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Serological Tests for Diagnosis of the Parasites *Giardia duodenalis, Entamoeba histolytica*, and *Cryprosporidium pavium* in Diarrheal Children in Baquba City, Diyala Province, Iraq

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ABSTRACT: Diarrheal illnesses pose a significant public health issue in developing nations. Diarrheal illness is transmitted through food or water that has been polluted, or through direct contact between individuals due to inadequate hygiene practices. The present study evaluated the prevalence of different type of parasite in diarrheal infection and assessment level of Malondialdehyde (MDA), Glutathione (GSH), vitamin K and vitamin B12 in parasitic infection. The current study included a sample of 310 patients children experiencing symptoms of abdominal discomfort, diarrhea, and vomiting, as well as 45 healthy individuals as a control group, with aged from 1 to 6 years of both sexes. All participants were recruited between November 2023 to April 2024 were taken from people with diarrhea who visit specialist doctors in Batoul Hospital, outpatient clinics and health centers in Diyala city, all samples were diagnosed to detect the presence of the *Giardia lamblia*, *E.histolytica*, and *Cryprosporidium pavium*. By using Enzyme Linked Immunosorbent Assay ELISA. The result of this study showed that from total 310 diarrheal disease, about 149(48.1%) were positive for parasitic infection, 52(16.8%) of them positive for *Giardia* lambelia, 51(16.5%) for *E.histolytica* and 46(14.8%)*were* positive *for C.pavium*. there is significant reduction in Glutathion, Vitamin B12, Vitamin K that were (19.96± 6.52, 3.25 ± 0.43, 135.54 ± 122.5) as compared with control (30.75±4.01, 3.7 ± 0.3, 337.04 ± 351.3) , at p-value <0.05. While increase in MDA level in diarrheal patient with parasitic infection as compared with control at p-value <0.05. This study concluded increase malondialdehyde in parasitic infection . While decrease vitamins and antioxidant such as glutathione in parasitic infection.

Keywords: *Giardia* lambelia, *E.histolytica* , *C.pavium*, malondialdehyde, glutathione, vitamins.

1. INTRODUCTION

Diarrhea is a universal occurrence that everybody inevitably encounters at some juncture in their lives. Each patient may have a unique definition of diarrhea. A stool weight of less than 200g per day is regarded normal, while a stool weight above 200g per day is termed diarrhea according to an objective criterion. Approximately 20% of individuals with diarrhea have a stool weight that is below 200g per day, and these cases may not be included in this particular criteria (1, 2). The World Health Organization (WHO) has defined diarrhea as the occurrence of three or more instances of loose or liquid stools per day, or a higher frequency of bowel movements than what

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is considered normal for the individual (2). According to the Global Health Estimates of 2020 by the World Health Organization (WHO), diarrhea is ranked eighth among the top ten global causes of mortality. In low middle income countries, it is the fifth most prevalent cause of death(3) . The regular occurrence of well-formed stools or the passage of loose, semi-solid stools by breastfed infants should not be classified as diarrhea (4). Diarrhea can be caused by either noninfectious factors or by an infectious cause. Diarrhea is categorized into different types depending on the length of time the symptoms continue (2,3). These types include acute diarrhea (lasting less than 2 weeks), persistent diarrhea (lasting more than 2 weeks but less than 4 weeks), and chronic diarrhea (lasting more than 4 weeks). Diagnosing chronic diarrhea and parasite diseases poses significant problems (5). Diarrheal disease is a significant contributor to mortality rates in numerous developing nations. In the majority of regions, it is also the most prevalent triggering factor in protein energy malnutrition (PEM) and a significant contributor to other nutritional deficits(6). Parasitic infections that result in diarrhea are of particular significance in young children, individuals with weakened immune systems, and those residing in environments characterized by inadequate sanitation and hygiene, as well as dangerous food and water sources (1). Travelers from industrialized countries are a vulnerable demographic, especially prone to parasite pathogens due to their lack of established protective immunity from prior exposure. Some of these organisms have zoonotic origins, meaning they can be transmitted between animals and humans. This poses potential concerns for those who work with or engage in leisure activities involving these organisms (7), *Giardia lamblia*, *Cryptosporidium spp*., *Entamoeba histolytica* and *Dientamoeba fragilis* are the most important diarrhoea-causing protozoans(8). *Giardia lamblia* infections (synonym *G. Intestinalis, G.* and *duodenales*) in developed countries occur approximately 6-8% among children and 2% among adults. Additionally, about 33% of the population is contaminated with protozoans (8). In the United States, the Centers for Disease Control and Prevention (CDC) has estimated that there are around 77,000 cases of Giardiasis reported each year (9). *Entamoeba histolytica* is the sole human pathogenic species of the genus Entamoeba and is responsible for causing amoebiasis (5). Amebiasis is a disease that is found all over the world. It is caused by the trophozoites of the *Entamoeba histolytica* parasite. The clinical signs may occur during a period of up to 4 months following exposure to contaminated food or materials. Approximately 90% of instances of the condition may progress without showing any symptoms, resolve on their own, and have recurring episodes. Other people may exhibit varying degrees of clinical severity, including acute colitis (characterized by bloody diarrhea, fever, and abdominal pain), severe colitis (marked by fulminant symptoms and abscesses), and chronic forms (such as ameboma and inflammatory bowel disease). The etiological diagnosis relies on

the microscopic identification of cysts and trophozoites in the patients' feces, as well as the detection of particular antibodies and antigens (9). Amoebiasis affects around 50 million individuals globally annually, leading to 100,000 fatalities every year (10). Therefore, The present study aimed to evaluated the prevalence of different type of parasite in diarrheal infection and assessment level of Malondialdehyde (MDA), Glutathione (GSH), vitamin K and vitamin B12 in parasitic infection

