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Haematological and Demographic Study in Children Infected with Enterobiasis in Al Diwaniyah Province, Iraq

Baraa Ali Mohammed Dohan, Harith Saeed Al-Warid*

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

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Abstract

Enterobius vermicularis infection is considered as one of the important causes of anaemia and malnutrition among children. This topic has recently received an increased amount of attention. The objective of this study is to evaluate the demographical, anthropometrical, nutritional, and haematological status of *E. vermicularis* infection among children. This study was conducted in Al Diwaniyah province, south of Iraq, for the period of October 2020 to the end of January 2021. The study included 122 children from both genders (males, n= 61, and females, n=61) and their ages ranged between 1 and 14 years. Nutritional status, body mass index (BMI), BMI percentile, and weight- for- age Z score were evaluated for some participants. In addition, samples of cellophane tape and blood were collected from all participants. The cellophane tape samples were examined microscopically for *E. vermicularis* detection, whereas blood samples were processed to evaluate several parameters, which were hemoglobin, serum iron, and total iron binding capacity (TIBC), ferritin, and vitamin B12. The results revealed a high infection rate (73.77%) among the studied population. The results also showed a significant correlation between gender and the occurrence of *E. vermicularis* infection. Males showed a higher risk of enterobiasis than females. While no association was noticed between each of age, nutritional status, and enterobiasis. Also, a significant correlation was recorded between *E. vermicularis* positive status and anaemia. The results showed that each of BMI, weight- for- age Z score, haemoglobin, serum iron, total iron binding capacity (TIBC), and ferritin had significantly lower values among those who were positive for *E. vermicularis*. Finally, the level of vitamin B12 was insignificantly decreased in children with enterobiasis. These findings indicate that more hematological changes are expected to occur in children with enterobiasis. Therefore, further consideration and investigation would be required on this topic in Iraq.

Keywords: Anaemia, children, *Enterobius vermicularis*

دراسة العوامل الدموية و الديموغرافية للأطفال المصابين بعدوى الديدان الدبوسية في محافظة الديوانية

براء علي محمد دوحان, حارث سعيد الورد*

قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

من المحتمل ان يكون الخمج بالدودة الدبوسية *Enterobius vermicularis* احد الاسباب المهمة التي تقترن مع فقر الدم و سوء التغذية بين الاطفال. لذلك فان هذا الموضوع نال شيئاً من الاهتمام خصوصاً في

*Email: harith.saeed@sc.uobaghdad.edu.iq

الأونة الأخيرة. هدفت الدراسة الى تقييم بعض العوامل الديموغرافية، القياسات البشرية، الحالة التغذوية والدموية للخمج بالدودة الدبوسية لدى الأطفال. اجريت الدراسة في محافظة الديوانية جنوب العراق ضمن المدة الواقعة بين تشرين الأول من العام 2020 و لغاية كانون الثاني من العام 2021. شملت الدراسة 122 طفلاً من كلا الجنسين (الذكور=61، الاناث=61) تراوحت اعمارهم من 1-14 سنة. تم تقييم كل من الحالة التغذوية، مؤشر الكتلة الحيوية. النسبة المئوية لمؤشر الكتلة الحيوية و قيمة (Z) الخاصة بالوزن الى العمر. لبعض المشتركين بالدراسة. كما تم جمع كل من عينات الشريط اللاصق وعينات الدم من جميع الاطفال المشاركين. تم فحص الشريط اللاصق تحت المجهر للتحري عن الاصابة بالديدان الدبوسية بينما خضعت عينات الدم الى جملة من الفحوصات للتحري عن كل من الهيموكلوبين ، المستوى المصلي للحديد، سعة الارتباط بالحديد، الفيريتين و فيتامين ب-12. أظهرت النتائج معدلاً مرتفعاً للخمج بالدودة الدبوسية بين الاطفال المشتركين في الدراسة والتي بلغت (73.77%). كما بينت الدراسة وجود علاقة معنوية بين الجنس و وقوع الاصابة بالديدان الدبوسية حيث اظهر الذكور مخاطر اعلى من الاناث للخمج بهذا الديدان. بينما لم يلاحظ اي ارتباط معنوي بين كل من العمر والحالة الغذائية مع الخمج بهذه الديدان. الا ان النتائج سجلت وجود علاقة معنوية بين الخمج بالديدان الدبوسية و فقر الدم لدى الاطفال المشاركين. في حين كانت معدلات كل من مؤشر الكتلة الحيوية، قيمة (Z) الخاصة بالوزن الى العمر، الهيموكلوبين، المستوى المصلي للحديد، سعة الارتباط بالحديد و الفيريتين واطئة و بشكل معنوي لدى الموجبين لفحص الديدان الدبوسية. بينما سجلت النتائج انخفاضاً غير معنوي في فيتامين ب-12. لدى الموجبين لفحص الديدان الدبوسية. خلُصت نتائج هذا البحث الى ان العديد من التغيرات في العوامل الدموية ان تكون متوقعه بشكل كبير بين الاطفال الذين لديهم خمجاً بالديدان الدبوسية. لذلك فان كثيراً من الاعتبار و مزيداً من الدراسة يمكن ان يولي للموضوع في العراق.

