DOI: https://dx.doi.org/10.21123/bsj.2021.18.4(Suppl.).1485

# Anemia and The Level of IL-6 in Children Infected with Entamoeba histolytica and Giardia lamblia

Israa N. Zeki <sup>1</sup>

Harith Saeed Al-Warid \*20

<sup>1</sup>Central Organization for Standardization and Quality Control Baghdad, Iraq. <sup>2</sup>Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. \*Corresponding author: <u>harithalward@yahoo.com</u> \*, <u>harith.saeed@sc.uobaghdad.edu.iq</u>. E-mails: <u>israal216@gmail.com</u>

Received 1/6/2020, Accepted 6/4/2021, Published 20/12/2021

This work is licensed under a <u>Creative Commons Attribution 4.0 International License</u>.

### Abstract:

The relation between anemia and inflammatory immune response has lately had much attention. This research was conducted from October 2018 until April 2019, including (110) children below 12 years from both gender in some Hospitals, Primary Health care centers, Public Primary Schools and Kindergarten in Baghdad, Iraq. The objective of this study is to determine the possible correlation between iron deficiency anemia and inflammatory immune response among children infected with *Entamoeba histolytica* or *Giardia lamblia*. Blood samples were taken from all groups to measure hemoglobin level, serum iron, total iron binding capacity (TIBC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), while the inflammatory related immune response was evaluated by measuring IL-6 and ferritin. Student T-Test was used to compare between means. The results showed that both hemoglobin and iron concentrations were significantly (P) < 0.01 lower in infected children compared with control, as well as both IL-6 and ferritin levels were significant where (P) <0.05 amplified among infected children compared to control. Microcytic hypochromic anemia was observed in the majority of infected children, while normocytic normochromic RBCs was recorded in the majority of control children.

Keywords: Children, E. histolytica, G lamblia, , IL-6, microcytic anemia.

# **Introduction:**

Childhood anaemia is a condition where a child has abnormal and insufficient hemoglobin level to provide adequate oxygen to the body tissues <sup>1</sup>. Anemia in children is considered as global public health challenge which is often numerous potential etiologies<sup>2</sup> but the major frequent cause of anemia globally is Iron Deficiency Anemia (IDA)<sup>3</sup>. IDA anemia is the most public micronutrient deficiency that affects nearly 35% of the world's population and 1.2 billion individuals worldwide <sup>4,5</sup>. Many influences contribute to anemia which are raising energy outflow, irregularly eating habit, poor maternal attention, and acquired infection mainly intestinal parasitic infections showed to be very common among school children<sup>6</sup> Intestinal parasites infection and their prevalence remain among the major health problems especially in the developing countries. The World Health Organization (WHO) reported that about 3.5 billion people worldwide are still affected by intestinal parasitic infections<sup>7</sup>. E.

histolytica and G. lamblia are among the most important and broadly prevalent intestinal protozoan parasites all over the world causing diarrhea, intestinal diseases and the most dominant cause of intestinal morbidity in children<sup>8,9</sup>. The common route of transmission is through contaminated food, drinking water, as well as person to person through fecal oral contact<sup>10</sup>. Protozoan parasites interfere with anemia by destructing the mucosa layer of intestine that effects on micronutrients absorption, such as iron<sup>11</sup>. Interaction of the parasite with host cells affect both of hosts' nutrition status and immunity<sup>12</sup> by inducing some cytokines that stimulate the innate and adaptive immune responses to eliminate the parasites . Some of which are: IL-1β, IL-6, IL-8, IL-12, IFN- $\gamma$ , and TNF- $\alpha$ , they induce the activation of a Th1 type response <sup>13</sup>. Some papers from Iraq focused on prevalence rates of intestinal parasites in many governorates <sup>13</sup>, while some other studies were conducted to determine the association between iron deficiency anemia (IDA) and gastrointestinal parasitic infection in children <sup>14</sup>. Little is known about the relation between gastro intestinal parasites and inflammatory immune response especially in anemic children. The objective of this study is to determine the possible correlation between iron deficiency anemia and inflammatory immune response among children infected with *E. histolytica* or *G. lamblia* in Baghdad- Iraq.

