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Nutritional Status and Lipid Profile Among Children Infected with *Giardia lamblia* and *Cryptosporidium*

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Abstract

Some intestinal parasites might impact a child's nutritional condition and lipid profile. It has recently been revealed that these parasites have a link to biometric data and lipid profiles. As a result, the current study aims to see how *Giardia lamblia* and *Cryptosporidium* affect nutritional status and lipid profile. From October 2021 to March 2022, this study was done in Baghdad. The participants in the study were 110 children of both sexes, ranging in age from 2 to 17. According to the results of the stool examination, the children were separated into three groups: those infected with *Giardia lamblia* (n=47), those infected with *Cryptosporidium* (n=43), and those who appeared to be healthy (n=20). All participating children were subjected to some biometric measurements (body mass index -BMI, weight and height for age percentile) and lipid profile assessment. BMI and height for age percentile were not significantly related to the *G. lamblia* and *Cryptosporidium* infections. Results showed that weight for age percentile was decreased significantly among children who had giardiasis and cryptosporidiosis compared to control group. Cholesterol levels were significantly increased in *G. lamblia* infected group and triglyceride was significantly increased in *Cryptosporidium* infected group. HDL was significantly raised up in both *G. lambli* and *Cryptospordium* infected group comparing to control. While non-significant differences were notice regarding LDL among groups

Keywords: Biometric measurments, Cryptosporidium, Giardia, Lipid profile

الحالة التغذوية و مستوى الدهون لدى الأطفال المصابين بطفيلي الجيارديا و طفيلي داء الابواغ الخبيئة

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الخلاصة

الاخماج ببعض الطفليات المعوية يمكن ان يكون لها تاثير واضح على كل من الحالة التغذوية و نسبه الدهون. ولقد ظهرا حديثا ان هناك علاقة بين الاخماج بهذه الطفيليات مع كل من القياسات البايومترية و نسبة الدهون. اجري هذا البحث في بغداد في الفترة من تشرين الاول 2021 الى آذار 2022. اشتمل البحث على 110 اطفال من كلا الجنسين تتراوح اعمارهم بين (2–17) سنة. تم تقسيم هؤلاء الاطفال ، وفقًا لفحص البراز

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، إلى مصابين بطفيلي الجيارديا لامبليا (العدد = 47) ، و مصابين بطفيلي الابواغ الخبيئة (العدد = 43) وأطفال اصحاء غير مصابين باي من الطفيليات المعوية, حيث تم اعتبارهم مجموعه سيطرة (العدد = 20). خضع جميع المرضى الى قياسات بايومترية (مؤشر كتلة الجسم –BMI ، الوزن والطول بالنسبة للفئة المئوية للعمر). كما تم تقييم مستوى الدهون لدى كل الاطفال المشمولين بالدراسة. اظهرت النتائج عدم ارتباط كل من مؤشر الكتلة الحيوية و نسبة الطول الى العمر مع الاخماج بطفيلي الجيارديا و طفيلي الابواغ الخبيئة. بينت النتائج انخفاضا معنوياً في نسبة الوزن الى العمر لدى الاطفال الذين لديهم خمجا بالجيارديا لامبيليا و داء الابواغ الخبيئة مقارنة مع السيطرة. كان هناك ارتفاعا معنويا في مستوى الكولسترول لدى الاطفال المصلبين بالجيارديا و ارتفاعا معنويا للدهون الثلاثية لدى الاطفال المصلبين بطفيلي الابواغ الخبيئة مقارنه مع مجموعة السيطرة. اما البروتينات الشحمية عالية الكثافة فقد اظهرا ارتفاعا معنويا في كل من الاطفال الذين لديهم اخماجا بالجيارديا و الابواغ الخبيئة. بينما لم تُظهر البروتينات الشحمية قليلة الكثافة اي اختلافا معنويا بينالمجموعات المختلفة.

