IHJPAS. 35(4)2022

Ibn Al-Haitham Journal for Pure and Applied Sciences

Journal homepage: http://jih.uobaghdad.edu.iq/index.php/j/index

Investigation of the ADA Enzyme and Lipid Profile in Children Infected with *Enterobius Vermicularis*

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Article history: Received 29 June 2022, Accepted 11 August 2022, Published in October 2022.

Doi: 10.30526/35.4.2925

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Adenosine deaminase (ADA) and lipid profile may be associated with some diseases. Parasitic diseases have also been reported to be associated with ADA and lipid profiles. This study aimed to evaluate ADA, lipid profile, and total protein among children infected with E.vermicularis. This research was carried out in Baghdad province from October 2021 to the end of March 2022. One hundred samples were collected from both sexes, ranging in age from (3-16) years. They all underwent examination for ADA, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total protein levels. Results showed no significant differences between E.vermicularis positive group and E.vermicularis negative group regarding ADA, LDL, and triglycerides, although their levels were lower in children with E. vermicularis infection versus the control. In contrast, results showed significant differences ($P<0.05$) in cholesterol, HDL, and total protein between E.vermicularis positive group and E.vermicularis negative group. All these values decreased significantly compared to the control group.

Keywords: Adenosine deaminase; Enterobius vermicularis; lipid, protein

1. Introduction

One of the most prevalent human parasitic helminths, Enterobius vermicularis (pinworms), is thought to infect 200 million individuals globally, with children aged 5 to 10 making up more than 30% of cases [1]. Pinworm infection can be transmitted easier by a few things, such as inadequate personal or group cleanliness and overcrowding in preschools, schools, orphanages, and family groups, regardless of one's specific socioeconomic status, race, or culture [2, 3]. These circumstances encourage the spread of pinworm eggs from one person to another, either directly through the anus-to-mouth route and finger contamination or indirectly through contaminated objects, such as toys, school tables, chairs, or the floor [4, 5]. Preschoolers who live in crowded settings, such as kindergartens, are the most likely to have pinworm infections because personal cleanliness and exposure are major transmission factors [6]. Cells are used to create the host

immune system's defense against parasites. Cytotoxic substances, reactive oxygen, and intermediate nitrogen products play significant roles in activated cells. These items are oxidizing chemicals that negatively impact the viability of parasites [7, 8]. In mammals, adenosine, two deoxyadenosine, and known ribozids are all catalyzed by adenosine deaminase (ADA), aminohydrolase involved in the breakdown of nucleotides [9].

On the other hand, lymphocytic cells have ten times greater ADA activity than erythrocytes. Researchers have identified ADA serum levels for various illnesses, including typhoid, acute pneumonia, tuberculosis, sarcoidosis, liver diseases, acute leukemia, various malignity, rheumatoid arthritis, systemic lupus erythematous (SLE), and Behcet's syndrome [10, 11]. ADA can be thought of as an immunity indicator. These infections included parasitic illnesses as well. Some researchers looked into this enzyme's activity in people with parasite illnesses. (Karman et al., 2009) looked at the prevalence of ADA in individuals with giardiasis and toxoplasmosis [12]. Patients with visceral leishmaniasis with various clinical states were investigated for serum ADA activity by [13]. The current study aimed to evaluate ADA, lipid profile, and total protein levels between Enterobius vermicularis positive children and control children, taking into account ADA as cellular and humoral immunity signal and as it acts on glucose and lipid metabolism.

2. Materials and Methods

2.1. Study design and subjects

Between October 2021 and March 2022, case-control research was conducted. Samples were collected from children in public elementary schools, primary health care clinics, and an orphanage in Baghdad, Iraq, recruited for this study. One hundred children of both sexes (male, n=28; female, n=72) were included. Their age ranged from 3 to 16 years. Forty-three were between 3 and 9, and 57 were between 10 and 16. The Ministry approved the study protocol of Health and Environment in Baghdad, Iraq, and the local ethics commission (Ref.:CSEC/0122/0009), Department of Biology, College of Science, University of Baghdad. The administrators of the primary school and the orphan care facility also gave their consent. Cellophane tape was used to examine each subject (Scotch, USA) for E.vermicularis infection. Before attaching the tape to a glass slide, the adhesive side of the tape was applied to the participant's anal and perianal areas 1-2 times [14]; with the support of the children's guardian, this procedure was made possible. The procedure was carried out early morning or night before defecation. The slides were delivered to the lab, where a light microscope was used to examine them (1000x). Based on their Cellophane tape for the E. vermicularis investigation, the 100 participants were split into two groups. Children in the first group (n=50) were shown to have the infection (E. vermicularis positive group), while children in the second group (n=50) were thought to be free of E. vermicularis (E.vermicularis negative group, control)

