

Study of some hematological and immunological parameters associated with the infection of intestinal parasites in the holy city of Kerbala, Iraq

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Abstract

The current study aimed at finding the relationship between infection of intestinal parasites and some hematological (complete blood count) and immunological parameters (histamine, interleukin-5, and IgE) in children visiting and infertile at Karbala Children's Hospital in Kerbala, Iraq. Two thousand and five hundred and forty-one fecessamples examined using both direct smear and flotation methods using a saturated salt solution for children aged 1 to 15 for the period from December 2017 to November 2018.

The results showed that the total infection percentage of intestinal parasites was 18.93%. Five species of intestinal parasites recorder: *Entamoebahistolytica* (14.60%), *Giardia lamblia* (3.90%), *Trichomonas hominis* (0.16%), *Hymenolepis nana* (0.59%) and *Enterobius vermicularis* (0.08%). The percentage of infection innmale was higher than that of females and the percentage of intestinal parasitic infection fluctuated during the study period. The incidence of infection in the age groups of infected children also affected. The statistical analysis showed significant differences ($P < 0.05$) between the percentage of intestinal parasitic infection with sex and age of children as well as study months and species of parasites recorded.

To conduct the hematological and immunological study, 59 infected children selected (30 males, 29 females) and 31 non-infected children (20 males and 11 females). The blood and serumsof these children obtained and examined by a blood analyzer for the hematological parameters and ELIZA method for immunological parameters.

The hematological parameters included RBCs, Hb, PCV, WBCS, Neu., Ba., Es., Ly., Mo., Plt., MCV, MHC, and MCHC. The results of the current study showed the effect of the infection of intestinal parasites on some of the hematological parameters, whether increase or decrease ($P < 0.05$). The results also showed that other hematological parameters did not affect that infection.

The results showed a significant increase ($P < 0.05$) in IgE, IL-5 and histamineconcentrations in children infected with intestinal parasites compared to non-infected children

We conclude from the results of the present study that the infection of intestinal parasites plays an important role in stimulating the immune mechanisms of the body and thus the production of immune materials can harm the body's various tissues.

Keywords: Intestinal parasite, hematological and immunological parameters, Iraq

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Introduction:

Intestinal parasitic infection is a major health problem in developing countries, especially in tropical and subtropical regions. It estimated that it affects about 3.5 billion people worldwide and believed that 450 million people suffer from these diseases, mostly children (1).

The main intestinal parasites affecting humans include *Entamoeba histolytica*, *Giardialamblia*, *Hymenolepis nana*, *Cryptosporidium* sp., *Taenia saginata*, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Trichuris Trichur* and Hookworms (1). Children are more likely to infect with parasites and intestinal parasites in particular for many reasons, including lack of health awareness and lack of hygiene, as well as decreased immune response compared to adults (2).

The prevalence of intestinal parasites differ from one region to another and its prevalence related to several factors such as geographical factors, climate, poverty, malnutrition, high population density, and personal and community hygiene. Also, optimal conditions for the growth and spread of intestinal parasites, the absence of potable water and the poor state of health play a major role in the spread of these parasites. Research has indicated that school-age children are most vulnerable to intestinal parasitic diseases, and their effect on children is different and dangerous (3).

Most of the parasitic infections transmitted through food and contaminated water. These two sources are one of the most important factors leading to increased intestinal parasites. More than 72 species of parasites have been isolated from water and food, most of which are humanly infected (4, 5, 6& 7).

Intestinal parasites are an important cause of many pathological effects such as Diarrhea, Abdominal pain, Anorexia, Weight loss, Flatulence, Vomiting, Nausea, Fever, and Bowel blockage (8). Some intestinal parasites can also impede the absorption of digested nutrients such as carbohydrates, proteins, vitamins, and minerals important for human health (9).

Anemia occurs in children due to iron deficiency, but the parasitic infection is another cause of it. Hemoglobin is the red dye that found within the red cell. Losses occur in certain pathological conditions including parasitic infection, malnutrition, blood loss, and chronic infection, which expressed as anemia. White blood cells are one of the essential components of blood in peripheral blood, protecting against germs, parasites, tumors, and other diseases. Five types of white blood cells differ in size, proportions and functions that are as follows: neutrophil, eosinophils, basophils cells, lymphocytes and monocytes (10).