1. Introduction

Gastrointestinal parasitic diseases are highly prevalent all over the world, especially in the developing countries [1]. These diseases pose public health problems of high, not only medical, but also social and economic importance [2]. These diseases display high prevalence in poor communities and especially in school and nursery children as a general population, since such conditions produce severe health problems and are associated with growth deficiency, anaemia, vitamins deficiency, and diminished general health status [3, 4]. Intestinal parasitic diseases can be caused by either protozoa or helminths, both causing anemia [5, 6] by reducing iron uptake from the intestine or by destructing the intestinal mucosa, which affects the absorption of iron and other elements [7]. Enterobiasis, a disease caused by *Enterobius vermicularis* (*E. vermicularis*), is among those helminthic infections that known to be related to such kind of morbidity [8]. *E. vermicularis* is commonly known as pinworm. It is one of the helminths that has a wide geographical distribution. It has been assessed to infect about 200 million people worldwide, [9]. People from tropical and sub-tropical climates are the most susceptible categories [10, 11]. Infection with *E. vermicularis* is caused by the ingestion of the pinworm eggs. Transmission occurs most commonly through the fecal-oral route [12]. High prevalence rate of *E. vermicularis* infection is associated with inadequate personal hygiene, consuming contaminated food or water, improper environmental sanitation, and living with infected individuals [13]. In Iraq, *E. vermicularis* was found to be the predominant helminth parasite [14, 15]. There have been many studies that reported the epidemiological and demographic status of *E. vermicularis* among the population from different parts of Iraq [16-18]. In addition, some other investigators focused on enterobiasis and its relation to enuresis, anaemia, biochemical parameters of the blood, and vitamin deficiencies [19-21]. Although several studies were achieved regarding *E. vermicularis*, updating our knowledge is still needed about its potential influence on the haematological, nutritional status, and immunological status of the host infected with *E. vermicularis*. Hence,

the purpose of this study is to evaluate the demographic, anthropometric, nutritional, and haematological status of *E. vermicularis* among children in Al Diwaniyah province.

2. Materials and Methods

2.1. Study design and subjects

A case control study was conducted in Al Diwaniyah province from October 2020 to January 2021. One hundred and twenty two children participated in this study. The mean age of them was 7.4 ± 3.2 years old (range: 1-14 years). Both sexes were included (male, n= 61; female, n=61). All the included participants fitted the inclusion criteria. The inclusion criteria were children aged ≤ 14 years, no anti-parasitic medication in the previous few months prior to the sampling, and no history of asthma, atopic dermatitis, immunodeficiency, malignancies, rheumatic disease, and other infections. Parents' authorization for participation in this study was obtained. The selected locations for sampling included different districts (Sumer, Al Dagara, Afag, Al Shafaya, Al Hamza) and the city center of Al Diwaniyah province, Iraq.

