- Serological levels of zinc, copper and iron elements in *G. lamblia* and *E. histolytica* infected children.**

Minerals Infections	Zinc ±SE (µg/dl)	Copper ±SE (µg/dl)	Iron ±SE (µg/dl)
Non-infected	96±7	125±11	87±10
<i>G. lamblia</i>	79±15*	110±19	54±6*
<i>E. histolytica</i>	78±13*	65±7**	68±11*

\*mean significant decrease at (p<0.05).

\*\*mean significant decrease at (p<0.01).

**Nutritional status**

As shown in table (3) *G. lamblia* and *E. histolytica* infections were prevalent in malnourished children and the rate

of underweight (WFA) and stunted (HFA) were higher in infected males (48.64%, 30.55%) than in females (27.27%, 19.04%) respectively. Also

the rate of underweight (WFA) were higher in non-infected males (16.12%) than females (8.69%), but In reverse to all other cases the rate of stunting (HFA) was higher in females (17.39%) than in males (10.71%). Rates of

malnutrition among infected children were 40.67%, 26.37%, and 4.79% for WFA, HFA, and WFH and non-infected children 12.96%, 13.72%, and 3.26% for WFA, HFA, and WFH respectively.

**Table (3):- The rate of malnutrition among children infected with *G. lamblia* and *E. histolytica* and non-infected children.**

Infection Parameter	Sex	Infected children		Non-infected children		Total rate of malnutrition	
		No.	%	No.	%	No.	%
WFA	Male	56	48.64	47	16.12	103	33.82
	Female	33	27.27	35	8.69	68	17.77
	Total	89	40.67	82	12.96	171	27.45
HFA	Male	55	30.55	42	10.71	97	21.87
	Female	32	19.04	35	17.39	67	18.18
	Total	87	26.37	77	13.72	164	19.44
WFH	Male	52	4.8	28	3.57	80	4.3
	Female	29	4.75	18	2.77	47	3.94
	Total	81	4.79	46	3.26	127	4.4

As shown in table (4) a relation was indicated between nutritional status and each parasite alone, the rates of underweight (WFA) were equally prevalent among *G. lamblia* and *E. histolytica* infected children, recorded (40.9%) and (40.54%) respectively, while the rate of stunting were higher among *G. lamblia* (fig. 3-7) (33.33%) than *E. histolytica* (22.22%) infected children. The rate of malnutrition, underweight (WFA) were higher in *G.*

*lamblia* and *E. histolytica* infected males (42.85% and 52.17%) than in females (37.5% and 21.42%) respectively, and the rates of stunting were higher in infected males with *E. histolytica* (31.81%) than in females (7.14%), while the rate of stunting were lower in infected males with *G. lamblia* (28.57%) than in females (42.85%).

**Table (4):- The rate of malnutrition among *G. lamblia* and *E. histolytica* infected children.**

Malnutrition	Sex	WFA		HFA		WFH	
		No.	%	No.	%	No.	%
<i>G. lamblia</i>	Male	21	42.85	21	28.57	20	4.9
	Female	12	37.5	11	42.85	11	3.46
	Total	33	40.9	32	33.33	31	3.73
<i>E. histolytica</i>	Male	35	52.17	33	31.81	32	5.47
	Female	21	21.42	21	7.14	18	5.55
	Total	56	40.54	54	22.22	50	6.3

## Discussion

### Biochemical parameters

Protein-energy malnutrition and intestinal parasitic infections are common problem in those populations characterized by low socioeconomic status and low level of public health sanitation. *G. lamblia* and *E. histolytica* infections were related to deleterious consequences to protein-energy nutritional status. Two major consequences may result from protein-energy malnutrition: deficient growth and intellectual performance (2). The total serum protein and albumin were significantly lower in both infections. In Kirkuk (15) recorded significantly lower level of total serum protein in *G. lamblia* infected patients  $5.83 \pm 0.65$  gm/dl in comparison to control ones  $6.73 \pm 0.45$  gm/dl. In Erbil (16), recorded no significant differences in the level of total serum protein and albumin between *G. lamblia* infected and non-infected group ( $6.82 \pm 0.59$  gm/dl,  $3.8 \pm 0.3$  gm/dl), ( $7.27 \pm 0.4$  gm/dl,  $3.82 \pm 0.27$  gm/dl), respectively. This may be due to her study was on

the general population which includes a large proportion of asymptomatic patients which can not be affected as heavy as symptomatic children who visit hospitals. Higher concentration of serum globulin among *G. lamblia* infected patients than non-infected ones is identical to study obtained by Baqai (17) who recorded significantly higher concentration of total globulin in serum of *G. lamblia* infected  $2.57$  gm/dl compared to control  $1.83$  gm/dl. In this study the concentration of serum globulin in Entamoeba did not vary significantly between infected and non infected ones.

