

The effect of infection with the parasite *Giardia lamblia* on some physiological, hormonal and biochemical variables

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Abstract

The study was conducted on patients infected with *Giardia lamblia*, who were admitted to Samarra General Hospital and some private laboratories in the city of Samarra / belonging to Salah al-Din Governorate, the age groups ranged from (1-40) years, the work was completed during the period from 10/2/2021 to 2022 /5/1. Cases of parasite infection were diagnosed by examining faeces samples by direct swab method using a light microscope to detect the trophic and cystic phases. The current study examined 585 faecal samples, and 76 were infected. The study includes its effect on some biochemical variables. Three aspects include: the first side is hormones (cholecystokinin and somatostatin hormone), the second side is vitamins (Vitamin B12 and (Vitamin A), and the third side includes elements including sodium and potassium. The results showed that there was a significant increase in the levels of each of Colcytokinin 156.0 ± 15.5 and Somatostatin 122.7 ± 18.1 compared with the control group. The results recorded a significant decrease in the concentration of each of sodium ions 81.8 ± 13.8 Na + and potassium 1.737 ± 0.285 compared to the control group. The results showed that there was a significant decrease in the concentration of vitamin B12 in the group of patients with 5.25 ± 1.79 compared with the control group, while there was no significant difference in the concentration of vitamin A among the patients with 1.149 ± 0.455 compared to the control group.

Keywords: *Giardia lamblia*, giardiasis, vitamin B12, cholecystokinin, somatostatin.

INTRODUCTION

Diarrhea is one of the top ten causes of death in all age groups, and therefore it is an important threat to global health. Since it is the second leading cause of death related to infectious diseases, it is important to control the pathogens that lead to diarrhea, including giardiasis, to reduce the possibility of increasing diarrhea-related deaths (1). It is the most common parasitic diarrheal disease that affects humans and more than 40 species of other mammals, as the World Health Organization has classified *Giardia* as the eleventh most important parasite in the world, and Giardiasis has a higher rate among children under the age of 5 years. Also, adults aged 30-40 years are more at risk (2). The causative agent of Giardiasis, therefore, is a major cause of diarrhea worldwide, affecting 280 million people annually, and is most prevalent in areas that suffer from poverty and lack of sanitation services, as is the case in many low-income countries as well as in high-income countries and regions Industrial (3). It is one of the eukaryote flagellate parasites (4), and it causes Giardiasis (5), so it is a common disease between humans and animals. (6)

The parasite infects the small intestine (the jejunum and ileum) of humans. The infection occurs after eating the parasite bags. And the infection can cause great damage to the intestinal lining as a result of the parasite's attachment with the help of its bi-lobed sucking discs to the intestinal lining (7). Those most affected by giardiasis are immunocompromised individuals, as well as travelers to areas with high rates of infection (8). The infection may occur without symptoms or clinical symptoms usually appear in the form of foul-smelling diarrhea, abdominal pain and bloating, and infection may lead to long-term problems such as irritable bowel syndrome (IBS), chronic fatigue, and infection occurs by swallowing gastric cysts through the mouth. Which are infested with water and food (9).

Vitamin B12 is involved in the synthesis of important biochemical transmitters in the brain and nervous system. Vitamin B12 is involved in DNA synthesis. It is especially important during growth and cell proliferation. (10). Damage to the intestinal

epithelium is caused by the adherent trophozoites of the parasite. It has been suggested as an important mechanism in the pathogenesis of infection. Giardiasis can cause vitamin B12 deficiency and enteritis and interfere with folic acid absorption (11). Few studies have shown vitamin B12 deficiency due to Giardia (12). Vitamin A and zinc deficiencies, which are common in malnourished children, may be linked to a predisposition to giardiasis (13). As a result, several studies have attempted to assess the association of Giardia with poor growth and stunting in young children. Studies have found CCK to be elevated among a small number of patients with Giardia (14). CCK is released by enteroendocrine cells located in the small intestine and is indicative of the presence of fat in the lumen. CCK triggers the release of bile to aid in fat dissolution and absorption, although bile is readily used by Giardia progenitors during growth.

Somatostatin SS is a neuropeptide and an immune hormone also called growth-inhibiting hormone produced in many tissues of the body. It performs a wide range of physiological functions and inhibits the secretion of other hormones and has a role in slowing the emptying process of the stomach and intestines. Somatostatin produced by macrophages within the granulomatous inflammation associated with parasitic infections, reduces of inflammatory responses to disease (15).