### Materials and Methods: Study design and population

This research was conducted from October 2018 until April 2019. The population in this study was children from both genders attending some Hospitals, Primary Health care centers, Public Primary Schools and Kindergarten in Al-Khadimya, Al Sader city, Bab Al Moadam and Al-Shulaa Baghdad, Iraq. The sampling included (110) children who had inclusion criteria and had no exclusion criteria. The inclusion criteria were children below 12 years old, who were willing and had their parents' agreement to be involved in this research. The children must not take anti-protozoa or anti-helminth treatment in previous 180 days and had no history of other illnesses. All these information was obtained based on interviews with performed according parents. to prepared questionnaire. The questionnaire was also employed to collect some information regarding background characteristics of children as well as the children's past and present illnesses.

Exclusion criteria were, obesity, blood and stool samples that were impaired accidentally. This study protocol was approved by Ministry of Health and Environment, Baghdad, Iraq. A permission from kindergarten and primary school authority was also approved. The 110 participants were divided into two groups based on their microscopic general stool examination.

# **General stool examination (GSE)**

Stool was examined for consistency and presence of blood and mucus with certain intestinal parasites. Microscopic examination:-stool samples were prepared using direct normal saline (0.9% NaCl) wet smear, Lugols-Iodine wet smear and formal ether sedimentation technique<sup>15, 16</sup>. Stool samples were kept in 10% formalin for transferring parasites to a laboratory where they were examined under the microscope. Diagnosis was confirmed by seeing trophozoites or cysts of *E. histolytica* or *G. lamblia* under the microscope.

Based on GSE, the 110 children were divided in to two groups. The first group (infected children) were diagnosed as *E. histolytica* infected

or *G. lamblia* infected children (n= 68) while no parasites were detected in the stool samples of other group who were considered as healthy control children (n= 42). Blood samples were taken from all groups for measuring blood parameter, .

# Haematological Laboratory techniques:

About (4-5) ml of blood samples was taken using a vein puncture technique. About (0.5 ml) of blood samples was added immediately into labelled paediatric EDTA tubes for anticoagulant and kept in cooling box and transferred back to the laboratory. The blood samples were analysed within 12 hours after collection. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) and haemoglobin level were measured using Celtac Es MEK-7300K (NIHOB KODHEN, Germany) automatic haematology analyser. The rest of blood samples were added into vacuumed, clot & gel activator tubes and left for 30 minutes at room temperature to clot before all samples were centrifuged at 3000 (rpm) for 10 minutes, each sample was transferred by serum sterile micropipette into 3 sterile Eppendrof tubes for following different tests to avoid freezing and thawing that may influence the accuracy of results. Serum iron and total binding iron capacity (TBIC) were measured using Dimensions X pand plus (Siemens, Germany).

# Immunological tests

Serum ferritin was measured using IMMULITE 2000 XPi Immunoassay System (Siemens, Germany), while the IL-6 was measured in all patients and healthy children by using sandwich enzyme-linked immunosorbent assay (ELISA) based on the manufacturer's instructions IL-6 human kit (DE 4640), Demeditec/ Germany.

# Statistical Analysis:

Student T-Test was used to compare between means. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability) in this study <sup>17</sup>. All these calculations were done using Statistical Analysis System, v. 10.0. 2. (North Carolina USA) <sup>18</sup>.

# **Results:**

Results showed that the mean $\pm$ SE of hemoglobin concentration was significantly (*p*) < 0.01 lower 11.81 $\pm$ 0.15g/dl in infected children (*E. histolytica* or *G. lamblia*) versus high hemoglobin 13.04 $\pm$ 0.11g/dl in control group. The mean  $\pm$ SE of serum iron 34.2 $\pm$ 3.42µg/dl was significantly (*p*) < 0.01 lower in children infected with intestinal protozoan parasites (either *G. lamblia* or *E. histolytica*) compared with high level of serum iron  $67.28\pm4.51\mu g/dl$  in control group. Although mean  $\pm$ SE of total iron binding capacity levels was lower in infected group  $360.70\pm10.97\mu g/dl$  in contrast to mean of TIBC in control group  $366.24 \pm 11.03\mu g/dl$  but the results showed no-significant differences (*p*) > 0.05 between the two groups. The mean  $\pm$ SE of corpuscular volume (MCV) was highly significantly lower (*p*) < 0.01 in infected children 77.89  $\pm$  0.92fl compared with high mean corpuscular volume