2.2. Biometric measurements

All of the kids' heights and weights were measured using a portable stadiometer and an electronic scale. Each participant had to stand still while their weight was being calculated. 0.1 kg and 0.5 cm of accuracy are used in the weighing and measuring. The kids were weighed without shoes and in only the most basic clothing. By combining weight (kg) and height squared, the Body Mass Index (BMI) is computed (m). Based on their BMI, the children were given the designations of malnutrition (BMI ≥18.5 kg/m2) and normal BMI (BMI = 18.5-25 kg/m2) [15].

2.3. Blood collection and serum preparation

About 5–6 mL of blood was drawn from each participant using a venipuncture technique. Once the blood samples were collected, they were immediately put into EDTA tubes for anticoagulation, preserved in a cooling box, and delivered, within an hour, to the lab for hematological analysis. The rest of the blood samples were put in suction, clot, and gel activator tubes and centrifuged for

10 minutes at 3000 rpm after allowing them to coagulate for around 30 minutes at room temperature. Five equal portions of each serum sample were divided. Each portion was placed into a sterile Eppendorf tube for biochemical analysis using a sterile micropipette. Within 10 hours of being drawn, all blood samples underwent testing.

2.4. Laboratory analyses

In this study, total protein, lipid profile, and Adenosine deaminase (ADA) quantitative measures were assessed for each participant. According to the directions provided by the maker of the ADA kits, ADA was evaluated using an enzyme-linked immunosorbent assay (ELISA) (Shanghai YL Biotech., Co., Ltd.). The ELISA reader was then used to read the results at 450 nm. At the same time, the spectrophotometer was used to quantify triglycerides, cholesterol, and high-density lipoprotein (HDL) (Human Diagnostics, Germany). The manufacturer's instructions were followed for each of these tests.

2.5. Statistical Analysis

Statistical data analysis was made 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 ADA, cholesterol, LDL, HDL, triglycerides, and total protein. One-way ANOVA was also used to determine the differences regarding the same previously mentioned parameters among E.vermicularis +ve with normal BMI, E.vermicularis +ve with malnutrition, E.vermicularis -ve with normal BMI, and E.vermicularis -ve with malnutrition. All data are expressed as mean± Standard Deviation (SD).

3. Results

The results of ADA are illustrated in **Table (1)**. No significant differences were noticed in the level of ADA between E.vermicularis +ve and E.vermicularis –ve groups. Although the mean of ADA concentration was slightly lower in E.vermicularis +ve (2.06±3.29 ng/ml) than E.vermicularis –ve group, their mean of ADA concentration was (2.47±4.85 ng/ml). Furthermore, nutritional status had no significant influence on ADA in both E.vermicularis +ve and E.vermicularis –ve groups. *E.vermicularis* -ve who had malnutrition showed the highest ADA level (3.07±1.02 ng/ml) compared with other groups (E.vermicularis +ve with normal BMI, E.vermicularis +ve with malnutrition, and E.vermicularis –ve with normal BMI) who showed lower ADA levels were as follow: (2.64±1.19 ng/ml), (1.85±0.4 ng/ml) and (1.39±0.4 ng/ml), respectively.

A significant difference ($P<0.05$) was noticed in cholesterol levels between the infected group versus the control. The cholesterol concentration was decreased significantly in E.vermicularis +ve children (132.27±20.24 mg/dl) versus an increase in cholesterol in the control group (143.14±27.77 mg/dl) (**Table 2**). In contrast, non-significant differences in cholesterol levels were noticed between groups (E.vermicularis +ve with normal BMI, E.vermicularis +ve with malnutrition and E.vermicularis –ve with normal BMI and E.vermicularis –ve with malnutrition), although the highest cholesterol level was detected among E.vermicularis –ve with normal BMI group (**Table 2**).

Non-significant differences were noticed between groups regarding LDL, although its level was lower in children who had E.vermicularis infection (1.9±0.45 mmol/l) versus high concentration in the control group (2.2±0.67 mmol/l) (**Table 3**). Non-significant differences were noticed among (E.vermicularis +ve with normal BMI, E.vermicularis +ve with malnutrition and E.vermicularis –ve with normal BMI, and E.vermicularis –ve with malnutrition), although its concentration was increased in E.vermicularis -ve with normal BMI group.

