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Parasitological and Hematological Study of Children Infected with Intestinal Parasites in Baquba City

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Abstract

The intestinal parasite infection is a significant health issue in developing countries, particularly in tropical and subtropical areas. About 3.7 billion individuals are thought to be affected annually, most of cases are children, including children in Iraq, and Baquba city. According to researchers, the main intestinal parasites affecting humans include *Entamoeba histolytica*, and *Giardia lambellia*. The present research was conducted to evaluate the prevalence of intestinal parasitic infections that causes serious problems among children aged (>1 – 15 years old) and to investigate the relationship between parasitic infestation among children and their immunity (i.e., IgE), the levels of Vit D3, and Zinc. About 300 samples were collected from hospitalized and non-hospitalized children and 100 control. A fresh stool and blood sample were taken from each child during the period from 1st of November 2021 to 31st of May 2022. The collected samples were examined microscopically for identifying the parasite and examined for immunological and hematological parameters. The result showed a significant variation among children from different age groups, the age group (1>-5) years old had the highest infection with intestinal parasites. In terms of gender, males had the highest intestinal parasitic infections than females. The levels of Vitamin D3 and Zinc decreased in infected children with intestinal parasitic comparing to the control. While IgE indicated higher levels in infected children than in the controls

Keywords: Endo-parasite, Intestinal parasite, Blood parameters, Vittamin D, Zinc, IgE

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دراسة طفيليات ودموية للأطفال المصابين بالطفيليات المعوية في مدينة بعقوبة

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الخلاصة

تعد عدوى الطفيليات المعوية مشكلة صحية كبيرة في الدول النامية، لا سيما في المناطق الاستوائية وشبه الاستوائية. يُعتقد أن حوالي ٣,٧ مليار شخص يتأثرون سنويًا، معظمهم من الأطفال، بما في ذلك الأطفال في العراق ومدينة بعقوبة. وفقًا للبحث، تشمل الطفيليات المعوية الرئيسية التي تصيب الإنسان هي المتحولة الحالة للنسيج *Entamoeba histolytica* والجياردية اللمبلية *Giardia lambilia* . تم إجراء هذا البحث لتقييم انتشار الالتهابات الطفيلية المعوية التي تسبب مشاكل خطيرة بين الأطفال الذين تتراوح أعمارهم (< ١-١٥ سنة) وللتحقق من العلاقة بين الإصابة الطفيلية لدى الأطفال ومناعتهم (أي:IgE)، ومستويات فيتامين د٣ والزنك. تم جمع حوالي ٣٠٠ عينة من الأطفال في المستشفيات وغير الراقدين بالمستشفى و١٠٠عينة من مجموعة السيطرة. تم أخذ عينة من الدم والبراز من كل طفل خلال الفترة من ١ تشرين الثاني ٢٠٢١ إلى ٣١ ايار ٢٠٢٢. تم فحص العينات التي تم جمعها مجهريًا للتعرف على الطفيل وفحص العوامل المناعية والدموية. أظهرت النتائج وجود تباين معنوي بين الأطفال من مختلف الفئات العمرية، حيث سجلت الفئة العمرية (١< -٥) سنوات أعلى إصابة بالطفيليات المعوية. من حيث الجنس، كان لدى الذكور أعلى نسبة من العدوى الطفيلية المعوية مقارنة بالإناث. كانت مستويات كل من فيتامين د٣ والزنك أقل في الأطفال المصابين بالطفيليات المعوية عنها في مجموعة السيطرة. بينما أشار IgEإلى مستويات أعلى في الأطفال المصابين مقارنة بمجموعة السيطرة.

الكلمات المفتاحية: طفيلي داخلي، طفيلي معوي، بارامترات الدم، فيتامين د، زنك،IgE .