1. Introduction

Cryptosporidiosis and giardiasis are eukaryotic protozoan parasitic illnesses caused by *Cryptosporidium* and *Giardia lamblia*, respectively. These protozoan parasites are a major public health issue, particularly in developing nations [1]. Person to person, zoonotic, waterborne, foodborne, and airborne (for *Cryptosporidium*) transmission of these two parasites occurs via the faecal–oral pathway following direct or indirect contact with the transmissive stages (*Cryptosporidium* oocysts and *Giardia* cysts) [2].Infections caused by those parasites continue to be a severe public health concern, as they can result in a variety of clinical consequences, particularly in children [3]. These diseases are linked to a high rate of morbidity and mortality, including diarrhea, malnutrition, iron deficiency, and anemia [4, 5]. Intestinal parasite prevalence is influenced by socioeconomic and health factors, as well as education and attitudes about traditional health practices, the presence of domestic animals in the home, and water and food contamination [6]. Protozoa infection has been proven to impact blood lipid levels in some people. The presence of blood lipids in the bloodstream is critical for host defense during the acute phase of inducible immune response. Infection and inflammatory response have been shown to affect total cholesterol and triglycerides in the blood [7]. Alteration in the lipid profile are observed in individuals with active parasite infections [8]. As a result, membrane proteins are thought to be engaged in such functions. Although all parasites can metabolize cholesterol, the exact link between pathogenic mechanisms and cholesterol metabolism is uncertain [9]. Cholesterol is an essential component of eukaryotic membrane structure, dynamics, and function. It's mainly seen in non-randomly distributed membrane domains [9]. Several research [10-13] have recently shown that focusing on gastrointestinal parasites has expanded considerably in Iraq. The link between parasite infections and cholesterol, triglycerides, high density lipoprotein (HDL), very low density lipoprotein (VLDL), and low density lipoprotein (LDL) levels, on the other hand, is poorly understood. This study aims to find out how children with cryptosporidiosis or giardiasis eat and what their lipid profile looks like.

2. Materials and methods

2.1.Study Area/Subject Selection

Between the period extended from October 2021 to March 2022, the study was conducted at four hospitals in Baghdad (Central Teaching Hospital for Children, Mansour Teaching Hospital for Children, Kadhimiya Hospital for Children, and Al-Karkh Hospital). The study comprised a total of 110 children ranging in age from 2 to 17 years old.

2.2. Ethical Approval

The Ministry of Health and Environment in Baghdad, Iraq, granted permission, and the study was approved by the local ethics commission (Ref.:CSEC/0222/0024) at the College of Science, University of Baghdad. Permission was also gotten from the parents of the children.

2.3. Biometric measurements

A portable stadiometer and electronic scale were used to measure the height and weight of all the children. Each participant was required to remain completely still while their weight was calculated. Weighing and measuring to a precision of 0.1 kg and 0.5 cm. The children were weighed without shoes and wearing only the most basic attire. BMI is calculated by multiplying weight (kg) by height squared (m). Malnutrition (BMI 18.5 kg/m^2) and normal BMI (BMI = 18.5-25 kg/m^2) were assigned to the children based on their BMI [14]. https://reference.medscape.com/calculator/ was used to calculate weight and height for age percentiles.

2.4. Stool Sample Collection and examination

Patients' stool samples were collected and delivered to the laboratory in sterile disposable plastic tubs that were labelled with the subject's identification. The materials were concentrated using the formalin-ether method [15]. A drop of each deposit was taken and smeared on a glass slide using a sterile pipette. A modified acid-fast procedure was used to produce and stain another smear [16]. A light microscope with a 100 objective was used to examine all of the smears. According to the results of their stool examinations, the 110 individuals were divided into three groups. The first group involved *G. lamblia*-infected children (n=47), the second *Cryptosporidium*-infected children (n=43), and the third group (n=20) contained no parasites.

2.5. Blood Sample Collection and Preparation

An antecubital vein was punctured with a sterile needle and syringe, and roughly 5-6 ml of blood was drawn into an EDTA anticoagulant tube. The blood samples were spun for 15 minutes at 3500 rpm in a bench centrifuge (Hittich EBA 20/ Germany). Serum was separated immediately into simple tubes and kept at -20 ºC until analysis.

2.6. Lipid profile

Total cholesterol, LDL cholesterol, and HDL cholesterol were determined using a standard cholesterol LDL precipitating reagent kit (Randox Laboratories Limited, United Kingdom), whereas triglyceride was estimated using a triglyceride specific assay (Randox Laboratories Limited, United Kingdom) (Biosystems Reagents and Instruments, Biosystems S.A. Costa Brava 30, Barcelona Spain). All of these tests were completed according to the manufacturer's guidelines.