While the result of HDL was completely different as its concentration was increased significantly ($P<0.05$) in children with E.vermicularis infection compared to the control group with lower HDL concentration value (**Table 4**). HDL levels varied significantly ($P<0.5$) among groups

(E.vermicularis +ve with normal BMI, E.vermicularis +ve with malnutrition and E.vermicularis –ve with normal BMI, and E.vermicularis –ve with malnutrition). The highest level of HDL levels was noticed among E.vermicularis –ve with normal BMI and E.vermicularis –ve with malnutrition. The level of their HDL was (53.89±6.8 mg/dl) and (50.3 ±8.11 mg/dl) respectively (**Table 4**).
Triglyceride level was not significantly varied between E.vermicularis +ve group and the control group. However, its level was slightly lower in the Enterobiasis group (107±49.82 mg/dl) compared with the control group (108±24.3 mg/dl). Triglyceride levels were insignificantly varied among (E.vermicularis +ve with normal BMI, E.vermicularis +ve with malnutrition, E.vermicularis –ve with normal BMI, and E.vermicularis –ve with malnutrition). In contrast, total protein was significantly ($P<0.05$) varied between groups (**Table 5**).
The mean value of the total protein was significantly decreased in E.vermicularis + group (63.95±6.67g/l) while the mean concentration of total protein was increased significantly (72.93±6.33 g/l). Total protein also showed significant differences between groups (E.vermicularis +ve with normal BMI, E.vermicularis +ve with malnutrition, and E.vermicularis –ve with normal BMI and E.vermicularis –ve with malnutrition). The highest total protein level was noticed among E.vermicularis –ve with normal BMI (72.97±7.4 g/l) and E.vermicularis –ve with malnutrition (72.91±5.7 g/l). (**Table 6**)

Table 1. Adenosine deaminase levels among different studied groups

Groups	**ADA ng/ml (Mean±SD)**	**P value**	
E.vermicularis +ve children (n=50)	2.06±3.29	0.4	T-test=0.3
E.vermicularis -ve children (n=50)	2.47±4.85		
E.vermicularis +ve with normal BMI (n=13)	2.64±1.19	0.4	F-test=0.8
E.vermicularis +ve with malnutrition(n=37)	1.85±0.4		
E.vermicularis -ve with normal BMI (n=18)	1.39±0.4		
E.vermicularis -ve with malnutrition (n=32)	3.07±1.02		

Table 2. Cholesterol levels among different studied groups

Groups	**Cholesterol mg/dl (Mean±SD)**	**P value**	
E.vermicularis +ve children (n=50)	132.27±20.24	0.01	T-test=2.23
E.vermicularis -ve children (n=50)	143.14±27.77		
E.vermicularis +ve with normal BMI (n=13)	131.99± 5.7		
E.vermicularis +ve with malnutrition(n=37)	123.37± 3.35	0.7	F-test=2.4
E.vermicularis -ve with normal BMI (n=18)	149.91± 6.98		
E.vermicularis -ve with malnutrition (n=32)	139.33± 4.66		

Table 3. Low density lipoprotein levels among different studied groups

Groups	LDL mmol/l (Mean±SD)	P value	
E.vermicularis +ve children (n=50)	1.9±0.45	0.1	T-test=1.4
E.vermicularis -ve children (n=50)	2.2±0.67		
E.vermicularis +ve with normal BMI (n=13)	1.87±0.13	0.3	F-test=1.07
E.vermicularis +ve with malnutrition(n=37)	1.9±0.07		
E.vermicularis -ve with normal BMI (n=18)	2.17±0.1		
E.vermicularis -ve with malnutrition (n=32)	1.93±0.11		

Table 4. High density lipoprotein levels among different studied groups

Groups	HDL mg/dl (Mean±SD)	P value	
E.vermicularis +ve children (n=50)	44.95±10.45	0.00024	T-test=3.6
E.vermicularis -ve children (n=50)	51.6±7.81		
E.vermicularis +ve with normal BMI (n=13)	47.8±7.9	0.0015	F-test=5.45
E.vermicularis +ve with malnutrition(n=37)	43.95±11.1		
E.vermicularis -ve with normal BMI (n=18)	53.89±6.8		
E.vermicularis -ve with malnutrition (n=32)	50.3±8.11		

