Protozoa Infections and Celiac disease: Relationship and hematological study among children under five years

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Abstract. The aims of this study was concerned on the evaluation the etiology of diarrhea (parasitic (E. histolytica and G. lamblia) and Celiac disease) in children under five years and its effectiveness on the some laboratory investigations which is the complete blood picture (CBP), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), the sero-prevalence of celiac disease; in which two types of autoantibodies was studied, anti-gliadin (IgG and IgA), and anti-tissutransglutaminase (IgG and IgA); A total of 67 children with diarrhea have been diagnostic, E. histolytica recorded the largest group 36 sample followed by G. lamblia 23 sample, and sero-positive celiac disease only 8 children. the E. histolytica was mostly at 4-5 years (36.1%) and G. lamblia was mostly in age 1-2 and 2-3 years (30.4%). we found that most of neutrophilia and eosinophilia cases were in E. histolytica and G. lamblia. Hemoglobin concentration show a significant decrease in patients with diarrhea. Abnormal ESR and CRP titer reading has been in shows higher mean values with E. histolytica and Sero-positive celiac disease then followed by G. lamblia.

Introduction:

Among the important intestinal protozoa, Giardia lamblia, which are distributed worldwide and are common in warm humid climates are microorganisms, whip, single cell, eukaryotes. The cause of diarrheal disease worldwide (1).

Giardia is one of the primary priorities that cause public health problems in most developing countries as well as some developed countries. G. is one of the causative agents of diarrhea in both children and adults (2).

Giardia is distribution worldwide. Prevalence rates are increasing in developing countries. Can reach 20% to 30% in the developing world, and from 2% to 5% in industrialized countries (3). Giardia epidemics have been reported on a number of occasions. While eating and bag-contaminated water is the most common method of infection, there may be direct transmission from person to person in children, male homosexuals, institutional personnel, camps, travelers, and an immunized patient.

Giardia is a highly contagious disease. If a family member becomes infected, the others will also be infected. Transmissions depend on the ingestion of mature bags. Prevention is based on a high level of sanitation. Cats, dogs and other animals can carry the infection, but their role in the epidemiology of Giardia has not been proven (4).

Entamoeba histolytica is estimated to spread around the world at about 500 million people, responsible for 40,000 to 100,000 deaths each year, especially in developing countries. (5) The incidence of E. histolytica varies among countries, socio-economic conditions, health conditions, and
population (6). Environmental, socio-economic, demographic and hygiene behavior are known to affect the transmission and distribution of intestinal parasitic infections (7). However, in the tropics, amoebic epidemiology is completely different and is more common among the general population, particularly among patients who go to health centers with diarrhea (8) and are distributed worldwide and pose a serious health threat in tropical and subtropical regions. Area Development (9).

Every day, 0.5 million tons of sewage are dumped in Iraqi rivers that pollute the country's main source of drinking water. This situation leads to the spread of epidemics from various diseases such as Giardia and Amebia (10). The spread of amoebiasis and the spread of Giardia infection generally occur in all parts of the country, especially in the summer months. (11) Diarrheal disease, either through direct contact or through contamination of food and water, and contaminated food and beverages with humans. For these pathogens and the use of waste for chemical fertilization, as well as the use of different sources of non-potable water are all contributing to the transmission of diarrheal disease.

There are factors that increase an individual's susceptibility to infectious pathogens, including: early age, vulnerability, malnutrition, lack of breastfeeding, lack of sanitation and hygiene to eat contaminated food and beverages for these nurses, and low level of motherhood. Coeliac disease is caused by a reaction to gliadin, a prolamin (gluten protein) found in wheat, and similar proteins found in the crops of the tribe Triticeae (which includes other common grains such as barley and rye). CD occurs almost exclusively in patients who express the MHC class II HLA-DQ2 and HLA-DQ8 molecules. The prevalence of celiac disease in the adult population varies between roughly one in 100 and one in 300 in most parts of the world (12, 13).

Materials and Methods:

This cross-sectional study was conducted in Karbala teaching hospital of children. Samples were collected from 67 children with diarrhea, their ages ranged between one month -five. From all children two types of samples were collected, stool and blood sample, the stool sample used in general stool examination (GSE) macroscopic and microscopic for protozoa detection (E. histolytica and G. lamblia). And 3-5 ml blood sample which divided into two tubes, one with anticoagulant (EDTA) used for CBP and ESR, and other without anticoagulant used for CRP titer, anti-gliadin (IgG, IgA), anti-tissutransglutaminase (IgG, IgA), and total IgG, IgA, and IgM and ABO blood groups. Statistical analysis was done using statistical package for social sciences (SPSS) version 10. Descriptive data analysis (statistical tables, mean value, standard Deviation, Standard Error, 95% Confidence Interval for population means value, min. and max. readings, contingency causes correlation ship coefficient for association tables, and graphical presentation by using:Bar charts) and Inferential data analysis (the Chi-Square test, the Binomial test, the One-Way ANOVA procedure, Levene test, and multiple Z-test).