Many of these parasitic infections protected by the response and activation of the Th₂ cells, resulting in the production of IgE antibodies and activation of the eosinophils. The cytokines produced by Th₂ cells promote the activation of the eosinophils and recruit them in parasites sites where they release toxic granular proteins that destroy the parasites. These cytokines IL-5 plays an important role, especially for the proliferation, activation, and survival of eosinophils. The high levels of IgE in the serum, especially in people infected with intestinal parasites

engaged in the induction and release of histamine and other intermediates in the reaction of immediate hypersensitivity leading to the destruction of parasites (11). An allergic reaction occurs when the person's immune system produces IgE antibodies in response to a foreign antigen. IgE molecules bind tightly to the surface of the mast cells and the basophils cells. These cells contain a high proportion of histamine and other substances responsible for allergies. On subsequent exposure to the same antigen, the allergen binds IgE to the cell surfaces. This causes the metabolism of mast cells and thus the secretion of histamine and other active amines in the blood vessels to obtain an appropriate immune response to defend the body (12). Histamine, which secreted from mast cells, is an important factor in the intestinal tract and increases the permeability of the blood vessels, which in turn increases the migration of cells to the affected area, especially neutrophils and monocytes. This leads to the expulsion of parasites and infection control (13).

Materials and Methods

Stool Sample Collection:

Stool Sample Collection during the period between the beginning of December 2017 and the end of November 2018, 2541 stool samples of children with diarrhea and some other gastrointestinal disorders collected and arrived at the Children's Hospital in the holy Kerbala province to investigate intestinal parasitic infections. Children ranged in age from 1-15 years. A special form organized for each reference, including date, sex, age and type of parasites.

The fecal samples collected using clean, dry and wide-open plastic containers with a sealed lid so that the stool sample can retain its moisture, prevent contamination with bacteria and dryness. Also note that the fecal sample not exposed to water that may contain some free-living organisms, As well as not receiving a sample of stool containing urine because it leads to the killing of the stages of vegetables and impedes the examination (14).

Fecal samples examined in less than half an hour or an hour, and when several stool samples obtained at the same time, the more soft samples containing mucus or blood were examined first and then the liquid samples (15).

Macroscopic Examination:

The stool samples examined primarily grossly, which included the observation of quantity, consistency, color and form, blood or mucus, and some live worms (16 & 17).

Microscopic Examination:

Stool samples were microscopically examined using direct mount method according to (18) and flotation method according (19). Intestinal parasites classified depending on the (20).

Hematological & Immunological Study:

For this purpose, 5 ml of blood samples were withdrawn following the sterilization of the methyl alcohol withdrawal zone (70%) of 59 children with intestinal parasites (30 males and 29 female), in addition to 31 blood samples from children without intestinal parasites (20 male blood and 11 female) with a sterile 5 ml disposable syringe. Put 3 ml of blood in a plastic tube 6 mL gel tube, disposable and without anticoagulation. It left in a vertical

position for a period. Then placed in the centrifuge at 5000 cycles/min for 10 minutes. The serum placed in a small tube of Eppendorf tubes and stored at 20 ° C for later use for immunological examination.

Also, 2 ml of blood drawn in a special tube containing the EDTA for the hematological parameters using the Sysmex (XN-350) dived from Japan.

Measurement of IgE concentration in serum: Used the ELIZA method by using the kit processed from Calbiotech Chinese Company to the measurement of IgE concentration (IU/ml) according to the method mentioned by this company. The standard curve is drawn for extracting antibody values in infected and uninfected children.

Measurement of IL-5 concentration in serum: Used the ELIZA method by using the kit processed from Wuhan USCN (Cloud- Clone Crop) Chinese Company to the measurement of IL-5 concentration (Pg. /ml) according to the method mentioned by this company. The standard curve is drawn for extracting antibody values in infected and uninfected children.

Measurement of Histamine concentration in serum: Used the ELIZA method by using the kit processed from Wuhan USCN (Cloud- Clone Crop) Chinese Company to the measurement of Histamine concentration (ng. /ml) according to the method mentioned by this company. The standard curve is drawn for extracting antibody values in infected and uninfected children.

