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

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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 anemia is a condition where a child has abnormal and insufficient hemoglobin level to provide adequate oxygen to the body tissues 1. Anemia in children is considered as global public health challenge which is often numerous potential etiologies2 but the major frequent cause of anemia globally is Iron Deficiency Anemia (IDA)3. IDA anemia is the most public micronutrient deficiency that affects nearly 35% of the world’s population and 1.2 billion individuals worldwide 4,5. 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 children6. 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 infections7. 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 children8,9. The common route of transmission is through contaminated food, drinking water, as well as person to person through fecal oral contact10. Protozoan parasites interfere with anemia by destructing the mucosa layer of intestine that effects on micronutrients absorption, such as iron11. Interaction of the parasite with host cells affect both of hosts’ nutrition status and immunity12 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-γ, and TNF-α, they induce the activation of a Th1 type response 13. Some papers from Iraq focused on prevalence rates of intestinal parasites in many governorates 13, while some other studies were conducted to determine the association
between iron deficiency anemia (IDA) and gastro-intestinal parasitic infection in children \(^{14}\). 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-helmint treatment in previous 180 days and had no history of other illnesses. All these information was obtained based on interviews with parents, performed according 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\(^ {15, 16}\). 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 serum sample was transferred by 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 \(^ {17}\). All these calculations were done using Statistical Analysis System, v. 10.0. 2. (North Carolina USA) \(^ {18}\).

### Results:

Results showed that the mean±SE of hemoglobin concentration was significantly \((p) < 0.01\) lower 11.81±0.15g/dl in infected children (*E. histolytica* or *G. lamblia*) versus high hemoglobin 13.04±0.11g/dl in control group. The mean ±SE of serum iron 34.2±3.42µg/dl was significantly \((p) < 0.01\) lower in children infected with intestinal parasitic infection.
protozoan parasites (either *G. lamblia* or *E. histolytica*) compared with high level of serum iron 67.28±4.51µg/dl in control group. Although mean ±SE of total iron binding capacity levels was lower in infected group 360.70±10.97µg/dl in contrast to mean of TIBC in control group 366.24±11.03µg/dl but the results showed no-significant differences (*p*) > 0.05 between the two groups. The mean ±SE of corpuscular volume (MCV) was highly significantly lower (*p*) < 0.01 in infected children 77.89 ± 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).

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients (infected with <em>E. histolytica</em> or <em>G. lamblia</em>)</td>
<td>Control</td>
</tr>
<tr>
<td>Iron (µg/ml)</td>
<td>34.25 ± 3.42</td>
<td>67.28 ± 4.51</td>
</tr>
<tr>
<td>T.I.B.C. (µg/dl)</td>
<td>360.70 ± 10.97</td>
<td>366.24 ± 11.03</td>
</tr>
<tr>
<td>Hb (g/ dl)</td>
<td>11.81 ± 0.15</td>
<td>13.04 ± 0.11</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>77.89 ± 0.92</td>
<td>87.02 ± 0.55</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>25.72 ± 0.36</td>
<td>29.05 ± 0.21</td>
</tr>
<tr>
<td>MCHC (mg/ dl)</td>
<td>32.98 ± 0.17</td>
<td>33.37 ± 0.07</td>
</tr>
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* (p) <0.05,  ** (p) <0.01,  NS: Non-Significant.  

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*) < 0.01 between the type of anemia and the infection with *E. histolytica* or *G. lamblia*. Only one patient of Macrocytic anemia (MCV> 98fl) was recognized 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±25.04 µg/ml in infected children versus low ferritin levels 23.69±2.54 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).

![Ferritin and IL-6 Levels](image)

**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 governorates13. Some other investigators studied the relation between anemia and parasitic infection14, 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 hypertrophy18. 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 acid19. 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 intestines20.

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. 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. 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. This result agrees with a previous study which showed a significant correlation (p)<0.05 between MCV, MCH, and anemia in children protozoan 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, but other recent studies have showed similar percentages of microcytic anemia in IA patients. 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. Serum ferritin concentration reflects the level of body iron stores, but it has to be interpreted because ferritin is an acute-phase reactant which increases in acute/chronic infection or inflammation. The WHO criteria proposed for higher threshold of 30 (μg/ml) are used in the presence of infection or inflammation. 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. An increase in some cytokines including IL-6 indicates the presence of inflammation. IL-6 is a pro-inflammatory cytokine featuring pleiotropic activity; it induces the synthesis of acute phase proteins such as hepcidin in hepatocytes by stimulating the transcription of the gene encoding hepcidin. Hepcidin hormone regulates iron homeostasis and serum iron concentrations, its synthesis is stimulated by high plasma iron concentrations, high hepatic iron stores and inflammation. 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.

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.

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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:

Fever and Feeding: a 6-0 Retorno in Children with Amoebic and Giardia Lamblia Infection

Sani Ye Zenki 1 and Harith Saeed Al-Ward 2

Abstract:

Recent interest in the relationship between anemia and immune response has increased. This study was conducted between October 2018 and April 2019. It included 111 children aged 21 years from different hospitals and specialized centers in Baghdad, Iraq. The study aimed to investigate any possible relationship between anemia and immune response in children with amoebic and Giardia lamblia infections. Blood samples were collected from the study group to measure hemoglobin, iron, iron saturation, mean corpuscular volume of red blood cells, serum ferritin, and interleukin-6. Student T-test was used to compare these levels. The results showed a significant decrease in hemoglobin and iron levels, while the iron saturation was not significantly different. The research also showed a significant increase in the levels of interleukin-6 and ferritin in the blood of kids with amoebic infections compared to the control group. Small red cell anemia is the most frequent type of anemia in children with amoebic infections and is significantly different from the control group.

Keywords: Children, amoebic infection, Giardia lamblia, interleukin-6, anemia of small red cells.