87.02±0.55fl in control group. The mean±SE of corpuscular hemoglobin (MCH) of red blood cells was significant lower (p) < 0.01 in infected children 25.72 ± 0.36 pg in comparison to control 29.05 ± 0.21 pg. Statistical analysis showed non-significant differences (p) > 0.05 although the mean±SE of corpuscular hemoglobin concentration (MCHC) was lower in infected group 32.98 ± 0.17 mg/ dl comparing to healthy children 33.37 ± 0.07 mg/ dl (Table 1).

Parameters	Mean ± SE		P-value	
	Patients (infected with	Control		
	E.histolytica or			
	G.lamblia)			
Iron (µg/ml)	$34.25\pm3.42$	$67.28 \pm 4.51$	0.0001	T test= $11.147$
				**
T.I.B.C. (µg/dl)	$360.70 \pm 10.97$	$366.24 \pm 11.03$	0.737	T  test = 32.594
				NS
Hb (g/ dl)	$11.81\pm0.15$	$13.04 \pm 0.11$	0.0001	T test = 0.435 **
MCV (fl)	$77.89 \pm 0.92$	$87.02 \pm 0.55$	0.0001	T test= 2.466 **
MCH (pg)	$25.72\pm0.36$	$29.05\pm0.21$	0.0001	T test= 1.749 **
MCHC (mg/ dl)	$32.98 \pm 0.17$	$33.37\pm0.07$	0.0865	T  test = 1.884
				NS
* (p) <0.05, ** (p)	<0.01, NS: Non-Significant.			

Table 1. The levels of Iron, TIBC, hemoglobin, MCV, MCH and MCHC in infected children (E.			
histolytica or G. lamblia) and healthy control.			

Microcytic anemia (MCV< 82fl) was remarked in the majority of infected children (70.5%) compared with a smaller number of infected subjects in control group (11.9%) with significant differences (p) < 0.01. On the other hand, normocytic RBCs (MCV= 82-98fl) was noticed in the majority of control group (88%) compared with less cases in infected children (27.9%). Statistical analysis showed that there was significant relation (p) < 0.01between the type of anemia and the infection with *E. histolytica* or *G. lamblia*. Only one patient of Macrocytic anemia (MCV> 98fl) was recgnized among infected and controls as manifested in Fig. 1.

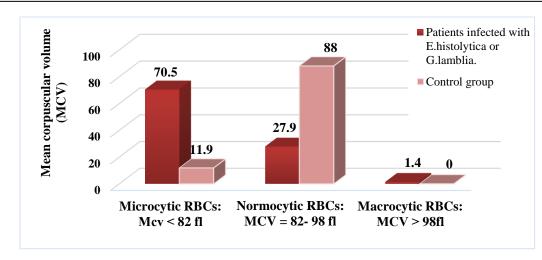


Figure 1. Types of anemia based on mean corpuscular volume (MCV) in patients infected with (*E histolytica* or G *lamblia*) and control.

# Inflammatory markers (serum ferritin, IL-6 levels)

In this study, it has been found that the mean±SE of ferritin level was significantly higher  $86.47\pm25.04$  µg/ml in infected children versus low ferritin levels  $23.69\pm2.54$  µg/ml in control group (*p*) <0.05, while the measurement of interleukin-6 indicated that the mean±SE was meaningfully greater (*p*) < 0.05 in infected children 192.76±122.48 pg/ml compared with low level 22.67±0.75 pg/ml in control group (Figs. 2 and 3).

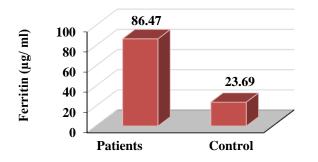


Figure 2. Comparison of ferritin levels between infected and control group.

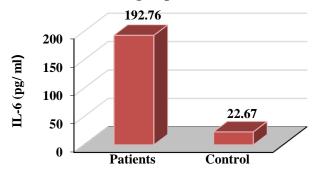


Figure 3. Comparison of IL-6 levels between infected and control groups.