Results and Discussion:

The study results show that children between 4 – 5 years has a higher present of Entameba histolytica infection (36.1%) in compare to the other age groups. The Giardia lamblia was high persent (30.4%) at 1 and 2 years age groups. But the Celiac disease corresponding to (62.5%) at age 4 – 5 year as explain in table 1. Infections of intestinal microorganisms, such as parasitic parasites, occur in tropical and subtropical regions of the world. These diseases are one of the most serious and leading causes of infant and child mortality, especially in developing countries with low economic and social status, increasing population density, Areas inhabited by people suffering from poverty, hunger and malnutrition, as well as the presence of many types of insects that help spread the incidence of micro-
organisms among people as the polluted environment and weak availability of drainage systems health (14).

Several studies and research carried out by Iraq and the world differed and agreed with the results of the present study. Including the study carried out by (15)

It has been shown that the infection of intestinal parasites in the province of Basra was higher, especially in the marshlands, while the results differed with other studies. Also the results differed with (16) Which recorded a high percentage of the total infection of intestinal parasites in Erbil, including the proportion of infection Ameba parasites condition of the tissue was higher than the results of our research. Results also differed with (17) of the age group most susceptible to infection were the ages of more than one year to ten years of patients with *G. lamblia* parasites. This was explained by the child's lack of adherence to the rules of hygiene as a situation to condemn oral movements of the random and non-washing and sterilization of the hands required and the frequent movement and random activity and their intermingling with animals Such as cats and birds, as well as the lack of full therapeutic doses for the difficulty of giving them to the child and also the weakness of the immune system (18). And also differed with the study (19) at the Kadhimiyah Hospital in Baghdad about the infection of amoeba parasites the condition of tissues for children from one month to twelve years, the highest rate of infection for children under the age of one year to two years and approached with what he said (20) in Ethiopia. The percentage of infection in the age group 10-9 years, which amounted to 31.5%, while also recorded the lowest rate of infection in the age group 14-13 years by 15.9%, as well as in Ethiopia (21) highest rate of infection in the age group 44-30 years, reaching 61.5% (22) in Thailand the highest Of injury to the age group 50-41)) a year in Thailand, amounting to 39.5% and category Alamrah30-21 years were at least where the injury rate of 5.9% recorded.

**Table 1** Distribution of the Observed frequencies, and percents of Age groups in the studied samples with comparison significant

| Age Groups | No. | %  | No. | %  | No. | %  |
|------------|-----|----|-----|----|-----|----|
| < 1 yr.    | 3   | 8.3| 0   | 0  | 2   | 25 |
| 1 - 6      | 6   | 16.7| 7 | 30.4| 1 | 12.5|
| 2 - 7      | 7   | 19.4| 7 | 30.4| 0 | 0  |
| 3 - 9      | 7   | 19.4| 4 | 17.4| 0 | 0  |
| 4 - 5      | 13  | 36.1| 5 | 21.7| 5 | 62.5|
| Total      | 36  | 100| 23  | 100| 8  | 100|
| Mean ± SD  | 2.99± 1.46| 2.58± 1.13| 2.16± 3.26|
| C.S.       | F=3.111| P=0.007|
| ANOVA      | HS   |     |     |     |     |

The study has also highlight on patients with Celiac disease and infection with parasite, table 2 show that 50% mixed cases. Many studies concluded that some cases were mixed with giardiasis or amebiasis (23).

**Table 2** Distribution of Mixed outcomes among different of the studied samples with cause’s correlation ships

| Groups       | No. and % | Mixed         |       |       |
|--------------|-----------|----------------|-------|-------|
|              | No.       | E. Histolytica | G. lamblia |
| Celiac disease | %Groups | 100% | 0.0% |       |
| % Mixed     | 50%      | 0.0% |       |       |
Table 3 explained the relationships between blood parameters and the study diseases. In which there is a high W.B.Cs mean value in *G. lamblia* cases, and eosinophilia with *E. histolytica* cases. But the Celiac disease show a lymphocytosis in most its cases. Also nearly most cases has anemia.

showed no significant difference in the percentage of white blood cells in those infected with Giardia lamplasia. This difference was explained by the fact that the Giardia parasite infection was at the beginning of the infection and the immune system was not yet in the process of stimulation, This is due to the immune response of the parasite's body (24).

The different results with the findings (26) and (25) With an increase in the number of white blood cells infected with the amoeba parasite histolytica. While the presence of inhibition of the formation of lymphocyte blood cells resulting in a low level of interleukin 2 IL.