Table 5. Triglyceride levels among different studied groups

Groups	Triglyceride mg/dl (Mean±SD)	P value	
E.vermicularis +ve children (n=50)	107±49.82	0.4	T-test=0.17
E.vermicularis -ve children (n=50)	108±24.3		
		0.7	T-test=0.34
E.vermicularis +ve with normal BMI (n=13)	102.72±7.9		
E.vermicularis +ve with malnutrition(n=37)	108.51±9.1		
E.vermicularis -ve with normal BMI (n=18)	99.62±7.6		
E.vermicularis -ve with malnutrition (n=32)	110.55±4.4		

Table 6. Total protein levels among different studied groups

Groups	Total protein g/l (Mean±SD)	P value	
E.vermicularis +ve children (n=50)	63.95±6.67	0.000001	T-test=6.9
E.vermicularis -ve children (n=50)	72.93±6.33		
E.vermicularis +ve with normal BMI (n=13)	62.99±3.6	0.0000002	T-test=15
E.vermicularis +ve with malnutrition(n=37)	64.28±7.4		
E.vermicularis -ve with normal BMI (n=18)	72.97±7.4		
E.vermicularis -ve with malnutrition (n=32)	72.91±5.7		

4. Discussion

The current study findings showed that although ADA levels were somewhat higher in the enterobiasis group, there were no statistically significant differences between children with enterobiasis and the control group. Adenosine deaminase's most significant biological function is defending lymphocytes from the harmful effects of 2-deoxyadenosine and deoxyadenosine triphosphate, which weaken the immune system [16]. The marginally elevated levels of ADA in the enterobiasis group compared to the control group implied that this was accompanied by a higher cellular immunological activation by the host to be protected against the parasites. The findings were in contrast to those of Karamanet al. (2014) [12] and Kiran et al. 2019 [17], which revealed that ADA dropped significantly in E. vermicular is infection patients compared to the control group. This investigation also showed that malnutrition had a negligible impact on ADA in both the E. vermicularis +ve and E. vermicularis -ve groups. Although, E.vermicularis -ve who had malnutrition showed the highest ADA compared with other groups (E.vermicularis +ve with normal BMI, E.vermicularis +ve with malnutrition, and E.vermicularis –ve with normal BMI) who showed lower ADA levels. Adenosine deaminase (ADA) activity may increase in the thymus, serum, and other fluids when there is a severe nutritional shortage [18]. On the other hand, cholesterol levels decreased significantly in those who had enterobiasis versus control. The findings are consistent with the theory that some parasitic helminths absorb cholesterol via a carrier-mediated mechanism. When the capacity of the medium's micellar phase is increased, cholesterol uptake is decreased, indicating that uptake includes an intermediary partitioning of sterol from micelles into the water phase of the medium [19]. The findings of this current study revealed a non-significant decrease in LDL in the Enterobiasis group compared to the control. But HDL was decreased significantly among enterobiasis children versus the control group. Direct lipid consumption by helminths is one potential route. Some helminths rely on the host's cholesterol supply because they cannot generate it [20]. On the other hand, malnutrition affected non-significantly the levels of both LDL and HDL in E.vermicularis +ve and E.vermicularis – subjects. Although E.vermicularis -ve with normal BMI showed the highest LDL and HDL concentration, decreased plasma albumin and total cholesterol concentrations in children with lower LDL-C might indicate underlying nutritional status. Therefore, malnutrition is linked to low LDL and HDL levels [21]. Triglyceride levels in the study's enterobiasis group did not change appreciably. These findings were in line with recent results [22] that showed an antagonistic relationship between HDL-C and the frequency of infection by several parasitic worm species, including hookworm, Strongyloidesstercoralis, and Trichuris trichiura. The mean serum total protein and serum iron levels in the enterobiasis-positive group were significantly lower than those in the enterobiasis-negative group. The results showed a significant decrease in total protein

concentration among E.vermicularis +ve with normal BMI E.vermicularis +ve with malnutrition. The enterobiais group's low protein levels may have been caused by increased nutritional loss from vomiting, diarrhea, or blood loss and decreased food intake. These consequences make anemia, protein energy deficiency, and other nutrient deficits worse [23]. These findings are consistent with those made public by other researchers [24, 25].

5. Conclusion

Based on the study findings, it is concluded that Enterobius vermicularis infection can alter some parameters related to lipid profile, but it may not be associated with ADA.

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