#### **Discussion:**

Anemia is the global health issue that disturbs many people in each socioeconomic status, gender and age all over the world. In Iraq, many papers presented different prevalence rates of intestinal parasites in many governorates<sup>13.</sup> Some other investigators studied the relation between anemia and parasitic infection<sup>14</sup>, but little was known about the correlation between inflammations caused by protozoan infection among anemic patients. The hemoglobin concentration was significantly lower in infected children (E. histolytica or G. lamblia) in comparison to control. Some gastrointestinal protozoan parasites interact with the mucous of the small intestine that makes villous atrophy in different level, besides triggering inflammatory infiltrate and crypt hypertrophy<sup>18</sup>. These processes disrupt the enterocytes and change bile acid metabolism that affects the absorption of most nutrients which are necessary for body function, for instance, vitamins, iron, zinc, and folic acid<sup>19</sup>. An abnormal deficiency of iron in the blood (Hypoferremia) represents an innate immune response to infection and inflammation as host defense for sequestering iron from pathogens. E. histolytica feeds directly on the erythrocytes and this leads to a defect in blood parameters, including erythrocytes numbers and hemoglobin. The Giardia disrupts the absorption of the essentials such as vitamins and important elements of the body in the intestines<sup>20</sup>.

A similar study indicated that prevalence of anemia was 21.6% for children infected with intestinal parasites. The serum iron concentration level decreases and the total iron-binding capacity (TIBC) increases without a change in the

hematocrit<sup>21</sup>.The hypoferraemic response developed to provide an increased capacity for transferrin to bind with iron that would be released during acute infection and during the associated destruction of tissues and erythrocytes, and so limit the generation of non-transferrin-bound iron that can be readily used by many microorganisms<sup>22</sup>. The results showed that microcytic hypochromic anemia was noticed in the majority of children infected with E. histolytica or G. lamblia compared with a small number of infected subjects in control group, while normocytic normochromic anemia was noticed in the majority of control group compared with high percentage in infected children. Only one individual of macrocytic anemia was detected among infected groups. Microcytic anemia is caused by iron deficiency, and it is the most common type of anemia during childhood, whereas macrocytic anemia is rare in children <sup>23</sup>. This result agrees with a previous study<sup>24</sup> which showed a significant correlation (p) < 0.05 between MCV, MCH, and anemia in children protozoa infection. Mean corpuscular volume results are fluctuated, some studies indicated that IDA is often microcytic, hypochromic anemia clearly differentiated it from inflammatory anemia (IA) which exhibits a mild to moderate normocytic, normochromic anemia<sup>25</sup>, but other recent studies have showed similar percentages of microcytic anemia in IA patients<sup>20, 21</sup> .These results are likely be a good indicator to possible interference between IDA and IA in infected individuals with gastrointestinal parasites in this study. Furthermore, in another study, a decline of iron levels was reflected by Hb and MCV was observed among patients' children. The result of this current study is consistent with another study where Hb and MCV are significantly reduced among patients with some inflammatory diseases<sup>20</sup>.Serum ferritin concentration reflects the level of body iron stores, but it has to be interpreted because ferritin is an acute-phase reactant which acute/chronic infection increases in or inflammation<sup>26, 27</sup>. The WHO criteria proposed for higher threshold of 30 (µg/ml) are used in the presence of infection or inflammation<sup>27</sup>. In this study, serum ferritin concentrations cross the threshold that is likely due to inflammation response. Serum levels of interleukin-6 indicated significant increase in children infected with intestinal parasites, this result may interpret that both intestinal protozoan parasites infect their hosts by ingestion of cysts and colonize the digestive tract. They attach to the intestinal epithelial surface of the duodenum/ ileum in patients with G. lamblia or colon in E. histolytica and stimulate an immune response involving interleukin IL-6 production by