Statistical Analysis: The results of the current study analyzed using the Chi-square test, t-test, and LSD test. Significant differences found below the probability level $P \leq 0.05$.

Results and Discussion:

Table (1) shows the total percentage of intestinal parasites infection among children in the holy city of Kerbala and the relation of this percentage to some epidemiological criteria. The results showed that the total percentage of intestinal parasitic infection was 18.93%. This percentage is near to that of other researchers, such as (21) in Iraq, (22) in Brazil and (23) in Thailand. On the other hand, the current total percentage of infection was less than recorded by (24) in Iraq, (25) in al-Yamen and (26) in Rwanda. While the current percentage was higher than that recorded by (27) in Turkey, (28) in Saudi Arabia and (29) in Iraq. This may be due to the different environments and areas from which the samples were collected, the size of the sample and the years of study, possibly due to the different living conditions, food, health, and climatic and economic conditions. As well as the different age groups, as well as the number of models studied and techniques used to detect the presence of intestinal parasites, such as using the direct method only and not using other methods of concentration Sedimentation or flotation in stool examination (27 & 30). Alternatively, perhaps through the impact of the level of sanitation and human behavior and laboratory diagnosis used to detect infections and health education among schoolchildren (31), or due to differences in awareness regarding the transmission and prevention of intestinal parasites, environmental and personal hygiene, the source of household water supply, and barefoot walking habits. This may also be due to the geographical status of the study area (32).

The percentage of intestinal parasitic infection in the males was higher (20.42%) than in the females (17.16%). Statistical analysis showed a significant difference in the percentage of intestinal parasitic infection between males

and females at $p \leq 0.05$. The results agree with the results of several studies such as the study of (33) in Iran, the study of (34) in Iraq and the study of (35) in Nigeria. The results of the present study differ from the results of other studies such as the study of (36) in Southwest Ethiopia, (24) in Iraq and (37) in Saudi Arabia.

The difference in the percentage of intestinal parasites infection between males and females in this study and previous studies, whether the percentage of infection of males higher than the percentage of female or vice versa, as well as the equal or rapprochement of these percentages in some studies due to the overlap of several factors. These factors including what depends on the habits of the community, some communities are masculine communities that make the male individual a priority in many things, whether playing outside the home or participating in agricultural work at an early age. Unlike other communities depended on females, especially in large families, at the farm we find so susceptible to various parasites including gastrointestinal (34 & 24). Animals in homes such as insects, rodents, and cattle play a very large role in the transmission of many parasites, especially intestinal, to humans as either intermediate hosts or reservoirhosts of these parasites. The physiological, nutritional and hormonal factors between the sexes and the source of infection have an important role in making one of the sexes more vulnerable to parasitic infection alone (38, 39 & 40). In addition to all these reasons, the social and economic situation and overcrowding are factors that significantly affect the percentage of intestinal parasitic infection.

The results of the current study explain the distribution of the percentage of intestinal parasitic infection according to age groups of children. It was found that the age group $1 > 5$ years is the age group with the highest percentage of intestinal parasitic infection (20.34%), Followed by the age group $5 > 10$ years (19.18%), While the lowest rate of infection in the age group 10 -15 year (13.99%). Statistical analysis showed significant differences between the age groups of children with percentages of intestinal parasitic infection ($p \leq 0.05$). The present results agree with results of (41) in the holy city of Kerbala/ Iraq, (42) in Duhok/ Iraq and (43) in Nepal. This result does not concur with the results of (44) in Samarra/ Iraq, (31) in the city of Tikrit/ Iraq and (35) in Nigeria.

The reason for the increase in percentage of infection in the age group $1 > 5$ years due to the weakness of immunity of this group, the children are more contact with the environment outside the home and play in places near the waste (45, 46 & 41). In addition, they eat the vegetables and fruits that not well washed (32). Some of these children have some bad habits, such as picking up some toys or other things and placing them inside the mouth, playing with soil that poses a risk of infection and they play with domestic animals such as cats and birds, which considered as intermediate or reservoir hosts for many parasites (44).

