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Evaluation of Hematological Factors and Micronutrients Among Children Infected with *Enterobius vermicularis*

Haneen Esam Akram, Harith Saeed Al-Warid*
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

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Abstract

Malnutrition, anemia, and micronutrient deficits may be associated with *Enterobius vermicularis* infection. Hence, the subject has recently received a lot of attention. The goal of this study was to analyse the nutritional, hematological and micronutrient status of children infected with *E. vermicularis*. This research was carried out in Baghdad from October 2021 to the end of March 2022. The study comprised 100 children of both sexes, ranging in age from 3-16 years. All individuals nutritional status was assessed using the weight-for-age Z score and the height-for-age Z score. As well as cellophane tape samples and blood samples were collected from all individuals. The cellophane tape samples were examined under microscope for *E. vermicularis* detection. Whereas blood samples were processed to assess many factors which were: haemoglobin, ferritin, total binding capacity (TIBC), serum iron, serum zinc and serum magnesium. Results revealed significant correlations between *E. vermicularis* infection occurrence and each of age and gender, even though most infections were noticed among females and among those who were between 3-9 years. The results also showed that the nutritional status, based on weight for age Z score, was significantly ($P<0.05$) related to *E. vermicularis* occurence. Moreover haemoglobin, ferritin, serum iron, serum zinc and serum magnesium were noted to have significantly ($P<0.05$) decreased among those who were *E. vermicularis* positive compared with *E. vermicularis* negative group. While total iron-binding capacity (TIBC) increased significantly among *E. vermicularis* positive children. The current investigation revealed that *E. vermicularis* infection had significant implications on several haematological elements, as well as stunting nutritional shortages. As a result, a lower prevalence of intestinal parasite infection (particularly enterobiasis) among children would almost certainly benefit their growth, development and educational outcomes.

Keywords: Children, *Enterobius vermicularis*, Hematological parameters, Micronutrients

تقييم مستوى العوامل الدموية و المغذيات الدقيقة لدى الاطفال المصابين بالدودة الدبوسية

حنين عصام اكرم، حـــارث سعيد الـورد*
قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

من الممكن ان يرتبط كل من سوء التغذية، فقر الدم و نقص المغذيات الدقيقه بالإصابة بالدودة الدبوسية. لذلك اخذ هذا الموضوع حيزا كبيرا من الاهتمام خصوصاً في الاونه الاخيرة. هدف البحث الحالي الى تحليل

*Email: harithalward@yahoo.com

كل من الحالة التغذوية، الحالة الدموية و مستوى المغذيات الدقيقة لدى الاطفال المصابين بالدودة الدبوسية. اجري هذا البحث في بغداد للمدة من تشرين الاول من العام 2021 الى نهاية اذار من العام 2022. شملت الدراسة مائة طفل من كلا الجنسين والذين تراوحت اعمارهم بين (3–16) سنة. تم تقييم الحالة التغذوية لجميع الاطفال المشاركين بالدراسة من خلال حساب قيمة (Z) الخاصة بالوزن الى العمر و قيمة (Z) الخاصة بالطول الى العمر. كما جمعت عينات الشريط اللاصق وعينات الدم من جميع المشاركين. تم فحص الشريط مجهرياً للكشف عن الاصابة بالديدان الدبوسية كما خضعت عينات الدم الى عدد من الفحوصات للتحري عن كل من الهيموكلوبين، الفيرتين، سعة الارتباط بالحديد، المستوى المصلي للحديد، المستوى المصلي للزنك والمستوى المصلي للمغنيسيوم. اظهرت النتائج عدم وجود علاقة معنوية بين كل من الجنس والعمر مع حدوث الأصابة بالدودة الدبوسية على الرغم ان غالبية الاصابات كانت لدى الاناث الذين تتراوح اعمارهم بين (3–9) سنوات. اظهرت النتائج ايضا وجود علاقة معنوية بين الحالة التغذوية من خلال حساب قيمة (Z) الخاصة بالوزن الى العمر و الأصابة بالدودة الدبوسية. كان هناك انخفاضاً معنوياً لكل من من الهيموكلوبين، الفيرتين، المستوى المصلي للحديد، المستوى المصلي للزنك والمستوى المصلي للمغنيسيوم لدى الموجبين لفحص الدودة الدبوسيه. بينما اظهرت سعة الارتباط بالحديد ازديادا معنويا لدى الموجبين لفحص الدودة الدبوسيه. كشفت الدراسة الحالية أن هناك آثاراً كبيرة للدودة الدبوسيه على العديد من عناصر الدم ، بالإضافة إلى نقص التغذية. نتيجة لذلك ، فأن انخفاض معدل انتشار عدوى الطفيليات المعوية (خاصة الديدان الدبوسيه) بين الأطفال سوف يفيد نموهم وتطورهم ونتائجهم المدرسية.