T-cells, dendritic cells and mast cells<sup>28</sup>. An increase in some cytokines including IL-6 indicates the presence of inflammation<sup>29</sup>. IL-6 is a pro inflammatory cytokine featuring pleiotropic activity; it induces the synthesis of acute phase proteins such as hepcidin in hepatocytes<sup>30</sup> by stimulating the transcription of the gene encoding hepcidin<sup>22</sup>. Hepcidin hormone regulates iron homeostasis and serum iron concentrations, its synthesis is stimulated by high plasma iron concentrations, high hepatic iron stores and inflammation<sup>31</sup>. High ferritin and IL-6 levels in this study may be interpreted by possible inflammatory anemia presence in protozoan infected children, this results in consist with another study which indicated that patients with anemia of chronic diseases (ACD) in Rheumatoid Arthritis had higher significant concentration of both ferritin and IL-6 in compared with IDA group  $^{20}$ .

### **Conclusion:**

Each of *E.histolytica* or *G.lamblia* infection stimulates inflammation represented by considerable elevation of IL-6. This elevation has been directly correlated with alteration in some blood parameters. Therefore IL-6, ferritin and MCV estimation may be related to iron deficiency anemia (IDA) and inflammatory anemia (IA) in children infected with intestinal protozoan parasites.

# Acknowledgment:

The cooperation of the medical staff at the Hospitals and Primary Health care centers in Baghdad is appreciated. Special thank is due to the technical staff of AL- Jawadeen primary health care center for their help.

### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

# Authors' contributions:

I.Z and H.S.W contributed equally to the design and implementation of the research, to the analysis of the results and to the writing and improving f the manuscript.

### **References:**

- 1. World Health Organization. The global prevalence of anaemia in 2011. Geneva: World Health Organization; 2015. p. 43.
- Allali S, Brousse V, Sacri A-S, Chalumeau M, de Montalembert M. Anemia in children: prevalence, causes, diagnostic work-up, and long-term consequences. Expert Rev. Hematol. 2017;10(11):1023-28.
- 3. Kassebaum NJ. The global burden of Anemia Hematol Oncol Clinics. 2016;30(2):247-308.
- Camaschella C. Iron-deficiency anemia. N Engl J Med. 2015;372(19):1832-43.
- 5. Auerbach M, Schrier S. Treatment of iron deficiency is getting trendy. Lancet Haematol. 2017;4(11):e500.
- Akeredolu IA, Oguntona BE, Okafor C, Osisanya OJ. Iron, zinc and copper malnutrition among primary school children in Lagos, Nigeria. Food Nutr Sci; 2011;2(10): 1063-70.
- 7.Mezeid N, Shaldoum F, Al-Hindi AI, Mohamed FS, Darwish ZE. Prevalence of intestinal parasites among the population of the Gaza Strip, Palestine. Ann Parasitol. 2014;60(40): 281- 89.
- Zebardast N, Yeganeh F, Gharavi MJ, Abadi A, Tabaei SJS, Haghighi A. Simultaneous detection and differentiation of Entamoeba histolytica, E. dispar, E. moshkovskii, Giardia lamblia and Cryptosporidium spp. in human fecal samples using multiplex PCR and qPCR-MCA. Acta tropica. 2016;162:233-38.
- 9. Hailegebriel T. Prevalence of intestinal parasitic infections and associated risk factors among students at Dona Berber primary school, Bahir Dar, Ethiopia. BMC infectious diseases. 2017;17(1):362-68.
- 10. Duc PP, Nguyen-Viet H, Hattendorf J, Zinsstag J, Cam PD, Odermatt P. Risk factors for Entamoeba histolytica infection in an agricultural community in Hanam province, Vietnam. Parasit Vectors. 2011;4(1):102-111.
- 11. Gopalakrishnan S, Eashwar VA, Muthulakshmi M, Geetha A. Intestinal parasitic infestations and anemia among urban female school children in Kancheepuram district, Tamil Nadu. J Family Med Prim Care. 2018;7(6):1395- 400.
- 12. Mohammadi R, Hosseini-Safa A, Ardakani MJE, Rostami-Nejad M. The relationship between intestinal parasites and some immune-mediated intestinal conditions. Gastroenterol Hepatol Bed Bench. 2015;8(2):123-31.
- 13. Al Saqur IM, Al-Warid HS, Albahadely HS. The prevalence of Giardia lamblia and Entamoeba histolytica/dispar among Iraqi provinces. KIJOMS. 2017;3(2):93-96.
- Al-Warid HS, Musa IS, Al-Qadhi BN. Iron deficiency and anthropometry in children infected with Entamoeba histolytica. Int J Recent Sci Res. (2013); 4(6): 823-26.
- 15. Truant AL, Elliott SH, Kelly MT, Smith JH. Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate flotation techniques for detection of intestinal parasites. JCM. 1981;13(5):882-84.