2.7. Statistical analysis

The data was statistically analyzed using the Statistical Analysis System - version 9.1. The Chi-square test was done to see if there was a link between nutritional status and parasite infestations. The means of BMI, weight for age percentile, height for age percentile, cholesterol, triglycerides, LDL, HDL, and VLDL parameters were compared between the *G.lamblia* infected group, the *Cryptosporidium* infected group, and the control group using a one-way ANOVA test. The significance of differences between pairs of group means was determined using a tukey kramer post hoc test. A P value of less than 0.05 is considered

statistically significant. The mean and standard deviation (mean SD) were used to express all of the data.

3. Results

Table 1 illustrated the nutritional status among children infected with *G. lamblia*, children infected with *Cryptosporidium* and control group. Normal BMI was noticed in 8.51% of *G. lamblia* infected group and 16.27% of *Cryptosporidium* infected group while BMI was normal in 30% of the control group. Although non-significant relation were noticed between BMI and infections with *G. lamblia* and *Cryptosporidium* parasites. Results also showed non-significant differences among groups regarding height for age percentile, although its value was higher in control group (47.39±4.5) comparing to *G. lamblia* infected children (41.55±3.11) and *Cryptosporidium* infected children (45.95±3.47) (Table 2). Alternatively, the results showed significant differences ($P<0.05$) among group regarding weight for age percentiles. The highest weight for age percentile was noticed in control group (35.40±2.50), comparing to *G. lamblia* infected children and *Cryptosporidium* infected children who showed lower means of weight for age percentile (24.28±3.14) and (35.14±3.46) respectively (Table 3). Tukey kramer post hoc test showed significant different regarding weight for age percentile between *G. lamblia* infected children and *Cryptosporidium* infected children. While insignificant differences were reported between *Giardia* infected group versus control and between *Cryptosporidium* infected versus control. Results showed significant differences ($P<0.05$) among groups regarding cholesterol levels. Children who had giardiasis showed the highest mean of cholesterol (235±9.5 mg/dL) compared to children who had Cryptosporidiosis and control group, their cholesterol level were (197±9.8 mg/dL) and (184±9.9 mg/dL) respectively (Table 4). Tukey kramer post hoc analysis showed significant differences ($P<0.05$) between *G. lamblia* infected group versus *Cryptosporidium* infected group. As well as significant difference ($P<0.05$) was noticed between *G. lamblia* infected children versus control children.

The results of triglyceride were also varied significantly ($P<0.05$) among groups. *Cryptosporidium* infected group showed the highest mean of triglycerides (246.62±23.6 mg/dl) compared to other groups, *G. lamblia* infected group (184.42±13.5 mg/dL) and control group (194.24±4.6 mg/dl) (Table 5). Tukey kramer post hoc test showed significant different regarding triglycerides between each pair goup. Results showed that HDL level was increased significantly in control group, their HDL level was (58.7±9.2 mg/dL). While HDL levels in *G.lamblia* infected group and *Cryptosporidium* infected group were reported to be lower than control group, their HDL values were (51.21±5.3 mg/dL) and (37.72±2.3 mg/dL) respectively (Table 6). Tukey kramer post hoc test revealed insignificant differences ($P<0.05$) regarding HDL between *G.lamblia* infected group and *Cryptosporidium* infected group and insignificant differences between *G.lamblia* infected group versus control. The only significant difference ($P<0.05$) for HDL was noticed between *Cryptosporidium* infected group versus control group. The results of LDL showed insignificant differences among group although *G.lamblia* infected children and *Cryptosporidium* infected children had the highest mean of LDL (117±6.8 mg/dL), (105±6.9 mg/dL) respectively compared to control children who showed low level (99.9±4.3 mg/dL) of LDL (Table 7). VLDL values were differed significantly ($P<0.05$) among groups. VLDL was increased significantly ($P<0.05$) in *Cryptosporidium* infected children (41.43±3.6 mg/dL) while it was lower in both *G.lamblia* infected children and control group, their VLDL means were (36.19±1.9 mg/dL) and (21.25±1.2 mg/dL) respectively (Table 8). Post hoc Tukey HSD analysis showed significant differences ($P<0.05$) between children with cryptosporidiosis versus control and between

children with giardiasis versus control. While no significant difference was noticed between cryptosporidiosis group and giardiasis group