The results also differed with (27), which found that there was no significant difference in the level of lymphocytes, neutrophils, macrophages and monovalent cells in both lamipa and jeridia lamplasia. There was a significant difference in the level of neutrophils in the acute and transitional stages of amoeba parasites And there was a significant difference between them and those infected Giardia lymphelia in the chronic phase and confirmed that there is a significant difference in the level of C-reactive protein (CRP) These results were agreed with or indicated that there is an increase of the cells of the neutrophil when infected with Amphibia The state of weaving also agreed with the results (28).

**Table 3** Summary Statistics of blood components parameters according to different of the studied samples

| B.C. | Groups          | No. | Mean  | Std. Dev. | Std. Error | 95% C.I. for Mean Lower Bound | 95% C.I. for Mean Upper Bound | Min.   | Max.   |
|------|-----------------|-----|-------|-----------|------------|-------------------------------|-------------------------------|--------|--------|
| W.B.C| E.H.            | 36  | 9.91  | 3.62      | 0.60       | 8.68                          | 11.13                         | 3.72   | 20.90  |
|      | Giardia lamblia | 23  | 12.1  | 5.71      | 1.19       | 9.68                          | 14.61                         | 5.00   | 25.30  |
|      | Celiac disease  | 8   | 11.8  | 4.82      | 1.71       | 7.77                          | 15.83                         | 6.80   | 18.90  |
| Neutrophil | E.H.        | 36  | 0.53  | 0.17      | 0.03       | 0.48                          | 0.59                          | 0.22   | 0.86   |
|      | Giardia lamblia | 23  | 0.53  | 0.14      | 0.03       | 0.46                          | 0.59                          | 0.29   | 0.85   |
|      | Celiac disease  | 8   | 0.49  | 0.16      | 0.06       | 0.36                          | 0.63                          | 0.24   | 0.79   |
| Lymphocyte | E.H.         | 36  | 0.33  | 0.14      | 0.02       | 0.28                          | 0.37                          | 0.08   | 0.63   |
|      | Giardia lamblia | 23  | 0.36  | 0.11      | 0.02       | 0.31                          | 0.41                          | 0.08   | 0.57   |
|      | Celiac disease  | 8   | 0.41  | 0.15      | 0.05       | 0.29                          | 0.53                          | 0.14   | 0.63   |
| Monocyte  | E.H.          | 36  | 0.05  | 0.06      | 0.01       | 0.03                          | 0.07                          | 0.00   | 0.23   |
|      | Giardia lamblia | 23  | 0.05  | 0.03      | 0.01       | 0.03                          | 0.06                          | 0.01   | 0.11   |
|      | Celiac disease  | 8   | 0.06  | 0.05      | 0.02       | 0.01                          | 0.10                          | 0.00   | 0.14   |
| Eosinophile | E.H.        | 36  | 0.08  | 0.07      | 0.01       | 0.05                          | 0.10                          | 0.00   | 0.31   |
|      | Giardia lamblia | 23  | 0.06  | 0.06      | 0.01       | 0.03                          | 0.08                          | 0.00   | 0.19   |
|      | Celiac disease  | 8   | 0.03  | 0.02      | 0.01       | 0.01                          | 0.05                          | 0.01   | 0.07   |
|      | E.H.          | 36  | 0.01  | 0.01      | 0.00       | 0.01                          | 0.01                          | 0.00   | 0.03   |
|      | Giardia lamblia | 23  | 0.01  | 0.00      | 0.00       | 0.01                          | 0.01                          | 0.00   | 0.02   |
|      | Celiac disease  | 8   | 0.01  | 0.01      | 0.00       | 0.02                          | 0.02                          | 0.00   | 0.02   |
| Basophile   | E.H.         | 36  | 10.6  | 2.01      | 0.34       | 9.94                          | 11.30                         | 4.10   | 14.60  |
|      | Giardia lamblia | 23  | 10.0  | 1.53      | 0.32       | 9.44                          | 10.75                         | 7.10   | 12.50  |
|      | Celiac disease  | 8   | 10.5  | 1.58      | 0.56       | 9.20                          | 11.85                         | 7.70   | 11.90  |
The ESR and CRP indicators has excellent relationships with diseases as show in table 4. The *G. lamblia* cases show a high mean value with ESR test, but there were a very high mean value of CRP with *E. histolytica* and Celiac disease respectively.

The statistical results confirmed in Table (4) showed a significant difference in the level of CRP for patients with amoeba parasites, the condition of tissues and infected Giardia lamplasia, wheat allergy (5.9%). The highest mean values of CRP were *E. histolytica* (58.18%), wheat sensitivity (56.29%), followed by *G. lamblia* (18.89%) and rotavirus (7.51%).