Due to the lack of studies that focused on the side effects of Giardia lamblia infection, this study was conducted with the aim of knowing the effect of infection with the G. lamblia parasite on hormones, including Cholecystokinin and Somatostatin, and the effect of parasite infection on some vitamins and elements such as vitamin B12 and vitamin A, and on some elements such as potassium K⁺ and Na⁺.

Materials and method

Examination of blood samples

5 ml of blood was drawn from the patients who tested positive for Giardia lamblia, in addition to the non-infected persons in the control groups. The blood was separated and serum was isolated from it to perform some biochemical and hormonal tests. The remaining part of the blood (8 ml) was placed in a sterile 10 ml Plain tube that does not contain an anticoagulant. It was left at laboratory temperature for 30 minutes, it was placed in a centrifuge at 2500 rpm for 10 minutes to obtain the blood serum, which was drawn by an automatic micro pipet, and distributed in (5) Eppendorf tubes to perform some tests:

Biochemical tests

For calculating the level of vitamins, including b12, A, lipid profile, and blood sugar. The tubes containing the serum were closed tightly and kept at a temperature of -20° C until they were used in conducting hormonal and biochemical tests. The levels of vitamin A, B12, Cholecystokinin, and Somatostatin were measured using an ELISA device, and sodium and potassium were measured with a Caretium Electrolyte Analyser.

Measuring the level of the hormone cholecystokinin using ELIZA technique

This kit is based on a competitive enzyme-linked immunosorbent assay technique (Appixa LTD- Cambridge, UK). An antibody is pre-coated on a 96-hole plate. Standards, test samples, and biotin-conjugated reagents are added to the pits and incubated. A competitive inhibition reaction occurs between biotin-labeled CCK and unlabeled CCK on the pre-coated antibody. Then the HRP-conjugated reagent is added, and the entire plate is incubated. Unbound conjugates are removed using washing buffer at each stage. The TMB substrate is used to measure the HRP enzymatic reaction. After adding the TMB substrate, pits containing only sufficient CCK will produce a blue colored product, which then changes to yellow after the addition of the acidic stopping solution. The intensity of yellow is inversely proportional to the amount of CCK associated with the plaque. OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the CCK concentration can be calculated.

Measuring the level of somatostatin hormone using ELIZA technique

This kit is based on competitive enzyme-linked immunosorbent assay technology (Appixa LTD- Cambridge, UK). An antibody is pre-coated on a 96-hole plate. Standards, test samples, and biotin-conjugated reagents are added to the pits and incubated. A competitive inhibition reaction occurs between biotin-labeled SST and unlabeled SST on the pre-coated antibody. Then the HRP-conjugated reagent is added, and the entire plate is incubated. Unbound complexes are removed using washing buffer at each stage. The TMB substrate is used to measure the HRP enzymatic reaction. After addition of the TMB substrate, only wells

containing sufficient SST will produce a blue colored product, which then changes to yellow after the addition of the acidic stopping solution. The intensity of yellow is inversely proportional to the amount of SST associated with the plate. OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the SST concentration can be calculated.

Vitamin A Level Measurement

Vitamin A is useful for diagnosing vitamin A deficiency and toxicity. Assessment of people with intestinal malabsorption of fats. Vitamin E is useful for diagnosing (or evaluating) individuals with motor and sensory neuropathies, monitoring the vitamin E status of premature infants requiring oxygenation, and evaluating those with malabsorption of fats in the intestine.

Vitamin B12 level measurement:

Vitamin B12 deficiency is a common condition. Between 1.5 and 15 percent of Americans have low levels of vitamin B12, according to the National Institutes of Health. Many people, especially the elderly and people with intestinal disorders, have trouble absorbing vitamin B12 from food and oral supplements.

Sodium concentration measurement:

The level of sodium concentration Na^+ was checked by taking a blood sample from the patient and obtaining the serum after being circulated in the centrifuge. The analysis was performed automatically with a Caretium Electrolyte Analyser (A-Lyte, India) and the results were read through the reading screen.

Potassium concentration measurement:

The potassium K^+ concentration was checked in the same way as the previous sodium test using a Caretium Electrolyte Analyser (A-Lyte, India) and the results were read through the reading screen.

Results

The results showed in Table (1) that there was a significant increase in the levels of CCK and SS in the blood of infected people with the parasite, compared with the control group, at a level of probability ($p = 0.05$).

Table (1): The effect of infection with *G. lamblia* parasite on the level of CCK and somatostatin SS.

