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Biochemical Study of Patients Infected with *Entamoeba Histolytica* in Al-Najaf Province

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ABSTRACT:
The current study was conducted to study some biochemical indicators in patients who suffer from diarrhea due to infection with the parasite Entamoeba histolytica. The aim was to study the effect of some vitamins and some blood characteristics on the body of the infected person. 520 stool samples were collected from diarrhea patients who arrived at hospitals in the province of Al-Najaf, ranging in age from 20 to 70.Blood samples were taken after confirming their infection with the parasite, as 70 people were found infected with the parasite, with a rate of 13.46%, and 20 blood samples were taken from healthy people. as a control group after ensuring that they are free of the parasite. The results of the current study showed that the percentage of parasite infection in the age group 20-30 years is the highest, reaching 54.29%. Our current study also showed a decrease in the concentrations of vitamins C, K, B9 and B12, with a significant difference as compared to control group, which it reached 0.031, 0.025, 0.001 and 0.015, respectively, at p-value 0.05.

Introduction

Intestinal parasite infections remain a significant health burden, particularly in developing countries where a large proportion of households have poor sanitation and lack access to adequate water. Many protozoan species in the genus *Entamoeba* colonize humans, but not all of them are associated with disease. *Entamoeba histolytica* is well known to be a pathogenic amoeba associated with intestinal and extraintestinal infections (Srinivasan *et al*., 2016). Parasitic infection can affect the nutritional status of infected persons, by modifying the key stages of food intake, digestion and absorption. Pathogenesis is particularly important in children with parasitic infection, ranging from malnutrition, anemia, growth retardation, irritability and cognitive impairment to increased susceptibility to other infections and severe complications. Protein malnutrition is another complication of parasitic infection that is a common problem in populations with low economic status and a low level of sanitation for general health, where the binding of activators on the intestinal mucosa impairs intestinal absorption of nutrients and produces a malabsorption and permeation syndrome in the intestinal mucosa. Which leads to diarrhea as well as malabsorption of nutrients and minerals (Kadir and Mhammad-Ali, 2011). The role of diet in the pathogenesis of IBD remains an open topic despite advances in understanding the microbiology of gastrointestinal and immune system physiology (Ghishan and Kiela, 2017). B vitamins are a group of water-soluble organic compounds that are essential for many physiological functions of nearly all living organisms (Kennedy *et al*., 2016). The functional roles of these micronutrients are varied. They act primarily as cofactors in a large number of enzymatic reactions, and one or more B vitamins are involved in all energy-producing cell reactions such as the mitochondrial citric acid cycle and cellular aerobic respiration (Hossain *et al*., 2022; Depeint *et al*., 2006).

They also play vital roles in immune function, neurotransmitter synthesis, carbon metabolism, cell signaling, and DNA biosynthesis (Rahman and Baumgartner, 2019). However, our gut also contains bacteria that produce B vitamins including biotin, cobalamin, folate, niacin, pantothenate, pyridoxine, riboflavin, and thiamine, but in limited amounts. Many gut bacteria also require certain vitamins for their growth. normal cellular diet and the development of many chronic diseases in humans. Therefore, B vitamins are essential not only for the ho st but also for the bacteria that live in the

Journal of Chemical Health Risks

www.jchr.org

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gut, and a dietary supply of these vitamins is necessary to meet the host's daily needs (Hossain *et al*., 2022).

Vitamin B9,12 has a role in the formation of red blood cells and their association with anemia associated with inflammatory bowel disease. Vitamin B12 (cobalamin) and folic acid play an important role in the synthesis of DNA and the formation of red blood cells during their differentiation, as red blood cells require both for reproduction, and their deficiency leads to a large size of red blood cells erythroid apoptosis and anemia, and the jejunum is the main site of vitamin B12 absorption (Battat *et al*., 2014). Vitamin K has been defined as an essential factor in blood clotting (Mladenka *et al*., 2022), and it is not a single compound but rather a term referring to many similar compounds that have the physiological function of this vitamin and share a common structure which is the nucleus 2-methyl-1,4- naphthoquinone, also known as menadione and the simpler form, which contains only the nucleolus, is known as vitamin K3. Unlike natural forms, K3 is hydrophilic and is not obtained through the diet. However, it does act as a mediator in the metabolism (Shearer and Newman 2014).

Vitamin K deficiency leads to abnormal prothrombin and gammacarboxyglutamic acid deficiency leads to serious bleeding and death. In IBD patients, vitamin K deficiency results in malabsorption resulting from vitamin K intestinal damage (Lai *et al*., 2022). In fact, levels of fat-soluble vitamins including A, D, E, and K are generally lower in patients with inflammatory bowel disease (Fabisiak *et al*., 2017). The prevalence of vitamin K deficiency in patients with Crohn's disease and IBD is higher. ulcerative colitis as the main forms of idiopathic inflammatory bowel disease, which are chronic inflammatory disorders of the gastrointestinal tract (Santoru *et al*., 2017) caused by altered interactions between gut bacteria and the immune system (Kostic *et al*., 2014).