Introduction

Endoparasite infection is a major health problem worldwide. It causes serious diarrhea, gastroenteritis, and malnutrition in different age groups and populations. The World Health Orgnization (WHO) in (2012) [1] indicated that children and poverty humaines were most vulnerable parasites due to their suscbitablity to contaminated soil in theior life more than others . Symptoms of intestinal parasite infection range from diarrhea to abdominal pain, and the more severe symptoms appear among immunocompromised and malnourished people [47]

United Naations International Childrens Emergency Fund (Unicef) confirmed in 2009 [2]. The intestinal parasites are among the most common causes of diarrhea, it is the leading cause of mortality and morbidity for under five years old children. In the same respect Long *et al* ., 2007 [3] mentioned that the age of the patient is an associated factor related to the individual's immunological status and behavioral patterns that contribute to a high prevalence of intestinal parasites among children than in adults.

Intestinal parasitic infections are also responsible for iron deficiency (anemia) [1]. More than that, inadequate intake of selected micronutrients can cause immune deficiency and increase susceptibility to microbial infection [4].

It was metioned by Zahra, (2017) [5] and Mabbott (2018) [6] that the specific immune response to parasites leads to the production of antibody. Infection by protozoan parasites is associated with the production of Ig classes them IgE is produced in response to intestinal protozoa, in addition to these specific T-dependent responses, a non-specific hypergammaglobulinaemia is present in many parasitic infections. Much of this non-specific antibody is the result of polyclonal B cell activation by released parasite antigens acting as mitogens. This response is ineffective at counteracting the parasite and can enhance the pathogenicity by causing the production of autoantibodies.

One of the vital components of blood, white blood cells guard against pathogens, parasites, cancers, and other disorders. The neutrophil, eosinophil, basophil, lymphocyte, and monocyte types of white blood cells all have different sizes, proportions, and roles. ALmaamory, (2015) [7] and Al-Mozan *et al*.,2017 [8], those researchers suggested that the creation of IgE antibodies and activation of eosinophils is caused by the reaction and activation of (TH2) cells (a type of differentiated T helper cells that are primarily involved in defending the body from external pathogens), which prevents parasitic infections. Eosinophils are activated by the cytokines (IL-5) released by TH2 cells, which attract them to the locations of the parasites where they release toxic granular proteins that kill the parasites. When an individual's immune system creates IgE antibodies in response to a foreign antigen, an allergic reaction takes place IgE molecules bind tightly to the surface of the mast cells and the basophil cells. High serum levels of IgE,

particularly in those with intestinal parasite infections, induced the production of histamine and other intermediates, which resulted in an acute hypersensitive reaction that destroyed the parasites. High levels of histamine and other allergen-causing chemicals are present in these cells. The allergen binds IgE to the cell surfaces on subsequent exposure to the same antigen. To get the right immunological response to defend the body, this triggers the mast cell metabolism and consequent release of histamine and other active amines in the blood vessels ALmaamory, (2015) [7].

Micronutrients with immune-modulating properties, such as vitamin A, vitamin B12, vitamin C, alpha-carotene, riboflavin, zinc, selenium, and iron, can affect how an infection develops (a laboratory study has demonstrated that vitamin deficiencies can result in parasitic infections. The presence of antibodies points to a potential relationship between vitamin deficiencies and the vulnerability to parasite infections [9,10].

Aims of the study

1. Investigating the prevalence of intestinal parasitic infection among children (>1 – 15 years old) who attended AL-Batool hospital in Baquba city.
2. Investigating the relationship between intestinal parasitic infection and some blood parameters, CBC, WBC count, and HB.
3. Examining the relationship between intestinal parasitic infection and some immunological parameters such as IgE, vitamins such as Vitamin D, and minerals such as Zinc.

Material and Methods

Three hundred children were considered in this study (visits ABTH, and health centers in Baquba city) that have symptoms of diarrhea two hundred thirteen samples were found to be positive for different intestinal parasites in the patient, and 100 samples as a control group taken from children who were visited hospitals but have negative for intestinal parasitic infection, the

children who complaining diarrhea were undergo screening for intestinal parasitic infection(any parasites stage eggs, cysts or trophozoite). Stool samples were collected in a plastic container the sample was taken with wooden sticks and diluted with distilled water or iodine then examined under the microscope to indicate the presence of any parasites stage. Blood samples were collected from the children to evaluate the blood parameters (WBC, HB, MCHC, PLT, RBC, MCH, MCV). This study was conducted during the period from First of October 2021 to the end of May 2022 in Al Batool Teaching Hospital laboratories and Medical Center.