2. MATERIALS AND METHODS

2.1 Stool samples collection

The study included a total of 310 patient children stool samples experiencing symptoms of abdominal discomfort, diarrhea, and vomiting, as well as 45 healthy individuals as a control group. The age range for both males and females was between 1 and 6 years. All participants were recruited between November 2023 to April 2024 were taken from people with diarrhea who visit specialist doctors in Batoul Hospital, outpatient clinics and health centers in Diyala city.

2.2 Microscopic examination

Stool samples were obtained using a sterile container with a wide mouth and screw cap. Fresh samples were then analyzed under a light microscope at a high magnification of 40X. A small volume (1-3 ml) of feces samples was collected in sterile screw cap containers and stored at a temperature of -20°C until they were analyzed using ELISA.

2.3 Diagnosis using ELISA technique to detect parasite antigens in stool samples.

-Microscopy positive samples were further examined by DRG ELISA based antigen detection of *(E. histolytica,* and *Giardia lambelia*), positive specimens were tested with TechLab *E. histolytica II* monoclonal ELISA based antigen detection. For *C. parvum* diagnosis, the method used in this test is Sandwich-ELISA (EDI Kit-Germany), according to the attached leaflet of the kit, the plate provided in this kit is pre-coated with an antibody specific for *C.parvum* antigen. The concentration of each of the standards in the samples was calculated by comparing the OD of the samples to the standard curve

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Figure 1. *E. histolytica* II (monoclonal ELISA for detection of *E. histolytica* adhesin in fecal specimen) (TechLAB Inc., Blacksburg, Virginia, USA).

2.4 Blood samples collection

Five ml of blood from patients who were proven to be infected with parasitic infection and suffer from diarrhea, in addition to the uninfected individuals in the control group was withdrawn. The blood serum was extracted from the blood by placing the blood in a sterile test tube with a 10 ml capacity, which did not contain an anticoagulant. The sample was incubated at room temperature for 30 minutes, then subjected to centrifugation at a speed of 2500 rpm for 10 minutes to separate the blood serum. The serum was collected using an automatic pipette (Micro pipet) and distributed into three Eppendorf tubes. The vials containing the serum were securely sealed and stored at a temperature of -20 ˚C until tested

2.5 Determination of serum MDA, GSH, vitamin K and B12

The levels of MDA, GSH, vitamin K, and vitamin B12 were determined using human Enzyme-Linked Immunosorbent Assay (ELISA) Kits from Sunlong firm in China.The ELISA kit employs the Sandwich-ELISA technique following the instructions provided by the manufacturer. The absorbance in each well was measured using an ELISA reader with a primary wavelength of 450 nm. The concentrations of MDA, GSH, vitamin K, and vitamin C were determined by utilizing the standard curve.

2.6 Statistical analysis

The data are presented as the Mean ± SEM. A unpaired t-test was utilized to compare the findings of research parameters between patients and control groups. The statistical studies were conducted using the SAS (2018) software package (11).

3. RESULTS AND DISCUSSION

The result of this study showed that from total 310 diarrheal disease, about 149(48.1%) were positive for parasitic infection, 52(16.8%) of them positive for *Giardia lambelia,* 51(16.5%) for *E.histolytica* and 46(14.8%)*were* positive *for C.pavium*, as shown in Table (1).

Table1: Prevalence of parasites in stool samples by using TechLab *and* EDI Kit ELISA

Types of parasites	Percent
Giardia lambelia	52(16.8%)
E.histolytica	51(16.5%)
C.pavium	46(14.8%)
Total	149(48.1%)

Parasitic diseases are characterized by malnutrition and a lack of necessary micronutrients, impaired digestion, decreased nutritional absorption, and persistent inflammation (12). The frequency of parasites in this study was similar to the percentage observed among children in Baghdad, which was 57.8%. Additionally, there was a significant occurrence of *E. histolytica* and *G. lamblia* (13). On the other hand, there were reduced levels of parasite occurrence in babies and children in several regions of Iraq, including Erbil, Wasit, and Kerbala, with prevalence rates of 30%, 34.6%, and 38.5% correspondingly (14, 15). In Iran, a much higher incidence rate (78.6%) of parasite infections among children has been recorded (16). The prevalence of parasites in this study was similar to the rate observed among youngsters in Saudi Arabia, which was 10.88% (17).