This was agreed with what he said (29) when he confirmed that infected Amoeba parasites the case of weaving with ages 1-6 years more catalysts for the protein C interactive. Most abnormal ESR readings were in Rotavirus, *E. histolytica* and *G. lamblia* because it is most often increased during acute infection (with increased fibrinogen concentration) and its value indicates inflammation severity. CRP such as ESR rises mostly during acute inflammation but its elevation is faster than ESR, in our cases CRP is predominantly positive and higher in *E. histolytica* and positive serous gastrointestinal disease, followed by *G. lamplasia* and rotavirus, which explained that there is acute inflammation due to infections, CRP is mainly used as a marker of inflammation (30-31).

showed that when these intestinal parasites occur, the immune response, especially IL-12 and TNF-β, is stimulated, particularly the cellular response. It stimulates the phagocytic process and increases the association of these protective cells with surfaces affected by the parasites. It has been characterized by parasites Giardia L. lamblia glands on the intestinal glands and secretion Alpha-gardins, a protein helps the parasite to reproduce and manufacture its needs and facilitate the process of adhesion in the intestinal mucosa and the flexibility of the body to stick with the transformation of the parasite into the bag, which leads to increase the proportion of protein C interactive We find Amoeba is a tissue condition with interferon-y-derived resistance by single-core peripheral cells.

Table 4 Summary Statistics of Infectious indicators parameters (ESR and CRP) according to different of the studied samples

| Inf. Ind. | Groups            | No. | Mean | Std. Dev. | Std. Error | 95% C.I. for Mean L.B. | U.B. | Min. | Max. |
|-----------|-------------------|-----|------|-----------|------------|------------------------|------|------|------|
| ESR mm/h  | *Entameba Histolytic* | 36  | 13.31| 8.45      | 1.41       | 10.45                  | 16.17| 4    | 37   |
|          | *G. lamblia*      | 23  | 14.17| 8.59      | 1.79       | 10.46                  | 17.89| 3    | 39   |
|          | Celiac disease    | 8   | 14.00| 10.41     | 3.68       | 5.30                   | 22.70| 2    | 28   |
| CRP mg/L  | *E. Histolytic*   | 36  | 58.18| 53.13     | 8.86       | 40.20                  | 76.15| 2    | 243.8|
|          | *G. lamblia*      | 23  | 18.89| 25.42     | 5.30       | 7.90                   | 29.88| 3.7  | 115.4|
|          | Celiac disease    | 8   | 56.29| 66.33     | 23.45      | 0.83                   | 111.74| 2.5  | 170.5|

The Rh and blood groups has been studied for *E. histolytica* and *G. lamblia* I which there was (91.6 and 86.9%) has Rh+ respectively. The O and A blood groups were the most common I studies sample, whereas E. histolytica show (30.5%) for A group and (36.1%) for the O group, but the G. lamblia show (34.8%) A group and (39.2%) O blood group.

The human blood groups are different for each individual because of certain antibodies in the blood cells and serum in humans and animals and classified in humans to the following main categories (O, B, AB, A), the results showed no significant difference on the effect of different blood groups in Patients with amoebic parasites and histopathology of Giardia lamplasia in increasing the incidence of intestinal parasites as shown in Table 5.

These results differed with the findings of the researchers (24) (25). Their results indicated that the different blood groups had an effect in changing the ratio of G. lamblia and increasing their resistance to the parasite. They showed that the AB blood group was more resistant, (26). This was explained by the different adhesion of red blood cells on the surface of the parasite, which is due to a
special mechanism to inhibit the inhibitions and adhesion enzymes on the parasite wall, and (25) by studying the relationship between blood groups and Giardia lamblia, The parasites are of the blood type A followed by the blood group O w B.

The results of the study showed a relationship between the high percentage of white blood cells of the acid and the equivalent of the two children studied with no difference in the proportion of infection of white blood cells while the increase in the proportion of white blood cells lymphatic when synchronized with the disease of wheat allergy also almost all cases have anemia These results were agreed with (27).

Table 5 Summary Statistics of blood groups and Rh factor according to different of the studied samples

| Rh Blood group | E histolytica | G lamblia |
|----------------|--------------|-----------|
| Rh +ve         | 33 91.6%     | 20 86.9%  |
| Rh -ve         | 3 8.4%       | 3 13.1%   |
| P value        | 0.283        |           |
| A              | 11 30.5%     | 8 34.8%   |
| B              | 10 27.7%     | 4 17.5%   |
| ABO Blood group|              |           |
| AB             | 2 5.5%       | 2 8.0%    |
| O              | 13 36.1%     | 9 39.2%   |
| P value        | 0.495        |           |

*Significant difference using Pearson Chi-square test at 0.05 level.

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