Hematological, Biochemical, and Immunological Study

For this, 60 children with intestinal parasites (39 males and 21 females) and another 30 blood samples from children not complaining of signs of intestinal parasites (18 males and 12 females) were put in a sterile 5 ml disposable syringe. A plastic 6 mL gel tube was filled with three milliliters of blood without the use of an anticoagulant. This gel tube was spun at 5000 cycles per minute for 10 minutes after being left upright for a while. The serum was then put into Eppendorf tubes and kept at 20 ° C to be utilized for an immunological test later on. Also, the other 2 ml of blood was drawn in a special tube containing the EDTA for the hematological parameters using the Sysmex (XN-300) dives from Japan.

Biochemical parameters (vitamin D and zinc)

Biochemical tests were performed for 60 patients and 30 healthy children by using the Cobas E 411 auto-electrolysis devices and Spectrophotometer.Which analyzes the results of the following laboratory tests of vitamin D and zinc parameters.

Statistical analysis

The statistical packages SPSS version 25.0 and Graph pad prism version were employed to investigate the data. The investigated IgE antibody, hematological parameters, and biochemical parameters were first tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk test). Parameters that fit both tests (no significant difference) were given as mean ± standard deviation (SD), and the parameters that did not fit the normality tests (significant difference) were given

as median and range, and a significant difference between median was assessed by Mann-Whitney (for comparison between two groups). The other parameters were given as percentage frequencies, and significant differences between frequencies were assessed by the Pearson-Chi-square test or two-tailed Fisher exact probability (*P*). Hematological parameters were estimated by a full automated cell-Dyn Ruby System which is used for the analysis and a count of hematological parameters was employed to carry out these analyses [11].

Results and Dscussion

Table 1: Frequency and percentage of age groups and gender

Group	Count	Percent	*P* value
1-5 year	101	47.4%	P<0.05*
6-10 year	75	35.2%	
11-15 year	37	17.3%	
Males	130	61%	P<0.05*
Females	83	38.9%	

Results of the present study show significant differences (p<0.05) among patients. The investigated IgE antibody, hematological parameters, and biochemical parameters were employed to carry out these analyses according to gender and age. Based on age groups, the 1-5 years old recorded the highest percentage (47.4% followed by 6-10 years old (35.2%) then 11-15 years old who recorded the least percentage (17.3%). The infections among males (61%) were higher percentage than in females (38.9%) (Table 1).

Table 2: Relation of parasitic infections with age of participants

		Age groups (years)			Total	P value
		1-5	6-10	11-15		
Entamoeba histolytica	N	70	55	25	150	P<0.01**
	%	46.6%	36.6%	15.3%	100.0%	
Giardia lamblia	N	11	9	7	27	P<0.01**
	%	40.7%	33.3%	25.9%	100.0%	

Parasitic infections and age groups

Results of our study show significant different ($p<0.05$) among patients according to parasitic infections and age groups. The 1-5 age groups scored highest percentage in patients with both parasites *Entamoeba histolytica,*and *Giardia lamblia*, compared to other age groups 6-10 and 11-15 years old see (Table 2).

Table 3: Comparative hematological parameters between study groups

Blood par		N	Mean	SD	*P* value
WBC	Patients	60	12.5	4.01	P<0.01**
	controls	30	7.13	2.37	
RBC	Patients	60	4.45	0.48	P>0.05
	controls	30	4.15	0.90	
HGB	Patients	60	9.82	1.13	P<0.01**
	controls	30	13.83	1.09	
MCV	Patients	60	64.85	7.13	P<0.001***
	Controls	30	91.83	7.38	
MCH	Patients	60	21.20	3.04	P<0.01**
	controls	30	30.7	1.95	
PLT	Patients	60	370.67	84.92	P<0.001**
	controls	30	293.27	59.71	

Hematological parameters and study groups

The results also showed significant differences ($p<0.05$) between the two study groups (patients and controls) in some hematological parameters whight blood cell count (WBC), haemoglobein (HGB), meav corpuscular volium (MCV), MCH (mean corpuscular hemoglobin) and PLT. WBC and PLT parameters were higher in patients (12.5±4.01 and 370.67±84.92) than in controls. In contrast, HGB, MCV, and MCH parameters showed lower in patients than in controls. Moreover, there were no significant differences between patients and controls in RBC (Table 3 Figure 1).