- Bhattachan B, Panta Y, Tiwari S, Magar DT, Sherchand J, Rai G, et al. Intestinal Parasitic Infection among School children in Chitwan district of Nepal. J Inst Med. 2015;37(2): 79-84.
- 17. SAS J,Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. Cary. North Carolina. USA: SAS Institute. Inc; 2012.
- Galván-Moroyoqui JM, Del Carmen Domínguez-Robles M, Meza I. Pathogenic bacteria prime the induction of Toll-like receptor signalling in human colonic cells by the Gal/GalNAc lectin Carbohydrate Recognition Domain of Entamoeba histolytica. Int J Parasitol. 2011;41(10):1101-12.
- Calvao F, Costa Gileno D, Malta JO, Vientini V, Anibal F. Anemia in patients with intestinal parasitic infection. Rev Ibero-Latinoam Parasitol. 2011;70(2):206-11.
- 20. Ali ET, Jabbar AS, Mohammed AN. A comparative study of interleukin 6, inflammatory markers, ferritin, and hematological profile in rheumatoid arthritis patients with anemia of chronic disease and iron deficiency anemia. Anemia. 2019; 2019(Article ID 3457347): 8 pages.
- 21. Perez Y, Presti K, Eden AN, Sandoval C. Iron-Deficiency Anemia During Childhood. InAnemia in the Young and Old 2019 (pp. 81-93). Springer, Cham.
- Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. Nat Rev Immunol. 2015;15(8):500-10.
- 23. Wang M. Iron deficiency and other types of anemia in infants and children. Am Fam Physician. 2016;93(4):270-78.
- 24. Darlan DM, Ananda FR, Sari MI, Arrasyid NK, Sari DI. Correlation between iron deficiency anemia and intestinal parasitic infection in school-age children in Medan. InIOP Conference Series: Earth and Environmental Science 2018 Mar 1; 125(1): 012059. IOP Publishing.
- 25. Longo DL, Camaschella C. Iron-deficiency anemia. N Engl J Med. 2015;372(19):1832-43.
- 26. Abdullah K, Birken CS, Maguire JL, Fehlings D, Hanley AJ, Thorpe KE, et al. Re-evaluation of serum ferritin cut-off values for the diagnosis of iron deficiency in children aged 12-36 months. J. Pediatr. 2017;188:287-90.
- 27. Lanzkowsky P, Lipton JM, Fish JD. Hematological reference values. In: Lanzkowsky's manual of pediatric hematology and oncology,6th edition. USA: Academic Press. p 709-29.
- Hemphill A, Müller N, Müller J. Comparative Pathobiology of the Intestinal Protozoan Parasites Giardia lamblia, Entamoeba histolytica, and Cryptosporidium parvum. Pathogens. 2019;8(3):8030116.
- Ma K, Zhang H, Baloch Z. Pathogenetic and therapeutic applications of tumor necrosis factor-α (TNF-α) in major depressive disorder: a systematic review. Int J Mol Sci. 2016;17(5):17050733.
- 30. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6(10):a016295.

 Sangkhae V, Nemeth E. Regulation of the iron homeostatic hormone hepcidin. Adv. Nutr 2017;8(1):126-36.

# فقر الدم ومستوى انترلوكين -6 في الاطفال المصابين بالامييبا الحالة للنسبج و الجيارديا اللامبلية

اسراء نایف زکی $^1$  حارث سعید الورد $^2$ 

<sup>1</sup>الجهاز المركزي للتقييس و السيطرة النوعية، بغداد، العراق. <sup>2</sup>قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.

الخلاصة:

الكلمات المفتاحية: الأطفال، الاميبا الحالة للنسج، الجيار ديا لامبلية، انترلوكين 6، فقر الدم الصغير الكريات.