1. Introduction

Malnutrition, anemia, micronutrient deficiencies and parasite infections continue to have an impact on children's nutrition and health in underdeveloped countries [1]. All of these outcomes happen frequently, and to poor and undeveloped communities [2]. Parasitic infections are thought to contribute to child malnutrition, anemia and micronutrient deficiencies through small impairments in digestion and absorption, prolonged inflammation and nutritional loss [3]. Dietary intake, digestion and absorption, metabolism and the maintenance of nutritional reserves can all be influenced by parasites [4]. The most prevalent parasites connected to nutritional status are intestinal parasites [5]. These parasites rely on their hosts for survival and they need food to fulfill essential functions like growth and reproduction [6]. *Enterobius vermicularis* (*E. vermicularis*) infection is a parasitic disease that is linked to malnutrition, anemia and nutritional deficiencies in children [7]. *E. vermicularis* is one of the gastrointestinal nematodes that are found all over the world. It is estimated that about 200 million persons are infected worldwide [8]. People living in tropical and sub-tropical regions are the most vulnerable [9]. The consumption of eggs causes Enterobiaisis. The fecal-oral route is the most common mode of transmission [10]. Inadequate personal hygiene, consuming contaminated food or water, poor environmental sanitation and living with infected individuals are all linked to a high prevalence rate of *E. vermicularis* infection [11]. *E. vermicularis* was reported to be the most common helminth parasite in Iraq [12, 13]. Many researchers have surveyed its epidemiology and demographic status in populations from various parts of Iraq [14-16]. Other researchers have looked at enterobiasis and its relationship to enuresis, anaemia, blood biochemical parameters and vitamin deficiencies [17-20]. Even though various research have been performed on *E. vermicularis*, we still need to update our understanding of its possible impact on the hematological, micro nutritional and antroponotic measurements of the infected children. The goal of this study was to assess nutritional, hematological and micronutrient status of *E. vermicularis* infected children in Baghdad.

2. MATERIALS AND METHODS

2.1. Study Design and Subjects

This was a case-control study that took place from October 2021 to March 2022. In this study, children of both genders were enrolled in primary health care clinics, public elementary schools and an orphanage in Baghdad, Iraq. The samples consisted of 100 children who met the inclusion criteria. Children under the age of 16 who were willing and had their guardians' permission to participate in the study met the inclusion criteria. They would not have taken anti-helminth or anti-protozoa medication in the four months prior to the trial, and they would not have had asthma, atopic dermatitis, immunodeficiency, malignancies, rheumatic disease, or other infections in the previous four months. Obesity was a criterion for exclusion, as were defective or missing stool and blood samples. The Ministry of Health and Environment in Baghdad, Iraq approved the study protocol which was also approved by the local ethics committee (Ref.: CSEC/0122/0009), Department of Biology, College of Science, University of Baghdad. Permissions from the authorities of the primary school and the orphan care center were also taken. The 100 participants were divided into two groups based on their cellophane tape for *E. vermicularis* examination which was achieved according to Dudlová *et al*. [9]. Cellophane tape (Scotch, USA) was placed to the anal and perianal regions of the participants using the sticky side of the tape for 2-3 times before putting the tape on to a glass slide. This procedure was made possible with the help of the children's guardians and the operation was performed either early morning or at night before defecation. The slides were then brought to the laboratory and examined under a light microscope (1000x). The first group was diagnosed as *E. vermicularis* infected children (n=50), while no *E. vermicularis* was detected in other group which was considered as *E. vermicularis* negative group (n=50).