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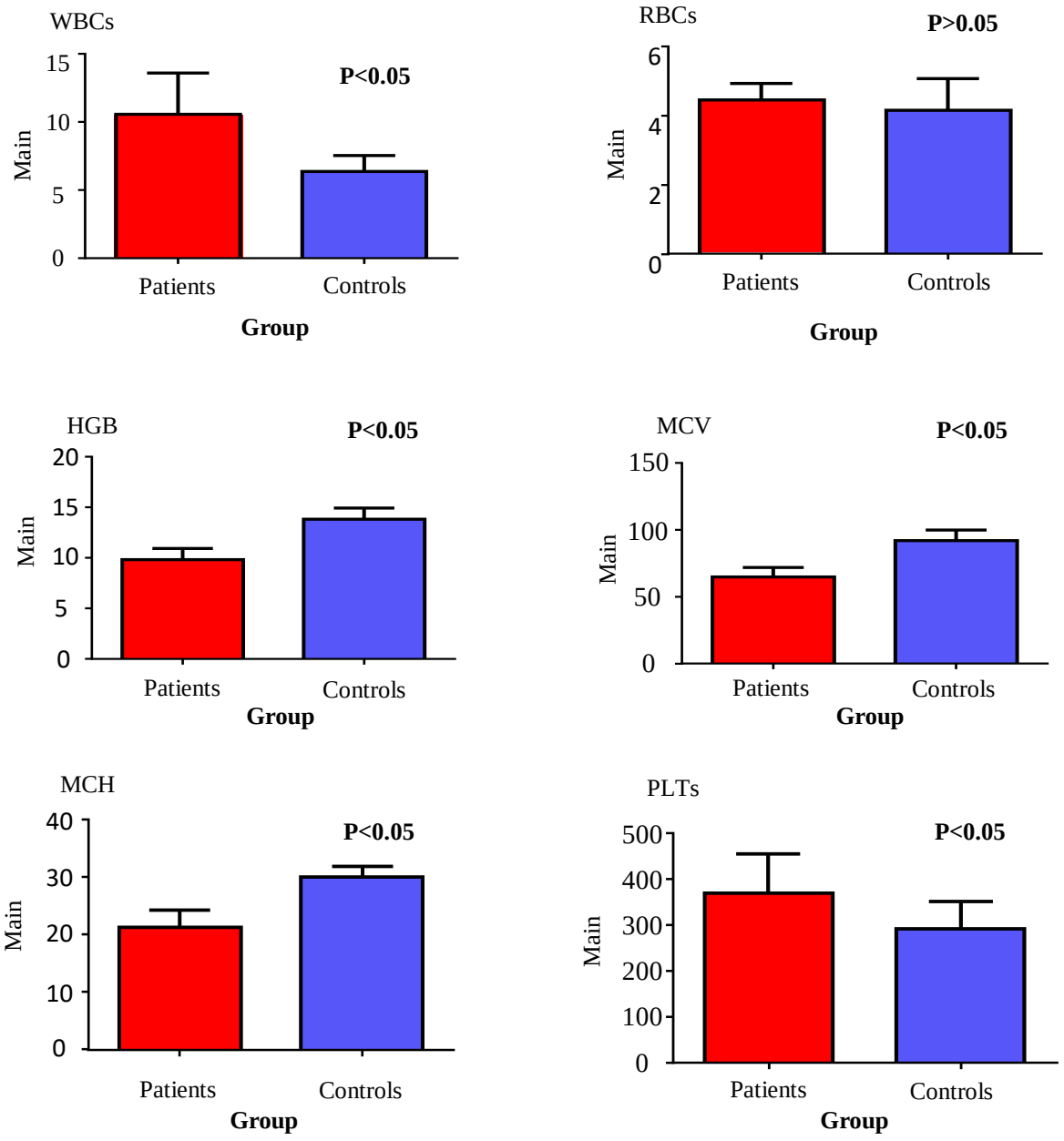