As shown in Tables (2, 4 and 5), there is significant reduction in Glutathion, Vitamin B12, Vitamin K that were (19.96± 6.52, 3.25 ± 0.43, 135.54 ± 122.5) as compared with control (30.75±4.01, 3.7 ± 0.3, 337.04 ± 351.3) , at p-value <0.05. While increase in MDA level in diarrheal patient with parasitic infection as compared with control at p-value <0.05, as shown in Table 3.

Table 2: Serum Glutathione level in patients and control groups

Glutathione (ng/mL)	Patients N = 310	Control N = 45	p-Value
Mean ± SD	19.96± 6.52	30.75±4.01	0.01
P Value is high significant at P ≤ 0.05			

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Table 3: Serum Malondialdehyde level in patients and control groups

Malondialdehyde (ng/mL)	Patients N = 310	Control N = 45	p-Value
Mean ± SD	59.98±4.18	27.34±6.33	0.001
P Value is high significant at P ≤ 0.05			

Table 4: Serum Vitamin B12 level in patients and control groups

Vitamin B12 (ng/mL)	Patients N = 310	Control N = 45	p- Value
Mean ± SD	3.15 ± 0.43	3.7 ±0.3	0.01
P Value is high significant at P ≤ 0.05			

Table 5: Serum Vitamin K level in patients and control groups

Vitamin K (ng/mL)	Patients N = 310	Control N = 45	p-Value
Mean ± SD	135.54 ± 122.5	337.04 ± 351.3	0.004
P Value is high significant at P ≤ 0.05			

Glutathione plays a crucial role in the body's antioxidant system and acts as a defense mechanism against many internal and environmental factors that might cause oxidative stress. Glutathione is an endogenous antioxidant that counteracts oxidants like some medications, cancer-causing substances, and other harmful damage. When glutathione levels are low, tissues become more susceptible to oxidation. This study showed decrease glutathione level in diarrhea patients that infected with different parasitic infection (18). The decrease in glutathione levels may be attributed to its role as a crucial cellular antioxidant. Glutathione protects cells from oxidative harm by reacting with free radicals generated by lipid peroxidation and peroxidase (18, 19). This study agree with MOHSIN in Kirkuk, that showed decrease glutathione in diarrheal patients that infected with *E. histolytica* infection (20). This result is consistent with two previous studies on *Toxoplasma gondii* infection in humans (21, 22), where they found that people with plantar fasciosis had lower activity of glutathione peroxidase (enzymes that convert H_2O_2 to water and alcohols using reduced glutathione) in serum and red blood cells compared to uninfected people. This study disagree with ALLAH that show increase glutathione in diarrheal patients that infected with *E. histolytica* infection(23). The results showed a significant increase in the levels of malondialdehyde (MDA) among those infected with the parasitic infection as compared to the control group. Their findings revealed that there was an increase in MDA levels, which is contrary to what was expected. The inconsistent findings may be

attributed to some elements of the immune response that trigger oxidative stress in the host organism (6). Antioxidant production may be significantly increased in response to various pathophysiological conditions such as inflammation, immune abnormalities, hypoxia, drug and alcohol metabolism, therapeutic radiation, and deficiency of antioxidant vitamins, which often damage cellular macromolecules (DNA, protein, and lipids) and other small antioxidant molecules (23).

In the present study, MDA concentrations were significantly elevated in the infected group compared to the uninfected group. The increase in MDA concentrations observed in this study may be due to increased production of free radicals and oxidants after infection or may be an indicator of decreased enzymatic activity of the antioxidant defense system (24). The results of this study indicate an inverse relationship between the level of malondialdehyde (MDA) and the level of glutathione (GSH) in the serum of parasite-infected individuals, which is consistent with other research.

Vitamin K shortage is reported in patients with diarrheal infection caused by parasite infection due to malabsorption resulting from intestinal injury. Additionally, a deficiency of vitamin K has been observed in individuals with persistent gastrointestinal diseases (24). This study supports previous findings that have demonstrated a reduction in vitamin K and B12 levels among individuals infected with *Entamoeba histolytica*. Patients with gastrointestinal infection typically have reduced levels of fat-soluble vitamins, such as A, D, E, and K (25). Vitamin K shortage worsens colitis induced by Dextran sulfate sodium and inhibits IL-6 generation by B cells.

Additionally, vitamin K deficiency exacerbates inflammatory illness. Studies conducted both in laboratory settings (in vitro) and in living organisms (in vivo) have demonstrated that vitamin K can suppress the production of pro-inflammatory cytokines, including IL6 and tumor necrosis factor-alpha (TNF-α) (24). The commensal bacteria in the intestine typically protect against enteric pathogens. However, in the case of histolytic infection, *enterobacteria* is necessary. Germ-free animals are resistant to *histolytica* infection, but the reintroduction of a single bacterial species restores the ability of the pathogen to cause amoebic diseases (26).

Folate deficiency causes an increase in the depth of the crypts in the intestinal mucosa of the duodenum and jejunum, as observed in a study by Klipstein *et al*.(27).

4. CONCLUSION

The findings indicate that infection with the parasite infection causes significant biochemical alterations, particularly in the levels of antioxidants and vitamins, resulting in a drop in their concentration in the patients' blood.

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