2.2. Data Collection

For each child in this study, a questionnaire form was created and completed by interviewing the children's guardians to get demographic information, background characteristics of the children, and information on the children's past and current illnesses. Using standardized protocols, the midpoint of duplicate measurements was utilized to quantify body weight and height [21]. When weighed, the children were dressed in the bare minimum of clothing and wore no shoes. The calculator at https://reference.medscape.com/calculator/ was used to determine the weight-for-age Z score and the height-for-age Z score.

2.3. Blood Collection and Serum Preparation

Venipuncture technique was used to obtain about 5-6 ml of blood from each participant. Then about 1-1.5 ml of the obtained blood samples were immediately placed in EDTA tubes for anticoagulation, kept in a cooling box and were, within one hour, sent to the laboratory for hematological analysis. The remaining blood samples were placed in suction, clot, and gel activator tubes and allowed to coagulate for roughly 30 minutes at room temperature before being centrifuged for 10 minutes at 3000 rpm. Each serum sample was split into five equal parts. Each portion was put into a sterile Eppendrof tube for biochemical analysis using a sterile micropipette. All blood samples were tested within 10 hours of being drawn.

2.4. Laboratory Analysis

Quantitative measurements for hemoglobin, ferritin, total iron binding capacity (TIBC), serum iron, serum zinc and serum magnesium were evaluated in this study for all participants. Hemoglobin concentration was measured using an automatic CBC-hematology analyzer (SPAINREACT, Spain). Ferritin concentration was measured by fluorescence immunoassay analyzer (FIA meter, FINCARE, China). While each total iron-binding capacity (TIBC),

serum iron, serum zinc and serum magnesium were measured by spectrophotometer (Human Diagnostics, Germany). All these tests were done according to their manufacturer's directions.

2.5. Statistical Analysis

Statistical analysis of data was made using SAS (Statistical Analysis System - version 9.1). Chi-square was used to analyse the differences between *E. vermicularis* +ve and –ve regarding the prevalence of anaemia, low ferritin and low serum iron levels. As well as student t-test was used to assess the significant differences between *E. vermicularis* +ve and –ve children regarding the means of weight-for-age Z score, height-for-age Z score, hemoglobin, ferritin, total iron binding capacity (TIBC), serum iron, serum zinc and serum magnesium. $P < 0.05$ was considered statistically significant. All data was expressed as mean± standard deviation (SD).

3. Results

3.1. Infection rates and nutritional status

One hundred children, aged between 3 to 16 years, were involved in this study, . Forty-three subjects were between 3 to 9 and 57 between 10 and 16 years. Table 1 demonstrates the infection rates of *E. vermicularis* in the surveyed population. D-shaped eggs of *E. vermicularis* were seen under a light microscope in all positive cases (Figure 1). Gender of children had no significant relation with the infection rates. Although the highest infection rate was recorded in females (52.77%) versus males who showed low infection rate (42.85%). As well as no significant differences were observed between the two age groups, although a high infection rate (58.13%) was recorded among those who were between 3-9 years (Table 1). The results of weight for age Z score showed that100% children who had severe malnutrition were infected with *E. vermicularis* as well as 90% of underweight children were infected with *E. vermicularis*. Chi-square analysis showed that the nutritional status, based on weight for age Z score, was significantly ($P<0.05$) related to *E. vermicularis* infection. On the other hand no significant ($P>0.05$) relation was noticed between nutritional status, based on height for age Z score, and positive rates of *E. vermicularis.* Although both severe and stunt cases of malnutrition were recorded in high percentages (80%) and (66.6%) respectively among children who had *E. vermicularis* infection (Table 2).