Figure 1: Comparative hematological parameters between study groups (patients and controls)

Table 4: Comparative biochemical and immunological parameters between study groups

Groups		N	Mean	SD	*P* value
VitD3	Patients	60	24.48	4.24	P<0.001***
	controls	30	70.30	21.87	
Zinc	Patients	60	73.68	12.51	P<0.01**
	controls	30	90.17	12.58	
IgE	Patients	60	60.55	18.32	P<0.001***
	controls	30	31.23	12.21	

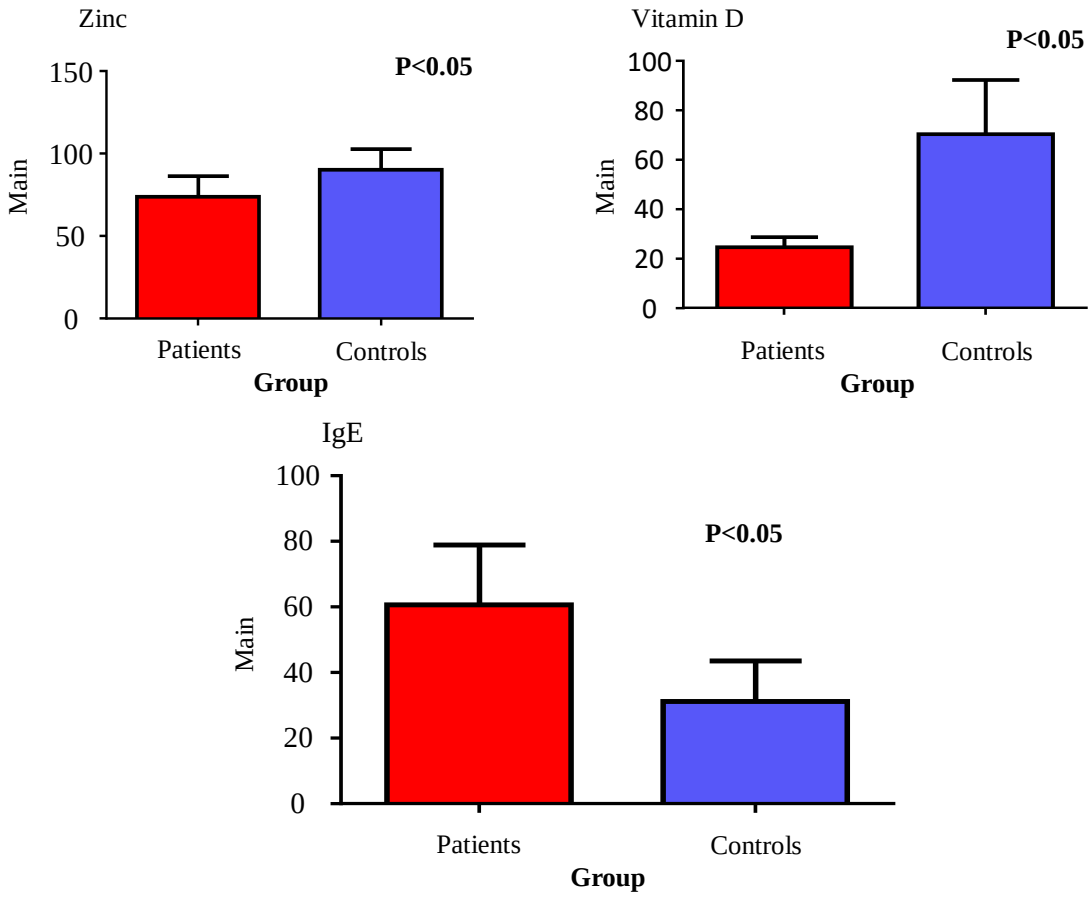

Figure 2: Comparative immunological parameters within study groups.