Table 1: Nutritional status of *Giardia lamblia* infected group, *Cryptosporidium* infected group and control

Nutritional status based on BMI	*Giardia* infected group N(%)	*Cryptosporidium* infected group N(%)	Control N(%)
Malnutrition (BMI < 18.5 kg/ m^2)	43(91.48%)	36 (83.72%)	14 (70%)
Normal BMI (BMI = 18.5 – 25 kg/ m^2)	4 (8.51%)	7(16.27)	6 (30%)
Total	47	43	20
X^2= 4.99, df= 2, p-value= 0.08			

Table 2: Height for age percentile of *Giardia lamblia* infected group, *Cryptosporidium* infected group and control

Group	Height for age percentile (Mean±SD)	P-value	
Giardia lamblia infected children (n=47)	41.55±3.11	0.5	F=0.65
Cryptosporidium infected children (n=43)	45.59±3.47		
Control (n=20)	47.39±4.5		

Table 3: Weight for age percentile of *Giardia lamblia* infected group, *Cryptosporidium* infected group and control

Group	Weight for age percentile (Mean±SD)	P-value	
Giardia lamblia infected children (n=47)	24.28± 3.4 [a]	0.033*	F=3.15
Cryptosporidium infected children (n=43)	35.14± 3.4 [b]		
Control (n=20)	35.4± 2.5 [a, b]		
Means with a different small letter in same column significantly different (P<0.05) using tukey kramer post hoc test			

Table 4: Cholesterol level of *Giardia lamblia* infected group, *Cryptosporidium* infected group and control

Group	Cholesterol level (Mean±SD) mg/dL	P-value	
Giardia lamblia infected children (n=47)	235± 9.5[a]	0.002*	F= 6.50
Cryptosporidium infected children (n=43)	197± 9.8[b]		
Control (n=20)	184± 9.9[b]		

Table 5: Triglyceride level of *Giardia lamblia* infected group, *Cryptosporidium* infected group and control

Group	Triglyceride level (Mean±SD) mg/dL	P-value	
***Giardia lamblia* infected children (n=47)**	184.42± 13.5[a]	0.00005*	F= 10.7
***Cryptosporidium* infected children (n=43)**	246.62± 23.6[b]		
Control (n=20)	194.24± 4.6[c]		
Means with a different small letter in same column significantly different (P<0.05) using tukey kramer post hoc test			

Table 6: HDL level of *Giardia lamblia* infected group, *Cryptosporidium* infected group and control

Group	HDL level (Mean±SD) mg/dL	P-value	
***Giardia lamblia* infected children (n=47)**	51.21± 5.3[a, b]	0.02	F= 3.69
***Cryptosporidium* infected children (n=43)**	37.72± 2.3[a]		
Control (n=20)	58.7± 9.2[b]		
Means with a different small letter in same column significantly different (P<0.05) using tukey kramer post hoc test			

Table 7: LDL level of *Giardia lamblia* infected group, *Cryptosporidium* infected group and control

Group	LDL level (Mean±SD) mg/dL	P-value	
***Giardia lamblia* infected children (n=47)**	117± 6.8	0.29	F=1.2
***Cryptosporidium* infected children (n=43)**	105.51± 6.9		
Control (n=20)	99.9± 4.3		

Table 8: VLDL level of *Giardia lamblia* infected group, *Cryptosporidium* infected group and control

Group	VLDL level (Mean±SD) mg/dL	P-value	
***Giardia lamblia* infected children (n=47)**	36.19± 1.9 [a]	0.0002	F= 9
***Cryptosporidium* infected children (n=43)**	41.43± 3.6 [a]		
Control (n=20)	21.25± 1.2 [b]		
Means with a different small letter in same column significantly different (P<0.05) using tukey kramer post hoc test			