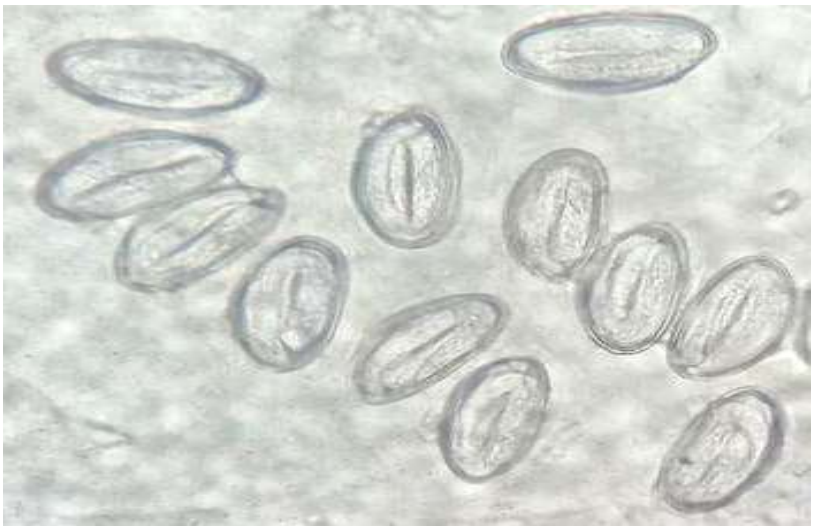

Figure 1: Ova of *Enterobius vermicularis* under light microscope (400X).

Table 1: An infection rate of *E. vermicularis* genders and age groups

Variables	*N*	*E. vermicularis* +ve Children N(%)	*E. vermicularis* -ve Children N(%)	X^2(P-value)
Gender				
Male	28	12 (42.85%)	16(57.14%)	0.7(0.3)
Female	72	38 (52.77%	43(59.72%)	
Age (years)				
3-9	43	25 (58.13%)	18(41.86%)	0.65(0.41)
10-14	57	25 (43.85%)	32(56.14%)	

Table 2: Nutritional status of the surveyed children based on weight for age- Z score and height for age- Z score

Variables	*N*	*E. vermicularis* +ve Children N(%)	X^2(P-value)
Weight for age- Z score			
Below -3 (Sever)	5	5 (100%)	14.69(0.0006)*
Below -2 (Underweight)	11	10 (90.9%)	
Normal & overweight	84	35 (41.66%)	
Height for age- Z score			
Below -3 (Sever)	5	4(80%)	4.26(0.11)
Below -2 (Stunted)	15	10 (66.6%)	
Normal	80	36 (45%)	

3.2. Hemoglobine Concentrations and *E.vermicularis* Infection

Results showed that 56 children out of the total participants (n=100) had anaemia (Hemoglobin concentration <11.5g/dl). The prevalence of anaemia among children with *E. vermicularis* infections was significantly greater (76.87%) than its prevalence (23.21%) in non-infected children. Statistical analysis showed that anemia was significantly ($P<0.05$) related to the occurrence of *E. vermicularis* (Table 3). Results also showed that hemoglobin concentrations had decreased significantly ($P < 0.05$) in *E. vermicularis* +ve children (10.66 ± 0.8 g/dl) compared to the children who tested –ve (12.37 ± 1.26 g/dl) (Table 4).

Table 3: Prevalence of anaemia in *E. vermicularis* +ve and *E. vermicularis* –ve groups

Variables	*N*	*E. vermicularis* +ve Children N(%)	*E. vermicularis* -ve Children N(%)	X^2(P-value)
Anaemic children (Hemoglobin concentration <11.5g/dl)	56	43 (76.78%)	13(23.21%)	36.52(0.00)*
Non- anemic children (Hemoglobin concentration >11.5 g/dl)	44	7 (15.9%)	37(84.09%)	

Table 4: Hemoglobin concentration *E. vermicularis* +ve and *E.vermicularis* –ve groups

Population	Hemoglobin g/dl (Mean±SD)	P value	
***E. vermicularis* +ve children (n=50)**	10.66±0.8	0.0000001	T-test= 7.8
***E. vermicularis* -ve children (n=50)**	12.37±1.26		

3.3 Ferritin Concentrations and *E.vermicularis* Infection

Results showed that of all the participants, low ferritin level (Ferritin concentration < 12 ng/ml) occurred in 8 children. The low ferritin was not significantly associated with the occurrence of *E. vermicularis*, although the prevalence of low ferritin levels was higher among the *E. vermicularis* +ve group (75%) (Table 5). On other hand, significant differences ($P < 0.05$) regarding ferritin concentration were observed between *E. vermicularis* +ve children and *E. vermicularis* –ve children. Highest level of serum ferritin (29.73± 15.54 ng/ml) was recorded among *E. vermicularis* +ve children while serum ferritin low level (51.67±46.56 ng/ml) was detected in *E. vermicularis* -ve children (Table 6).