Results show significant different (p<0.05) between levels of Vit D Zinc) and IgE in patients than controls. The Vit D (24.48 ±4.24), and zinc (73.68 ±12.51) parameters were lowest in

patients than in controls. While the IgE parameters (60.55±18.32) were higher in patients than in controls (Table 4, Figure 2).

The finding showed the levels of vitamin D3 and zinc elements was low in patient than control, IgE was high in value while Hb show lower level in patients than controls and WBC was higher ratio in most patients in children with parasitic infection compared to the healthy ones.

Total percentage of parasitic infectionamong the study sample

This study found that children admitted to Albatool Hospital frequently had gastrointestinal parasites. *E. histolytica* and *G. lamblia* infections were the most prevalent parasite infections in this investigation, these two parasites are prevalent in warm areas and have a global spread, this idea is supported by studies done by Al-Warid *et al*., 2013 [12] and Al-Saqur *et al.*,2017 [13]. The high prevalence of these parasite infections can be attributed to a variety of factors, such as the simple means of transmission and the nature of direct fecal-oral contamination, which can occur either directly from person to person or indirectly through the consumption of contaminated food and water , and because protozoans have a straightforward life cycle that does not require an intermediate host, many of them commonly live in the human intestine before turning pathogenic when the immune system is compromised [14]. In addition, the research area contains parasite cysts that are resistant to treatment. The results of our study were consistent with other studies that showed that these parasites are the most common in Iraq [15]. In a study conducted by Mbuh *et al*., 2010 [16] they found that the rate of protozoan infections *E. histolytica* and *G. lamblia*. (44.2%) (49.7 %) was higher than the rate of helminthic infections (6.74%). Moreover a study conducted in Diyala communities found protozoan infection higher than helminthic [17]. The prevalence of these protozoans is also common in some eastern countries. For example, in India, a study found protozoans more common than helminths (77.4% and 23% respectively) [18]. Similar results were found in Senegal, where protozoans (29.6%) were more prevalent than helminths (0.8%) [19] . However, in China it was found that the total prevalence of infection rate helminths was higher than the protozoan [20].

Effects of gender and age on the vulnerability of patients to be infected by intestinal parasites

The infection rate for *E. histolytica* was significantly higher than *G. lamblia*, which may be due to the activity of cysts under environmental conditions. Our results were in agreement with the results of other studies [17] [21] [22] However our results disagreed with the results of Al-Qadhi *et al.*, 2011 [21] who indicated that the incidence of *G. lamblia* was superior than the incidence of *E. histolytica*. The differences in the prevalence of these intestinal protozoal parasites between studies may due to several factors, including environmental, nutritional, socio-economic, and geographical conditions, as well as demographic and health-related behavior.

Based on both age and gender, there were disparities in parasitic infection rates. In other investigations, the prevalence of both *G. lamblia* and *E. histolyticain* was significantly influenced by age and gender [13] [23]. In a study to determine the frequency of protozoan the bulk of *G. lamblia* and *E. histolytica* positive samples, according to the findings, came from children aged 1 to 5, followed by those aged 6 to 10, and then 11 to 15[13] .The present findings are in line with those of other studies carried out in India Jad *et al.*, 2017 [23] and in Thi-Qar Province AlMosawi, 2016 [24], they discovered increased infection rates in children at the age more than 6 years, they ascribed that to ; because children in this category had a higher likelihood of consuming unclean and tainted food, particularly at this age when they had more contact with the ground and were more susceptible to parasites than older children were. Another possible explanation for the greater parasite infections in the young population is their poorer immunity [23] few studies have shown a correlation between parasite infection and elevated levels of IgE, Vitamin D, and Zinc.