The results of the study showed that the percentage of intestinal parasitic infection in the holy city of Kerbala during the months of the study was uneven. The highest percentage of infection in March (23.41%) and the lowest percentage was in July (15.11%), the percentage of infection in the remaining months of study ranged between these two percentages. The statistical analysis showed a significant difference among intestinal parasitic infection percentages during the study months ($p \leq 0.05$). These results are consistent with results that recorder by (47) in Babylon province (highest percentage of infection in March); while the current results ware similar to that recorded by (41) in the holy city of Kerbala (the highest percentage was recorded in October and November). This result is inconsistent with (44) in Samarra (the lowest percentage of infection in March and the highest percentage of infection in January). The prevalence of parasitic diseases during the months of the year may be due to several factors such as climatic conditions that vary according to latitude and longitude, Agriculture, water availability, high

temperatures, low rainfall and moisture and the presence and spread of insects and other animals, which considered as intermediate or reservoir hosts for many parasites (41).

Table (1): Distribution of intestinal parasitic infection percentage according to epidemiological factors.

Variable	Infection		Non-infection		Total		Significant
	No.	%	No.	%	No.	%	
Gender							
Male	282	20.42	1099	79.58	1381	54.34	χ^2 (P< 0.05)*
Female	199	17.16	961	82.85	1160	45.65	
Age groups							
1->5	285	20.34	1116	79.65	1401	55.13	χ^2 (P< 0.05)*
5->10	135	19.18	569	80.82	704	27.70	
10-25	61	13.99	375	86.00	436	17.10	
Months							
Des. 2017	22	18.97	94	81.03	116	4.56	t- test (P< 0.05)*
Jan. 2028	24	18.32	107	81.16	131	5.15	
Feb.	17	15.18	95	84.82	112	4.40	
Mar.	48	23.41	157	76.58	205	8.06	
Apr.	46	17.10	223	82.89	269	10.58	
May	72	21.36	265	78.63	337	13.26	
June	40	17.47	189	82.53	229	9.01	
July	47	15.11	264	84.88	311	12.23	
Aug.	36	21.30	133	78.69	169	6.65	
Sep.	39	23.08	130	76.92	169	6.65	
Oct.	40	15.50	218	84.49	258	10.15	
Nov.	50	21.28	185	78.72	235	9.24	
Total	481	18.93	2060	80.07	2541	100	

In the current study, five species of intestinal parasites recorded (table 2), three of which were protozoa, which included *E. histolytica* (14.60%), *G. lamblia* (3.90%), *T. hominis* (0.16%), and 2 worms, one of the worms was from Cestoda *H. nana* (0.59%) and one of the nematodes was the pinworm (*E. vermicularis* with percentage 0.08%). Statistical analysis showed significant differences between the percentages of infection with the different species of intestinal parasites recorded in this study (p≤ 0.05).

The prevalence of *E. histolytica* is widespread worldwide, especially in the tropical and subtropical regions such as Iraq(48). The wet climate provides the conditions for the maturity of the cysts of this parasite(49) and then their transmission to humans (50). It also transmitted from person to person directly and indirectly (fecal-oral) through food and water contaminated with mature cysts (10 & 51). As well as the lack of potable water that may be contaminated with cysts of this parasite, that not affected by the chlorination process for sterilizing drinking water (7). Some insects such as domestic flies play a role in transporting this parasite. The increase in infection in children is due to the slow development of their immune system and their lack of knowledge of good health habits (36).

The cause of *G. lamblia* parasites infection is due to the direct transmission of it by contaminated water and food with mature cysts. Animal waste and farming practices are important sources of water and food pollution (52), as well as a lack of interest in personal hygiene(53).

The reason for the low incidence of the *T. hominis* parasite maybe because it has only onestage(trophozoites) that cannot resist the inappropriate conditions and the infection occurs when the stage fed in the quiescentphase (54). This parasite infection may be by contaminated water and foods, especially fresh, non-washed foods (55).