Table 5: The prevalence of low and normal ferritin levels among studied groups

Variables	*N*	*E. vermicularis* +ve Children N(%)	*E. vermicularis* -ve Children N(%)	X^2(P-value)
Low ferritin level (Ferritin concentration < 12 ng/ml)	8	6 (75%)	2(25%)	2.17(0.14)
Normal ferritin level (Ferritin concentration >12 ng/ml)	92	44 (47.82%)	48(52.17%)	

Table 6: Serum ferritin concentration in *E. vermicularis* +ve and *E. vermicularis* –ve groups

Population	Serum fFrritin ng/ml (Mean±SD)	P-value	
***E. vermicularis* +ve children (n=50)**	29.73±15.54	0.001	T-test=3.16
***E. vermicularis* -ve children (n=50)**	51.67±46.56		

3.3. Total Iron-binding Capacity (TIBC) and *E. vermicularis* Infection

The results showed that the matter was different from the concentrations of the TIBC. *E. vermicularis* +ve group revealed a high level of TIBC (446.56±83.69 mg/dl) versus low level (332.56±69.89 mg/dl) in the *E. vermicularis* -ve group. Statistical analysis revealed a significant difference ($P < 0.05$) between *E. vermicularis* +ve and *E. vermicularis* –ve groups for the total iron binding capacity (TIBC) (Table 7).

Table 7: Total iron binding capacity concentration in *E. vermicularis* +ve and *E. vermicularis* –ve groups

Population	Total Iron Binding Capacity mg/dl (Mean±SD)	P-value	
***E. vermicularis* +ve children (n=50)**	446.56 ±83.69	0.0000001	T-test= 7.39
***E. vermicularis* -ve children (n=50)**	332.56±69.89		

3.4. Iron, Zinc and Magnesium Levels and *E. vermicularis* Infection

Significant relation (P<0.05) was observed between low iron levels (iron concentration <50 mg/dl) and *E. vermicularis* occurrence. The prevalence of low iron levels was noticed in the majority (75.43%) of those who were infected (Table 8). As well as serum iron levels had decreased (28.75±17.57 mg/dl) significantly (P<0.05) in children infected with *E. vermicularis* while the iron level stayed in its normal level among *E. vermicularis* –ve children (61.23±22.13 mg/dl) (Table 9).

The results of zinc did not differ from the results of iron, as the *E. vermicularis* -ve group showed low means of serum iron (53. 01±16.24 mg/dl) versus *E. vermicularis* -ve group who showed high means of serum iron (68.3±14.47 mg/dl) (Table 10). Finally, magnesium levels also showed significant differences between the *E. vermicularis* +ve group and the -ve group. Low levels of magnesium were observed in the *E. vermicularis* +ve group (1.89±0.23 mg/dl) versus the -ve group who showed high level of magnesium (2.05 ±0.35 mg/dl) (Table 11).

Table 8: The prevalence of low and normal serum iron levels among studied groups

Variables	*N*	*E. vermicularis* +ve Children N(%)	*E. vermicularis* -ve Children N(%)	X^2(P-value)
Low serum iron level (iron concentration <50 mg/dl)	57	43 (75.43%)	14(24.56%)	34.13(0.00)*
Normal serum iron level (iron concentration >50 mg/dl)	43	7(16.27%)	36(83.72%)	

Table 9: Serum iron concentration in *E. vermicularis* +ve and *E. vermicularis* –ve groups

Population	Serum Iron mg/dl (Mean±SD)	P value	
***E. vermicularis* +ve children (n=50)**	28.75±17.57	0.000001	T-test=8.12
***E. vermicularis* -ve children (n=50)**	61.23±22.13		

Table 10: Serum zinc concentration in *E. vermicularis* +ve and *E. vermicularis* –ve groups

Population	Serum Zinc mg/dl (Mean±SD)	P value	
***E. vermicularis* +ve children (n=50)**	53.01±16.24	0.000001	T-test=4.9
***E. vermicularis* -ve children (n=50)**	68.3±14.74		

Table 11: Serum magnesium concentration in *E. vermicularis* +ve and *E.vermicularis* –ve groups

Population	Serum Magnesium mg/dl (Mean±SD)	P value	
***E. vermicularis* +ve children (n=50)**	1.89±0.23	0.004	T-test=2.6
***E. vermicularis* -ve children (n=50)**	2.05±0.35		