In our study and many other studies, patient gender may be one of the major variables affecting the prevalence of intestinal parasites [13,25]. The incidence of protozoan parasites has been strongly influenced by gender in numerous local studies carried out in Baghdad AL-Kubaisy *et al*., 2014 [26], Kirkuk Obaid, 2014 [22] and Al Saqur *et al*., 2017 [13]. Males made up the vast

majority of the positive instances in this research. Although both genders (males and females) of different ages are susceptible to amoebiasis because they both shared the same environments and climates. The result of another studies showed that males were more vulnerableto intestinal parasitic infection than females. This may be because males are more exposed to unhygienic conditions (contaminated soil or playing with animals and swimming in contaminated water) in fields during outdoor activity females may spend more time indoors [25]

Hematological parameters: the current study found that there was significant different in the value of Hb between patients had been infected *Giardia lamblia* and/or *E. histolytica* only *E. histolytica* infections compared to controls. The results also showed that there is no difference in RBC levels, while the levels of MCV, MCH, and MCHC show significant differences between patients and control. Additionally, the PLT count significantly increased in both patients and there is a significant difference between patients and control. Cyntia *et al*.,2012 [27] looked at 81 individuals had helminth and *E. histolytica* infections and found that there was no improvement in the erythrocyte and hemoglobin levels in the parasite-infected patients.

Cyntia *et al*.,2012 [27] stated that patients with parasite infections had lower rates of erythrocytes and hemoglobin levels, and there had been no appreciable increase in platelet levels. He also said that there had been no change in the volume of blood cells or the concentration of hemoglobin within the cell, Because of competition for the source of food and the absorption of food from the stomach lining, levels of hemoglobin and B12 decrease, as well as vitamins and lower blood levels, also the patient's health, immunity and social status

The decomposition of the blood sample also affects the result of the analysis, so the results vary from one region to another, as well as between different studies while 207 patients infected with *G. lamblia*, *E. histolytica*, and harmful bacteria were evaluated in a study done by Obaid, 2014 [22] in Al Haweeja/Kirkuk, Iraq, there was no impact of the amoeba parasite on blood parameters. The researcher may have looked at patients whose parasite levels were insufficient to cause malabsorption or enough blood loss to have an impact on iron levels, which could account for the difference. Because of the bleeding brought on by invasive *E. histolytica*, research has shown that a high frequency of intestinal infections, particularly parasites, is linked

to anemia and a sharp increase in leukocyte rates as described by Salim, 2018 [28] and found the individuals with symptomatic *G. lamblia* and *E. vermicularis* infections have lower vitamin B12 levels than asymptomatic patients; this could indicate that the intestinal mucosa is more severely impacted. These findings may open the door to treating these parasite infections in symptomatic children who have *G. lamblia* and *E. vermicularis* infections, along with providing vitamin B12 supplements. The results of our study are compatible with previous studies in many regions in Iraq, in Kurdistan by Hama, A. A. & Rahemo, Z. I. 2014 [29] and hussein AL-MOUSAWI, & NEAMAH, 2021 [30]. They recorded significant increase of MCV, MCH, MCHC, Hb, and HCT in children patients more than in control. However, many studies disagree with Nsagha et al., 2015 [31] the direct link between intestinal parasite infection and the levels of blood parameters. and they attributed the low hemoglobin concentration, to malabsorption syndrome or Ferro-elective malabsorption.

Immunological parameters and parasitic infections:

Eosinophils in particular may also have increased in number as a result of their role in the immune system's response to the treatment and eradication of intestinal parasites. Eosinophilia is brought on by the activity of IL-5 generated by Th2 cells.The most significant cytokine in eosinophil growth and transformation is IL-5, which functions as a eosinophil activator. Parasitic disorders are one of the important factors contributing to the rise of eosinophils in the blood. Eosinophils are agents that attack parasite. A polarized immunological response of the type Th2, characterized by eosinophils, mast cells, and reactive cell types, is linked to the group of parasite illnesses [32]. Additionally, the presence of eosinophils for antibody receptors like IgA, which is crucial for guarding against parasite invasion on mucosal surfaces [33].