2.2. Data Collection, Anthropometry, and Nutritional status

A questionnaire form was arranged and used for each child in this study. The questionnaire was filled out by interviewing their parents to take information regarding some demographic information, background characteristics of children, and children's past and present sickness. The mean value of duplicate measurements of body weight and height was calculated using standardized procedures [22]. The children wore only the bare minimum of clothes and no shoes when they were weighed. Body Mass Index (BMI), which is the weight in kilograms of a person divided by the square of the height in meters, was considered to evaluate the nutritional status of the children. It was classified into the categories of severe malnutrition (BMI < 15.9 kg/m²), moderate and mild malnutrition (BMI = 16–18.4 kg/m²), and normal (BMI = 18.5-25 kg/m²) [22]. The nutritional status was evaluated only for those aged ≥ 5 years old. Weight-for-age Z score was calculated using the online calculator found in <https://reference.medscape.com/calculator/>. Both BMI and weight-for-age Z score were considered for those aged ≥ 2 years old.

2.3. Cellophane tape for *E. vermicularis* examination

E. vermicularis eggs were identified using cellophane tape (scotch, USA) by applying the sticky side of the tape for 3-4 times on the anal and perianal region of the participants and then sticking the tape on a glass slide. This method was achieved by the assistance of the children's parents. The procedure was conducted in the early morning or at night before defecation [23]. The slides were transported to the laboratory and examined under a light microscope (1000x).

2.4. Blood collection and serum preparation

About 4-5 mL of blood was collected from each participant, using the venipuncture technique. Then, about 1-1.5 mL of the collected blood sample was added immediately into EDTA tubes for anticoagulation, preserved in a cooling box, and transported to the laboratory within one hour for hematological analyses. The rest of blood sample was applied into vacuomed, clot, gel activator tubes and left for about 30 minutes at room temperature to clot. All samples were centrifuged at 3000 rpm for 10 minutes. Each serum sample was divided into 5 parts. Each part transferred by a sterile micropipette into a sterile Eppendorf tube for biochemical analyses. All blood samples were analyzed within 10 hours after blood collection.

2.5. Hematological analyses

Hemoglobin concentration was measured using automatic hematology analyzer (ABBOTT Diagnostic, USA). The analyses were performed according to the manufacturer's instructions.

2.6. Biochemical analyses

Biochemical quantitative measurements for serum iron, total iron binding capacity (TIBC),

ferritin and vitamin B12 were evaluated for all children. Serum iron was measured using an automated clinical chemistry analyzer system (Mindary BS-230, Mindary bio-medical electronics Co., Ltd., China). TIBC was analyzed by a spectrophotometer (BIOLAB specific kit, France). Both ferritin and vitamin B12 concentrations were measured by an automated immunoassay analyzer system (Beckman Coulter, Inc., USA). All these tests were conducted according to their manufacturer's directions.

2.7. Statistical Analysis

Statistical analysis of data was applied using SAS (Statistical Analysis System - version 9.1). Student t-test was used to assess the significant differences between *E. vermicularis* +ve and *E. vermicularis* -ve children regarding the means of weight-for-age Z score, BMI, BMI percentile, serum iron, Total Iron Binding Capacity (TIBC), ferritin, and vitamin B12. The infection rates were compared by using the Chi-square test. Differences at $P < 0.05$ were considered statistically significant. All data are expressed as mean \pm SD.

3. Results

One hundred and twenty two children were involved in this study. Their age ranged between 1 and 14 years. Fifty-three participants were between 1 to 6, while 69 were between 7 and 14 years old.