4. Discussion

Malnutrition, anemia and micronutrient deficiencies are health problems that affect children all over the world. Given that *E. vermicularis* infection is one of the most common

causes of anaemia and nutritional deficits in children, some hematological and micronutrients characteristics of *E. vermicularis* infection must be discussed. There were some difficulties and limitations in the present study which included the small sample size and the lack of cooperation from most guardians. No significant relation was noticed between gender and the infection of *E. vermicularis* in this current study, although the majority of pinworm positive cases were reported among females. This is possibly because children who attend school, regardless of gender can be exposed to the same conditions that make them vulnerable to pinworm infection. This result was consistent with the findings of Al-Daoody and Al-Bazzaz [20] who found no significant relationship between *E. vermicularis* prevalence and gender. While these findings disagreed with those of Dohan and Al-Warid, 2022 [19] who reported a significant link between gender and the presence of *E. vermicularis*. The overall incidence of enterobiasis is far greater among 3-9 years old compared to other age class (10-14 years). Although no significant differences were noticed between the two age groups. This high proportion of infection among young children may be attributed to direct contact transmission, which is especially common among kindergarten and primary school students [22]. In addition, schoolchildren of this age show variations in exposure to environments that promote the transmission of the infective stages of most helminths, including *E. vermicularis.* Another study discovered that this age group's hand-washing behaviour is quite poor [23]. Results also showed that bad nutritional statuses, scored by the weight-for-age Z score and the height-for-age Z score, were correlated significantly with *E. vermicularis* infection. The majority of severe malnutrition, underweight and stunted children were recorded among those children who had *E. vermicularis* infection. Intestinal parasitism can affect children's nutrition and cause them to "fail to thrive." Abnormalities in lipid, D-xylose, vitamin A and vitamin B12 absorption in the intestine have been linked to parasite infections [19, 20, 24]. This result agreed with the results of Nematian *et al*. [25]who found that the risk of stunting was significantly higher in children infected with *E. vermicularis*. The current investigation found a link between anemia and enterobiasis. This parasite had infected majority of the anemic children. In children with enterobiasis, hemoglobin concentration was significantly lower. The effect of parasitism is most likely to be responsible for the substantial connection between anemia and *E. vermicularis* infection. Intestinal parasites are predicted to reduce food intake and absorption, as well as an act through chronic diarrhea and enteropathies [26]. This result corroborated with findings from studies by(Kadir and Aziz [27] and Dohan and Al-Warid [19], both demonstrating a link between enterobiasis and hemoglobin deficiency.

Ferritin levels also varied between infected and non-infected children in the current study; non-infected children had higher levels, while infected children had lower levels. It is concluded that enterobiasis has a negative effect on iron stores which is indicated by serum ferritin. These findings supported those of Le *et al*. [28] who found that ferritin levels in children afflicted with intestinal parasites were low. On the other hand, the findings contradicted those of Silva *et al*. [29] who found that ferritin serum concentrations were higher in infected children with gastrointestinal parasites than in non-infected children. The parasite type and sample size analyzed in this study differed from those in others. The results of total binding capacity (TIBC) indicated that *E. vermicularis* +ve group had high TIBC level than that of *E. vermicularis* –ve group. The plasma concentrations of TIBC do not change fast, so TIBC can indicate a chronic *E. vermicularis* infection, but it is not a good predictor of early iron insufficiency because values do not alter until reserves are depleted [30].

It was observed in this study that iron, zinc and magnesium declined significantly among those who had pinworm infections compared with the children who had no pinworm infections. Low iron, zinc, and magnesium levels in the blood could damage cellular, physiological and enzymatic processes [31]. Zinc is not stored in considerable quantities in

the body. In children with poor zinc intake, serum zinc levels can quickly drop during illnesses. Zinc levels in the blood were often lower during protozoan and helminth infections [32]. In some other research, serum zinc levels in children with enterobiasis were found to have decreased [20, 33] because magnesium levels in human serum were shown to be significantly low in children who were diagnosed with this parasite. Magnesium might be utilized as a reliable and fast indication of *E. vermicularis* parasites inhabited in the intestines of children [33].The findings of the current study revealed that *E.vermicularis* infection had severe consequences on some hematological elements and stunted micronutrients deficiencies. So, reduced prevalence of intestinal parasite illness (especially enterobiasis) among children would most likely have a positive impact on child growth, development and educational outcomes.

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