For immunological suppression against a variety of parasites, neutrophils are essential (both internal and external). Despite having a short lifespan, they produce a large amount of immunosuppressive substances, such as histamine, cytokines, chemokines, and active lipids that support type II immune response (cellular immune response to toxicity). These cells are either directly triggered by parasite material or indirectly by strong IgE antibody binding to the

Fc RI receptor on the cell surface, which identifies parasite antigens. Because of various parasite infections, these cells are more abundant in tissues [34]

According to the type of parasite that causes infection, distinct immune responses and host defense mechanisms against parasites and toxins may explain why children with intestinal parasites have higher concentrations of IgE than children who are not affected [35]. According to Amâncio, F. A. M. 2012 [36], Th2-cell activation, IgE antibody production, and eosinophil activation all contribute to the defense against several parasitic diseases.

Al-Ibrahimi claimed in 2016 [37] that a rise in IgE levels frequently causes parasites and toxins to be expelled. As a result of IgE's interaction with its high-receptor FcRI on the surface of cells like mast cells and basophils, and the subsequent activation of these cells, which is essential for the expulsion of the parasite, histamine and a number of other active amines are generated and released. Another process that contributes in the expulsion of parasites is antibody-dependent cell-mediated cytotoxicity (ADCC) via IgE or IgG receptors.

Results of our reaserch in Baquba city governorate of Diyala agree with Al-Hasheme et al., 2020 [38]. There was a significant decrease in serum levels of Zn in infected children than in noninfected children. These alterations in serum level of Zn were more significant with *G. lamblia* infection than with other parasitic infections. Our results were in agreement with Al–Mousawi & NEAMAH, 2021 [30]. This could be explained by the antagonistic effect of Zn and Zn deficiency in the patient with parasitic infection. Zn antagonizes by inducing the synthesis of thionein, which has a higher affinity to Cu than Zn [39]

The declines in serum Zn levels in the infected group were consistent with findings from other research Al-wahab et al., 2009 [40] and Doaa *et al*., 2015 [41]. Thus, the body may use natural cytoplasmic superoxide dismutase (SOD) to scavenge the excess formation of reactive oxygen species as a result of parasite infection, which results in a lower serum Zn level. This could be related to dietary variables (intake of food poor in antioxidants).

Immune control and inflammatory processes are affected by vitamin D. All immune cells, as well as the majority of bodily tissues, contain vitamin D receptors Youssef *et al*.,2011 [42]. Few research have examined how vitamin D affects childhood diarrhea [43].

The information at hand points to a link between a lack of vitamin D and a higher incidence of GI and ear infections [44]. In our study serum, vitamin D levels were lower in the case group compared to the controls. Whether this finding was just an epiphenomenon or indeed represents a co-risk factor for diarrhea is unclear.

The present inconsistent findings may be connected to the debate over the threshold for determining low 25(OH) D levels [45]. A 20 ng/ml cut-off threshold is frequently used in modern literature, which is used for our investigation. However, given that it is linked to an increase in PTH levels, other writers contend that a level between 20 and 30 ng/mL still denotes a modest level of deficiency (insufficiency). This difficulty in identifying vitamin D shortage or sufficiency has spread to the literature and led to conflicting findings on how common vitamin D inadequacy is [46]. *G. lamblia* could cause malabsorption by covering the lumen of intestinal cells and deteriorating the physiological action of epithelial cells [47].

Additionally, inadequate intake of certain micronutrients can result in immune deficiencies and increase susceptibility to infection. Deficiencies in some nutrients can lower the host's immune function, impairing the body's resistance to infectious diseases and increasing susceptibility to intestinal parasites [9].

Conclusions:

In conclusion, our findings from this report reveal a higher prevalence of *E. histolytica* than *G. lamblia* , and endoparasitic infections has a direct or indirect impact on levels of blood parameters and immunity parameters (IgE, vit D, zinc) where a decrease in the levels of antioxidants such as vit D3and zinc was observed. On the other hand, there was an increase in the level of concentration of IgE, is the indicator of oxidative stress involved in the pathogenesis of several diseases.

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