Rats, mice and some other rodents are the natural final host of the dwarf tapeworm (*H. nana*) that directly or indirectly infected of humans (56 & 57). They are global-spread worm but are more prevalent in dry-climate hot regions (57). This worm characterized by its life cycle beingdirect without the need for the intermediate host or indirect, where some insects such as fleas and beetles are intermediatehosts and the spread of these insects play an important role in the transmission and spread of infection with this worm (58 & 59).

The low incidence of *E. vermicularis* worm is due to how the eggs of these worms diagnosed in the feces. The use of the direct smear method does not reveal that the worm eggs are good for the few eggs that this worm poses in the stool first and because it is lightweight. The number of eggs mixed with the exit relatively small and not observed when examining the feces (18). The Scotch Tape Method is more efficient than the detection of parasitic infection (60 & 61). The Vaseline-Paraffin Swab smear method is more efficient than the above methods in detecting pinworm eggs.

Table (2):Distribution of intestinal parasitic infection percentages in children according to species of the parasite (number examined = 2541).

Species of parasites	Infected number	Percentage (%)
<i>E. histolytica</i>	471	14.60
<i>G. lamblia</i>	99	3.90
<i>T. hominis</i>	15	0.59
<i>H. nana</i>	4	0.16
<i>E. vermicularis</i>	2	0.08
t-testt(P≤ 0.05)*		

Table (3) shows the means of hematological parameters in children infected and non-infected with intestinal parasites in the holy city of Kerbala. The results of this study showed asignificant decrease(p≤ 0.005)in the RBCs, Hb, and PCV in children infected with intestinal parasitescompared with non-infected children, but the results showed a significant increase in the WBCs, Neu., Ba.,Eo.andPLT in children infected with intestinal parasitescompared with non-infected children.

The reduction in some hematological parameters (RBCs, Hb, and PCV) is due to intestinal parasites that colonize the digestive system, especially duodenum and jejunum, iron absorption sites where infected intestinal parasites cause bad absorption of sugars, fats, vitamins (such as D and B12), folic acid, iron, and zinc.The weak absorption of iron causes iron deficiency, which causes anemia because it enters the composition of hemoglobin (62, 63 & 64). It due to the difference like the damage caused by each parasite species where worms usually have a

greater effect than protozoa on hematological parameters (10). The *E. histolytica* and *G. lamblia* infection lead to iron deficiency (65). The presence of *E. histolytica* in the intestines causes gastrointestinal disturbances, through the trophozoite, which attaches to the intestinal villi and absorbs nutrients (66). It also secretes the proteins that analyze the host's tissues and cells and feed on red blood cells (67). In addition, parasite infection may lead to necrosis of the intestinal mucosa, leading to the deterioration of absorption of essential substances and tissue damage; Intestinal parasites are strongly associated with the development of anemia because they cause malabsorption and undernutrition (68). The Giardia parasite causes damage to the gastrointestinal mucosa, causing malabsorption syndrome, especially vitamins (Vitamin B12) and iron (69, 70 & 71). Besides, other essential nutrients because it makes a barrier to the passage of these substances from the intestinal cavity to the bloodstream. The effect of the dwarf worms on the amount of hemoglobin, through the competition of the host on food, depending on the number and density of this parasite. The pinworm has little effect compared to other parasites because they are found in the small intestine and do not feed on blood but feed on secretions or host products and do not cause any damage to the intestinal wall (72).

The increasing number of certain types of white blood cells may be due to their role in the response of the immune system to the treatment and elimination of intestinal parasites, especially the eosinophils. Eosinophilia caused by the effect of IL-5 synthesized from the Th2 cells. IL-5 is the most important cytokine in the transformation and development of eosinophils, and acts as an "eosinophil activator". One of the significant causes of the increase in eosinophils in the blood is parasitic diseases. Eosinophils are effectors against parasitic targets. The set of parasitic diseases are associated with a polarized Th2-type immune response, typified by reactive cell types, eosinophils and mast cells (73). As well as the possession of eosinophils for antibody receptors such as IgA, which plays an important role in the protection of mucous surfaces susceptible to parasite invasion (74 & 75). The neutrophils play an important role in immunosuppression against various types of parasites (both internal and external). Despite their short life, they produce large amounts of immunosuppressive molecules such as histamine, cytokines, chemokines and active fats that stimulate immune response Type II (cellular immune response to toxicity). These cells activated directly by parasite substances or indirectly by distinguishing parasite antigens by strong binding of the IgE antibodies to the FcεRI receptor on the cell surface. Many parasitic infections increase the presence of these cells in tissues (76).