### Serum concentration of minerals in relation to *G. lamblia* and *E. histolytica* infections:

In agreement with other studies, serum zinc concentration decreased in both giardiasis and amoebiasis. Ertan *et al.* (6) recorded significantly lower serum zinc level among *G. lamblia* infected  $67 \pm 17$   $\mu$ g/dl children aged 2-15 years old compared to Non-infected  $145 \pm 9$   $\mu$ g/dl. Demirci *et al.* (7) showed

significantly lower serum zinc concentration among *G. lamblia* infected children  $109 \pm 10$   $\mu\text{g/dl}$  with comparison to control group  $136 \pm 10$   $\mu\text{g/dl}$ . Karakas *et al.* (18) reported a significant decrease in serum zinc levels in Turkish children with giardiasis and amoebiasis. Erbagci *et al.* (5) demonstrated that serum concentration of zinc is decreased in patients with acute *E. histolytica* rectocolitis  $64$   $\mu\text{g/dl}$  compared to healthy controls  $81$   $\mu\text{g/dl}$ . Although the concentration of copper in *Giardia* infected patients was lower than non infected ones but statistically there was no significant difference between them. Similar results was reported by Demerci *et al.* (7) who recorded no significant difference between *G. lamblia* infected children  $115 \pm 10$   $\mu\text{g/dl}$  compared to control group  $120 \pm 9$   $\mu\text{g/dl}$ . While results obtained by most other researchers disagree with this study as Ertan *et al.* (6) who showed significant increase in the serum copper level among *G. lamblia* infected  $198 \pm 39$   $\mu\text{g/dl}$  children compared to control group  $150 \pm 21$   $\mu\text{g/dl}$ . While the concentration of copper in *Entamoeba* infected patients was significantly lower than non-infected ones. Erbagci *et al.* (5) revealed that serum copper concentration of the patients with *E. histolytica* rectocolitis  $125$   $\mu\text{g/dl}$  and the control group  $77$   $\mu\text{g/dl}$  were not statistically different. It is suggested that acute phase response to *E. histolytica* infection upregulates ceruloplasmin gene and synthesis in liver and subsequently the level of ceruloplasmin-Cu complex in the blood. The ceruloplasmin contains approximately 95% of total serum copper, a concomitant increase in the serum copper concentration is an expending finding in infectious/inflammatory conditions like the results obtained by Erbagci *et al.* (5).

Alterations in concentration of serum iron, zinc and copper are commonly found in patients with gastrointestinal infections. Recent studies suggest that intracellular zinc, copper and iron elements participate in activation of immune cells. For example, deficiency of each of these trace metals decreases the synthesis and secretion of IL-2 (19). Iron deficiency is common among patients affected by malabsorptive states, such as giardiasis and amoebiasis (7). In this study, it was shown that the serum iron levels decreased significantly ( $p < 0.05$ ) in children with giardiasis  $54 \pm 6$   $\mu\text{g/dl}$  and amoebiasis  $68 \pm 11$  compared to the non-infected group  $87 \pm 10$   $\mu\text{g/dl}$ . Similar results have been obtained by Ertan *et al.* (4) who recorded significant difference in serum iron level among *G. lamblia* infected children  $87 \pm 18$   $\mu\text{g/dl}$  in comparison to controls  $160 \pm 45$   $\mu\text{g/dl}$ . Demerci *et al.* (7) showed significant difference between children infected with giardiasis  $95 \pm 7$   $\mu\text{g/dl}$  and healthy controls  $120 \pm 9$   $\mu\text{g/dl}$  in serum iron level point of view. Askari *et al.* (20) confirmed that serum iron level in patients with giardiasis  $77$   $\mu\text{g/dl}$  was less than the control group  $87$   $\mu\text{g/dl}$  and the differences were significant. Iron deficiency in children with giardiasis and amoebiasis may be protective because iron plays a role in the formation of hydroxyl radicals which in turn cause lipid peroxidation and abolishes membrane structure and function, for this reason, therapy should be initiated before treatment of iron deficiency in infected children.

### **Nutritional status**

In this study the prevalence of malnutrition among children was 27.43% (WFA), 19.44% (HFA) and 4.4% (WFH). *G. lamblia* and *E. histolytica* infections were more prevalent in underweight children (low

WFA), and stunted Alshishtawy and Anany (21) in Omman, revealed that parasitic infections were associated with underweight in 41.67% compared to 27.93% of normal weight people. Haque *et al.* (22) in Bangladesh, recorded 39% and 32% of underweight and stunting among 2-5 years old children infected with *E.histolytica*. Sadjjadi and Tanideh (23) in Iran, observed significantly higher degree of malnutrition, underweight (WFA) and stunted (HFA) among *G. lamblia* infected than non-infected children. Hama (24) in Erbil, found that the intestinal parasitic infections were more prevalent in underweight (low WFA), whereas wasted children (low HFA) was slightly associated with parasitic infections but there is no relation between stunted children (low HFA) and intestinal parasitic infections. Some studies have not found a relation between intestinal parasitic infection and nutritional status such as Al-Agha and Teodorscu (25) in Palestine, Abd-El-Aal *et al.* (26) in Egypt, recorded no significant difference between weight and height of *G. lamblia* and non-infected children, these may be due to the high standard of socio-economic level and better quality meals of the studied areas or intestinal parasites intensity and the type of infection have a notable role to reveal the symptoms as well as the signs of malnutrition. It is not worthy to compare studies conducted among acute infections, because it can not correlate the effect of parasites on nutritional status clearly because the period of infection is not efficient to assess the nutritional parameters change, but chronic and asymptomatic intestinal parasitic infections cause nutritional deficiency. Our data support the hypothesis that early treatment of *G. lamblia* and *E. histolytica* infections will improve the growth of children and decrease the morbidity and

mortality of the related illnesses. *It is concluded that both G. lamblia* and *E. histolytica* infections were more prevalent in underweight children (low WFA), and stunted children (low HFA), whereas in wasted children (WFH) had no relation with *both infections*.

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