Vitamin C is a water-soluble vitamin that is essential for all humans and some other mammals that lack the ability to biosynthesize the compound from glucose because they lack the enzyme gulonolactone oxidase. The term vitamin C refers to both ascorbic acid and dehydroascorbic acid. Both have anti-scorbutic activity and ascorbic acid is considered the primary and functional form in vivo of the vitamin. This compound is characterized by its acidic character and the availability of electrons for its function as a reductant and an antioxidant. The biological functions of ascorbic acid depend on its ability to provide reducing equivalents for a variety of biochemical reactions due to its ability to Reducing, as the vitamin can reduce most reactive oxygen species (Buettner, 1993). The vitamin also acts mainly as a cofactor for reactions that require deficiency of iron or the mineral copper and as a protective antioxidant both inside and outside cells (Halliwell and Whiteman, 1997). Due to its ability to donate electrons, it removes reactive nitrogen species such as hydroxyl, peroxyl, superoxide, peroxynitrite, nitroxide radicals as well as singlet oxygen and hypochlorite (Frei et al, 1989). Also indirectly antioxidant protection by regenerating other biological antioxidants such as glutathione and α-tocopherol to their active state (Jacob, 1995). The current study aimed to study the effect of parasite infection on the levels of water and fat soluble vitamins C, Vitamin B12, B9 and Vitamin K.

Materials and Methods

Study population:

The present study consisted of 520 patients samples suffering from abdominal pain, diarrhea, and vomiting, and 20 of healthy subjects as acontrol group , with ages from (20-70) years and for both sexes. All participants were recruited between November 2021 to April 2022 from Al-Hakim Teaching Hospital, Al-Sadr Teaching Hospital, Al-Najaf General Hospital, Al-Sajjad General Hospital, and Al-Furat Al-Awsat Hospital. Approval for the study was taken from these hospitals.

Serum sampling

Five ml of venous blood were obtained from each individual participating in this study and the serum separation from blood was performed by centrifugation at 3000 rpm for approximately 15 minutes. Then the serum was collected in sterile appendorf tube and the serum isolated, aliquoted and stored at -80°C until subsequent ELISA.

ELISA analysis of Vitamin C,K,B9,B12

Levels of Vitamin **C,K,B9 and B12** was estimated by using human Enzyme-Linked Immunosorbent Assay (ELISA) Kits (Sunlong company ,China).ELISA kit uses Sandwich-ELISA in accordance with the manufacturer's instructions. Absorbance in each well was read on an ELISA reader using 450 nm as the primary wavelength. Concentrations of Vitamin **C,K,B9 and B12** were estimated using the standard curve.

Statistical analysis

All statistical analyses were performed by using SPSS version 26 software program. (Paulsan, 2008). In this

Journal of Chemical Health Risks

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JCHR (2023) 13(4), 722-730 | ISSN:2251-6727

study, non-parametric tests were used using the manwhitney test to extract the values of the arithmetic mean and standard deviation at the level of significance ($p<0.05$) among the groups under study, because the data of the study are not subject to a normal distribution.

Results

The case study was conducted in Najaf province, specifically in Al-Hakim Teaching Hospital, Al-Sadr Teaching Hospital, Al-Najaf General Hospital, Al-Sajjad General Hospital, and Al-Furat Al-Awsat Hospital, where 520 patient samples were collected, suffering from abdominal pain, diarrhea, and vomiting, with ages from 20-70 years and for both sexes. 70 samples were positive for *Entamoeba histolytica*.

Studying the effect of age on the percentage of infection with *Entamoeba histolytica*.

The results of our current study showed that the percentage of parasite infection was higher in the 20-30 age group, followed by the 31-40 age group, as shown in Table (1).

Table (1) Percentage of infection with *Entamoeba histolytica* according to age groups

Age group	30-20	31-40	41-50	51-60	61-70	total samples
Positive	38	16	7	5	4	70
Percent	54.29	22.86	10	7.14	5.71	100

Through our current study, it was found that the average concentration of vitamin C,K,B9,B12 in patients was lower than in the control group, with a significant difference of .031, .025, .001, .015 respectively at a p-value 0.05 as shown in Table (2).

Table (2) Relationship of Vitamin C,k,B9,B12 concentration to infection with Entamoeba histolytica

Vitamins	Patient**S** **N=70** **M**± SD	**Control** **N=20** **M**± SD	**p-value**
C	2.0541± .73499	2.2750 ± .89436	.031
K	156.4429 ± 143.64034	312.4500 ± 353.59968	.025
B9	9.7116 ± 1.47637	12.0618 ± 4.28022	.001
B12	2.0880 ± .69314	2.6022 ± 1.16387	.015

***Significant differences at p 0.05 between patients and control.#**

Table (3) shows a positive correlation between the concentration of vitamin K with vitamin C, as well as vitamin K with vitamin B9 and vitamin 12, vitamin B9 with vitamin B12 among patients.