Table 1 illustrates the infection rates of *E. vermicularis* in the surveyed population. Ova of *E. vermicularis* were noticed under light microscope in all positive cases (Figure 1). The total infection rate was 73.77%, i.e. 90 out of 122 children were positive for *E. vermicularis* infection. The results showed a significant difference ($P=0.03$) between males and females regarding *E. vermicularis* infection. Males showed a higher infection rate (81.96%) in comparison with females (65.57%) (Table 1). While, no significant differences were noticed between age groups. Both age groups (1-6 and 7-14 years) showed very close infection rates (73.85% and 73.91%, respectively). Also, no significant differences were realized between age \times gender groups, although a high infection rate (85.29%) was recorded among those males who were 7-14 years old. The nutritional status was assessed in this study only for those aged ≥ 5 years old. No significant relation was noticed between nutritional status and positive rates of *E. vermicularis*, although the infection rates were slightly higher in children with moderate and mild malnutrition (Table 2). Additionally, both weight-for-age Z score and BMI were scored for participants who were ≥ 2 years old. Weight-for-age Z score was found to be significantly lower (0.2 ± 1.5) in children with *E. vermicularis* infection ($P=0.04$), whereas it was higher (0.9 ± 2.2) in children who had no Enterobiasis (Table 3). Moreover, significant differences ($P < 0.05$) were noticed between *E. vermicularis* +ve and *E. vermicularis* -ve groups regarding BMI. The BMI was higher ($20.26 \pm 5.6 \text{ kg/m}^2$) among *E. vermicularis* -ve compared with $17.3 \pm 5.6 \text{ kg/m}^2$ in *E. vermicularis* +ve group. While, no significant differences were recorded between *E. vermicularis* +ve and *E. vermicularis* -ve groups regarding BMI percentile, although it was higher in *E. vermicularis* -ve group (Table 3). Moreover, a significant correlation ($P < 0.05$) between anemia and the occurrence of *E. vermicularis* was noticed. All (100%) of the anemic children (Haemoglobin concentration $< 11.5 \text{ g/dl}$) were positive for *E. vermicularis* versus only 69.81% of non-anemic children (Haemoglobin concentration $> 11.5 \text{ g/dl}$) (Table 4).

The values of haemoglobine, serum iron, total iron binding capacity, ferretting and vitamin B12 in *Enterobius vermicularis* +ve and *Enterobius vermicularis* -ve groups are allustrated in Table 5. Heamoglobine concentrations were decreased significantly ($P < 0.05$) in *E. vermicularis* +ve ($12.34 \pm 1.15 \text{ g/dl}$) children compared with *E. vermicularis* -ve children ($13.25 \pm 0.7 \text{ g/dl}$). Significant differences ($P < 0.05$) were realized between *E. vermicularis* +ve children and *E. vermicularis* -ve children regarding serum iron. The highest level of serum iron ($17.65 \pm 4.7 \text{ Mmol/L}$) were recorded among *E. vermicularis* -ve children, while a lower level ($14.91 \pm 5.7 \text{ Mmol/L}$) was noticed in *E. vermicularis* +ve children. The

differences in TIBC were also found to be significant ($P < 0.05$) between *E. vermicularis* +ve children and *E. vermicularis* -ve children. TIBC was in its highest value (61.84 ± 13.5 Mmol/L) among those children who had no *E. vermicularis* infection, compared with a lower level (51.63 ± 15.83 Mmol/L) in *E. vermicularis* +ve children. More significant differences were noticed regarding ferritin concentration. It was low (25.13 ± 16.75 ng/ml) among *E. vermicularis* +ve children, while higher (40.35 ± 13.41 ng/ml) in *E. vermicularis* -ve children. On the other hand, no significant differences were detected between *E. vermicularis* +ve and *E. vermicularis* -ve groups in vitamin B12 levels, although *E. vermicularis* -ve group showed a higher level (316.16 ± 78.99 ng/ml) in comparison with *E. vermicularis* +ve group (282.23 ± 86.6 ng/ml).

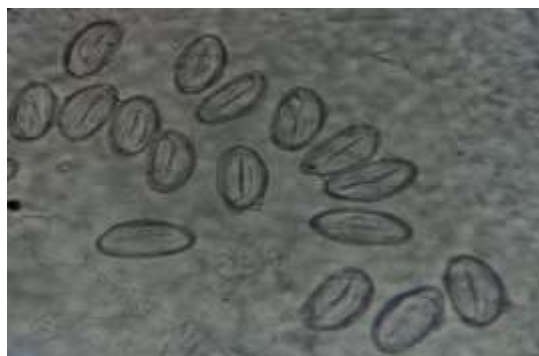


Figure 1- Ova of *E. vermicularis* under light microscope (400 X), diagnosed using using cellophane tape (scotch, USA)