4. Discussion

Malnutrition is a global health issue that affects people of all socioeconomic backgrounds all over the world. Malnutrition is a major public health concern in Iraq, especially in children [17]. According to their BMI, the majority of children infected with *G. lamblia* and *Cryptosporidium* were malnourished in the current study. In addition, in the *G. lamblia* infected group, there was a substantial fall in weight for age percentile. Giardiasis can cause steatorrhea, malnutrition, and carbohydrate and vitamin A and B12 malabsorption. The causes of absorptive dysfunction are unknown, but structural abnormalities of the intestinal mucosa

and/or bacterial overgrowth may be involved [18]. Some longitudinal studies have found a link between *G. lamblia* infection and malnutrition, whereas others have found no such link. A study in Brazil found that children with symptomatic infections had lower weight-for-age and height-for-age percentiles [19], while a study in Malaysia found that children with giardiasis were considerably underweight and wasting compared to those without the parasite [20]. Other researchers, on the other hand, found no significant link between malenutrition and parasite infection [21-23]. This could be due to the fact that these studies reported a lower prevalence of *G. lamblia* than the current study. On the other hand, the current study's findings found no link between parasite infection and height for age percentile It is commonly recognized that a person's genetic makeup has the greatest influence on their height. Many other factors, like as nutrition, hormones, exercise levels, and medical problems, also influence height during development [24]. The findings also revealed that infection with *Cryptosporidium* did not have a significant relationship with weight for age percentile. Because the diarrhea induced by this parasite can be more severe in infants and influence their weight more than in non-infants [25], there was no significant effect of parasite infection on children in the current investigation because all of the children were non-infants. In comparison to the control group, patients with Giardiasis or Cryptosporidiosis, in this current study, exhibited a considerable increase in cholesterol levels. Recent research has found higher levels of lipoproteins including total cholesterol in parasite infection patients [26]. The physical state of bile salt molecules in solution (monomers or micelles) is critical in determining cholesterol uptake by *Giardia*, according to Lujan *et al*.[27] and both bile salt excess and cholesterol deficiency promote encystation by blocking cholesterol uptake.The cholesterol results in this investigation contradicted those of Alhuchaimi, *et al.* [28] who found that *G. lamblia* infection can maintain low serum cholesterol. When compared to *G.lamblia* infected children and a control group, individuals with *Cryptosporidium* infection had considerably higher triglyceride levels. *Cryptosporidium* can harm enterocytes through a variety of ways, including direct cytotoxicity, induction of apoptosis in the host enterocyte, or beginning a phenotyp shift in the enterocyte [29]. These processes can cause an inflammatory immunological response, which can lead to changes in lipid metabolism, including triglyceride increases [30]. Infected children with *G. lamblia* did not demonstrate the same rise. This finding was consistent with that of Bansal *et al*. [31] who found that patients infected with *G. lamblia* had reduced levels of lipid markers, particularly triglycerides, when compared to a healthy control group. It also aligned with the findings of Ma'ani and Jabir [32], who discovered normal triglyceride levels in giardiasis patients compared to controls. The findings were contradicted by Eltayeb *et al*. [33] who found a significant increase in triglycerides in giardiasis patients. The results of this study also revealed that the tested groups had varying levels of HDL and VLDL. When compared to the control group, HDL levels in patients with Giardiais did not alter appreciably. This conclusion was consistent with the findings of Saki *et al*. [34] who found that HDL levels in *G.lamblia* cyst passers did not alter significantly. While the current findings revealed that HDL levels in the *Cryptosporidium*-infected group were considerably lower than in the control group. In young children, cryptosporidiosis causes local inflammation in the digestive tract, which can cause a range of changes in lipid metabolism, including a drop in serum HDL [35]. The findings were consistent with those of Abdulla *et al.*[36] who found that HDL levels were considerably lower in *Cryptosporidium*-infected patients. VLDL, on the other hand, was considerably elevated exclusively in the Cryptosporidiosis group. While Cryptosporidium has a Type I polyketide synthase (CpPKSl) that is likely involved in the creation of unknown polyketides from a fatty acid precursor [37], Giardia cannot generate fat on its own [38]. Based on these outcomes, it is determined that both *G. lamblia* and *Cryptosporidium* can affect some biometric measurements in children as well as they have ability to alter lipid profile.

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