Totals	CCK	SS
	S.D. $\pm$ M	
The injured	15.5 $\pm$ 156.0	122.7 $\pm$ 18.1
The control	16.8 $\pm$ 103.1	9.97 $\pm$ 38.71
Calculated tvalue=14.69* tabular t value=1.671		Calculated t value =18.16* tabular t value =1.698

(*) = Significant differences at the $0.05 < p$

The results in Table (2) showed a significant decrease in the concentration of vitamin B12 in the blood of those infected with the parasite compared with the control group, while there was no significant difference in the concentration of vitamin A between the infected and the control group.

Table (2): The effect of infection with *G. lamblia* parasite on the level of vitamin B12 and vitamin A in the blood

Totals	Vit B12	Vit A
	M±S.D.	
The injured	5.25± 1.79	1.149±0.455
The control	7.219 ± 0.379	86± 470
Calculated tvalue=8.05* tabular t value=1.668		Calculated t value = 1.02n.s tabular t value =1.696

(*) = Significant differences at the 0.05<p

(n.s) = No significant differences at the level of 0.05<p

The results in Table (3) showed a significant decrease in the concentration of potassium ions K + and Na + in those infected with the parasite compared with the control group at the probability level (p < 0.05).

Table (3): Effect of *G. lamblia* infestation on potassium K⁺ and Na⁺ levels.

Totals	K ⁺	Na ⁺
	M ± S.D.	
The injured	0.285 ± 1.737	81.8± 13.8
The control	0.479 ± 3.021	119.6± 26.3
Calculated tvalue=13.88* tabular t value=1.680		Calculated t value =6.89* tabular t value =1.686

(*) = Significant differences at the 0.05<p

Discussion:

Irregular intestinal contractions is a common symptom of giardiasis in humans, as infection with giardiasis in mice leads to changes in intestinal motility. Previous studies have shown smooth muscle contraction and that the neurotransmitter cholecystokinin (CCK) is a neurotransmitter and an excessive increase in bowel movement. (16) (17) also describes an increase in CCK levels in the colon of mice infected with *Giardia*, and previous studies have shown muscle contraction smooth; Colocystokinin (CCK) is a neurotransmitter and the bowel movement is increased excessively. CCK was found to be elevated among a small number of patients with *Giardia*. CCK is released by enteroendocrine cells located in the small intestine and indicates the presence of fat in the lumen. CCK typically results in the release of bile salts to aid in fat dissolution and absorption, although bile is readily used by *Giardia* protozoans during growth. Parasite-mediated CCK release should be beneficial to the parasite during infection.

Vitamin B12 is involved in the synthesis of important biochemical transmitters in the brain and nervous system. Vitamin B12 is also involved in DNA synthesis. It is particularly important during growth and cell proliferation. (10). Vitamin B12 is

important in transporting and storing folic acid in cells. Folic acid is also important in cell division and DNA synthesis. Folic acid must be digested by pancreatic juice in the duodenum, as it is normally colonized by *G. intestinalis* (18). Damage to the intestinal epithelium is caused by the adherent trophozoites of *G. intestinalis*. It has been suggested as an important mechanism in the pathogenesis of infection. *Giardia* can cause vitamin B12 deficiency and enteritis and interfere with folic acid absorption (11). Few studies have shown vitamin B12 deficiency due to *Giardia* (12). Deficiencies in protein, vitamin A, and zinc, which are common in malnourished children, may be a predisposition to giardiasis (13, 19). In contrast, *Giardia* may impair zinc absorption and make vitamin supplements ineffective (20,21)). As a result, several studies have attempted to assess the association of *Giardia* with poor growth in young children. Older studies also indicate that infection with *Giardia* at an early age was already a risk factor for stunting at age 2 (22, 23).

This study agreed with the study (24,25)). In patients with diarrhoea, stool osmosis is calculated almost entirely by electrolytes (Na^+ , K^+ and concomitant ions). In osmotic diarrhea, there is an unaccounted gap between fecal water electrolytes and measured osmosis. This gap is due to poorly absorbed molecules (eg lactose causes lactase deficiency) that draw fluid into the lumen (26). In patients with giardiasis, the levels of electrolytes would be expected to decrease due to lack of absorption. Gastrointestinal parasitism can lead to ipsilateral electrolyte deficiency as classically occurs in hyperkalemia and hyponatremia (27). For the purpose of acid-base balance as well as osmotic pressure, sodium (Na^+) and potassium (K^+) as electrolytes play an important role in this regard. Low levels of these ions can lead to severe complications and uncontrollable events in body functions. Deficiency of these macro elements can therefore lead to clinical disturbance, weight loss and even death, especially with parasite overload if untreated (28, 29).

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