Table 1- Prevalence of *E. vermicularis* regarding gender and age groups

Group	N	n(%) <i>E. vermicularis</i> positive individuals	X ² (P-value)
All	122	90 (73.77%)	-
Male	61	50(81.96%)	4.23 (0.03)*
Female	61	40 (65.57%)	
Age (years)			
1-6	53	39 (73.58%)	0.02 (0.9)
7-14	69	51 (73.91%)	
Male, 1-6 years	28	21 (75%)	4.55(0.2)
Female, 1-6 years	25	18 (72%)	
Male, 7-14 years	34	29(85.29%)	
Female, 7-14 years	35	22 (62.85%)	

Table 2- Prevalence of *E. vermicularis* among different groups regarding their nutritional status only for those aged ≥ 5 years old.

Malnutrition based on BMI	N	n(%) <i>E. vermicularis</i> positive individuals	X ² (P-value)
Severe malnutrition (BMI < 15.9 kg/m ²)	29	21 (72.4%)	0.6(0.7)
Moderate, Mild malnutrition (BMI = 16–18.4 kg/m ²)	41	32 (78.04%)	
Normal (BMI = 18.5-25 kg/m ²)	30	21 (70%)	

Table 3- Weight for age (WAZ), BMI and BMI percentile in *Enterobius vermicularis* +ve and *Enterobius vermicularis* -ve groups, only for those aged ≥ 2 years old.

Mean \pm SD	<i>E. vermicularis</i> +ve children (n=88)	<i>E. vermicularis</i> -ve children (n=31)	T(P-value)
Weight for age, Z score (WAZ)	0.2 \pm 1.5	0.9 \pm 2.2	2.1 (0.04)*
BMI (kg/m ²)	17.3 \pm 2.9	20.26 \pm 5.6	3.6 (0.0004)*
BMI percentile	59.07 \pm 31.07	63.06 \pm 31.7	0.6 (0.5)

Table 4- Prevalence of *Enterobius vermicularis* regarding anaemia status

Anaemia status based on haemoglobin concentration	N	n(%) <i>E. vermicularis</i> positive individuals	X ² (P-value)
Anaemic children (Haemoglobin concentration <11.5g/dl)	16	16 (100%)	6.54 (0.01)*
Non Anaemic children (Haemoglobin concentration >11.5 g/dl)	106	74 (69.81%)	

Table 5- The concentrations of haemoglobine, serum iron, total iron binding capacity, ferreting and vitamin B12 in *Enterobius vermicularis* +ve and *Enterobius vermicularis* -ve groups

Mean \pm SD	<i>E. vermicularis</i> +ve children (n=90)	<i>E. vermicularis</i> -ve children (n=30)	T(P-value)
Haemoglobin (g/dl)	12.34 \pm 1.15	13.25 \pm 0.7	3.73 (0.0003)*
Serum iron (Mmol/L)	14.91 \pm 5.7	17.65 \pm 4.7	2.4 (0.016)*
Total iron binding capacity (Mmol/L)	51.63 \pm 15.38	61.84 \pm 13.5	3.32 (0.0012)*
Ferritin (ng/ml)	25.13 \pm 13.41	40.35 \pm 16.75	5.15 (0.0001)*
Vitamin B12 (pg/ml)	282.23 \pm 86.8	316.16 \pm 78.99	1.9(0.054)