Table (3): Comparative between hematological parameters in infected and

Non – infected children with intestinal parasites.

Hematological parameters	Infected children M±SD	Non-infected children M±SD
RBCs (X106/mm ³)	4.45±0.42*	4.83±0.41*
Hb (g/dl)	11.74±1.8*	13.03±0.79*

PCV (%)	35.01±3.93*	38.66±2.42*
PLT (X10 ⁹ /mm ³)	371.41±130.38*	323.84±60.16*
MCV (FL)	78.99±7.95	80.01±4.06
MCH (pg.)	26.13±3.74	26.83±1.8
MCHC (g/dl)	33.17±2.17	33.81 ±1.17
WBCs (cells/mm ²)	12.12±5.06*	7.65±1.59*
Neutrophils (%)	60.71 ±18.18*	48.09±9.12*
Basophils (%)	1±0.34*	0.49±0.26*
Eosinophils (%)	4.06±3.43*	2.31 ±1.38*
Lymphocytes (%)	34.57±12.39	34.35 ±4.82
Monocytes (%)	5.53 ±3.77	5.51 2.07
*mean significant		

The results of the current study (Table 4) showed that the concentration of IgE, IL-5 and histamine in children infected with intestinal parasites was higher (56.63 IU / ml, 22.08 pg. / ml and 76.27 ng/ml, respectively) compared to non-infected children(42.76 IU / mL, 15.43 pg/ ml and 65.35 ng/ml, respectively). Statistical analysis using t-test showed a significant difference in the concentration of these parameters in sera of children infected with intestinal parasites compared to non-infected children.

The high concentration of IgE in children infected with intestinal parasites compare to non-infected children due to immune response and host defense mechanism against parasite and toxins,as can be different depending on the type of parasite that causes infection (77). Many parasitic infections defended by activating Th₂- cells, producing IgE antibodies and activating the role of eosinophils (11). The increase in IgE levels often leads to the expulsion of parasites and the elimination of toxins. The binding of IgE with its high-receptors FcεRI on the surface of cells such as mast cells and basophils and the subsequent activation of these cells results in the production and release of biologically active intermediates such as histamine and some other active amines that play an important role in the parasite expulsion. Another mechanism contributing to the expulsion of parasites is antibody-dependent cell-mediated cytotoxicity (ADCC) via IgE receptors or IgG (77).

The elevation in the concentration of IL-5 in sera of children infected with intestinal parasites compared with non-infected children was due to the host's immune response to intestinal parasites by Th₂ cells. These cells respond by producing high levels of interleukins IL-4, IL-5, IL-9, IL-10, IL-13, IL-21 and IL-33. These interleukins regulate the reaction of immediate hypersensitivity, which involves B-lymphocytes changing their production from antibodies to IgG4 and IgM only, high number of eosinophil, goblets cells, mast cells, and alternative activation of the macrophages and influx of inflammatory cells such as eosinophil that contribute to parasite killing (78, 79 & 80).

The increase in histamine concentrations in sera of children infected with intestinal parasites compared to the uninfected children, because of the host's immune response to intestinal parasites. This infection leads to increased

production of mast cells that produce histamine, an important factor in the intestinal tract as histamine participates in the inflammatory process (13). When an allergen or antigen enters the body, mast cells secrete histamine and other active amines in the blood vessels to obtain an appropriate immune response to the body (81). This result agreed with the results of (80) in the province of Baghdad, capital of Iraq.

Table (4): Comparison of immunological parameters concentration among children infected and non-infected with intestinal parasites.

Concentrations of immunological parameters	Infected children M±SD	Non-infected children M±SD
IgE(IU/ml)	56.63*±14.02	42.76*±12.04
IL-5(pg./ml)	22.08*±6.79	15.43*±3.19
Histamine(ng/ml)	76.27*±23.75	65.35*±15.7
*mean significant		

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