4. Discussion

Considering the point that *E. vermicularis* infection is likely among the important causes of anaemia and nutrients deficiencies in children, it is necessary to discuss some demographic, haematological, and biochemical aspects of *E. vermicularis* infection among children in Iraq, especially in rural areas. Most of the districts included in this study are considered to live in rural areas. The infectivity rate of *E. vermicularis* was relatively high (73.33%). This high infection rate can be explained by the inadequate personal hygiene that is more likely common in such kinds of communities [24]. Previous reports from some parts of Iraq have presented different infection rates of *E. vermicularis*. The study of Kadir, 2001, showed a lower infection rate than that of the current study. They showed 24.9% infection rate among 1008 children aged 1-12 years in Kalar/ Sulaimania province [25]. Also, the infection rate shown in the current study is higher than that obtained by Al-Daody *et al.* (2020) who showed 10.5% infection rate among 400 children aged 1-15 years in Dohuk province [26]. While, it is lower than the result of Hussein (2015) who showed 83.9% infection rate among 124 children aged 4-7 years in Najaf province [27]. It is also lower than the result of Al-Qhadi *et al.* (2011) who reported 84.3% infection rate among 51 orphan children aged 3-12 years in Baghdad [19]. The variations in infection rates of enterobiasis among different studies are likely due to some effects, including sample size, diagnostic technique used, living conditions, socio-economic situations, spatial changes, and demographic variations [28]. A significant relation was noticed between gender and the occurrence of *E. vermicularis* in the present study. Most *E. vermicularis* +ve cases were diagnosed in male individuals. This is possibly because males are more involved in outdoor activities [29], especially in Iraq, and therefore are highly exposed to be infected by playing with soil or contacting with infected children. This bias is nonetheless in agreement with other reports in Iraq [17, 25]. They showed high

infection rates in males compared with females. While, our results disagree with other investigations that presented higher infection rates of *E. vermicularis* among female children, such as the study of Al-Daody (2005) in Mosul [30] and the study of Hammadi (2012) in Al-Mahmoudyia area - Baghdad [31]. The study also revealed no association between the status of malnutrition and the occurrence of *E. vermicularis*, although the infection rates of *E. vermicularis* were high among severe, moderate, and mild malnutrition compared with subjects of normal BMI. While, both BMI and weight-for-age Z score were significantly declined among those who had *E. vermicularis* infection. Infections with some gastrointestinal parasites are associated with the reduction of nutrients and minerals uptake from the intestine by destructing the intestinal mucosa [5, 6]. Some intestinal parasites display adverse properties on weight gain, which may lead to insufficient food intake, which in turn may cause poor appetite and metabolic and clinical disturbances [32]. Low BMI and weight-for-age Z score among *E. vermicularis* +ve cases in the present study coincided with the study of Nematian *et al.* (2008) who stated that *E. vermicularis* is correlated with child growth. Their results also showed that *E. vermicularis* infection was significantly associated with a relatively low weight-for-age Z score [33]. On the other hand, the present study showed a significant relation between anaemia and the occurrence of enterobiasis. All the anaemic children had infections with this parasite. In addition, haemoglobin concentration was significantly decreased in children with enterobiasis. This significant correlation is likely because of the effect of parasitism. It is expected that intestinal parasites will compromise nutrient intake and absorption. They also act through persistent attacks of diarrhoea and enteropathies [34]. Serum iron declined significantly in *E. vermicularis* infected group as compared to non-infected group. The decrease in serum iron concentrations is likely because of appetite loss that may be a result of *E. vermicularis* infection [21]. This result is similar to that reported by others [35, 36]. The results of iron concentration were confirmed by TIBC analysis which indicated that *E. vermicularis* +ve group had less TIBC value than that of *E. vermicularis* -ve group. This result is likely considered as an indicator of very low iron concentration in the blood of infected children. Unlike serum iron, TIBC does not have rapidly changing concentrations in the plasma. However, it is not a useful marker of early iron deficiency, as the values do not change until stores are depleted [37]. Hence, the low TIBC can indicate a chronic *E. vermicularis* infection. Ferritin levels were also significantly varied in the current study, being higher in non-infected children. These results agree with the results of Le *et al.* (2007) who showed that ferritin was in its lowest level in children infected with some intestinal parasites [38]. On the other hand, the results disagree with the results of Silva *et al.* (2009) who showed that ferritin serum concentrations were higher in infected children with gastrointestinal parasite [39]. Differences between the results of the current study and those of others are likely due to the parasite species and sample size studied. While, vitamin B12 levels were non-significantly lower in *E. vermicularis* +ve group. These results agree with the findings of some other investigator [40, 41]. The deficiency of vitamin B12 is probably associated with anaemia. Many gastrointestinal parasites inhabit the intestine. Therefore, these parasites may lead to malabsorption and vitamin B12 deficiency [40].

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