Table (3): Correlation of vitamins concentration among patients

Spearman's rho Correlation Coefficient	**Vitamin C**	**Vitamin K**	**Vitamin B9**	**Vitamin B12**
Vitamin K	.274**	1.000	.216*	.315**
p-value	.009	.	.041	.003
N	90	90	90	90
Vitamin B9	.192	.216*	1.000	.214*
p-value	.070	.041	.	.043
N	90	90	90	90

****. Correlation is significant at the 0.01 level (2-tailed).**

***. Correlation is significant at the 0.05 level (2-tailed).**

Journal of Chemical Health Risks

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JCHR (2023) 13(4), 722-730 | ISSN:2251-6727

Discussion

The high incidence of infection in the age group 20-30 may be due to the fact that this age group is more likely to engage in many work activities that may expose individuals to infection through contaminated soil, water and food, and the presence of other family members infected with the parasite, all of which may lead to transmission of infection and incidence of disease (Wegayehu *et al.*, 2013) This result is also consistent with studies that showed more infections among individuals aged (15-44) years for some gastrointestinal parasitic infections compared to other age groups, as age is an important and significant factor that It affects the spread of most intestinal parasites, and this is consistent with (Vahedi *et al*., 2012). Vitamin C is a good indicator of the intake of fruits and vegetables, and the low concentration of vitamin C in the body may be attributed to the low intake of fresh fruits and vegetables, and this is consistent with (Rivera *et al*., 2003) Many IBD patients suffer from micronutrient and mineral deficiencies, including vitamins. These deficiencies may be caused by malabsorption associated with the underlying disease, such as severe diarrhea, vomiting caused by a parasite, not eating proper food during the illness, the length of the illness, or certain dietary changes, all of which factors that may affect vitamin absorption. Overall, up to 68% of Patients with inflammatory bowel disease limit their diet to try to control their disease and reduce symptoms. There are some possible explanations, including patients following doctors' advice to temporarily limit their fiber intake during the peak period of illness and patients either self-restricting or Patients receiving inappropriate dietary advice or patients who are unable to consume foods rich in vitamin C due to disease activity, this is consistent with (Dunleavy *et al*., 2021).In 2020, the International Organization for the Study of Inflammatory Bowel Diseases (IOIBD) published A report on dietary guidelines for patients with inflammatory bowel disease, and these recommendations included moderate to high consumption of fruits and vegetables in Crohn's patients, and no restrictions on fruits and vegetables for patients with ulcerative bowel disease (Levine *et al*.,2020) ,in patients with inflammatory bowel disease, vitamin K deficiency occurs due to malabsorption resulting from intestinal damage and deficiency has also been observed in chronic gastrointestinal disorders (Krasinski *et al*., 1985), including inflammatory bowel disease (Kuwabara *et al*., 2009) and irritable bowel syndromeshort (Krzyżanowska *et al*., 2012). In fact, levels of fat-soluble vitamins including A, D, E, and K are generally lower in patients with IBD (Fabisiak *et al*.,2017). Vitamin K deficiency also exacerbated colitis caused by Dextran sulfate sodium inhibits IL-6 production by B cells and vitamin K deficiency exacerbates inflammatory disease. In vitro and in vivo experiments revealed that vitamin K inhibits the production of pro-inflammatory cytokines, particularly IL-6 and tumor necrosis factor alpha (TNF-α) (Shiraishi *et al*., 2014).

As IL-15 is a cytokine that has pro-inflammatory properties and is upregulated by myeloid and non-myeloid cells under conditions of tissue stress and during infection, IL-15 is known to be overexpressed in the epithelium of the small intestine and colon of patients with wheat allergy, Crohn's disease and ulcerative colitis that changes in IL-15 levels alter the functional characteristics of the gut microbiota, which in turn affects its metabolic ability to produce host-protective molecules such as butyrate, a short-chain fatty acid that is created when gut bacteria ferment the fibers in our gut. Expression of IL-15 in the intestine can precede and precipitate complex intestinal and extraintestinal immune disorders, through its effect on the microbiota and in particular through its effect on butyrate-producing bacteria and luminal butyrate concentration through the contribution of IL-15 to disease. Noting its effect on immune cells and this is consistent with (Abadie and Jabri, 2014), IL-15 promotes microbial imbalance dysbiosis. Furthermore, our study puts forward the concept that the cytokine could lead to reduced abundance of butyrate-producing bacteria (S24-7 family). And a decrease in the concentration of intestinal butyrate, which is important given their association with intestinal infections and possibly autoimmunity (Arpaia *et al*., 2013) and this microbial imbalance leads to a loss of their ability to secrete vitamin K during the period of infection with intestinal parasites. The commensal bacteria in the intestine are generally protective against enteric pathogens, however, histolytica infection required the presence of enterobacteria as germ-free animals were resistant to histolytica infection, but the introduction of a single bacterial species reintroduced the pathogenesis of amoebic diseases (Haque *et al*. , 2003).

Bacteria exert critical effects on the onset and persistence of intestinal inflammation in inflammatory bowel disease (Taniuchi *et al*., 2013). Intestinal microorganisms or bacteria in food may produce a bacterially synthesized menaquinone that contributes to the fulfillment of K2

Journal of Chemical Health Risks

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JCHR (2023) 13(4), 722-730 | ISSN:2251-6727

requirements in humans (Teixeira *et al*., 2012). Bacterial overgrowth in the small intestine may not increase bacterial K2 biosynthesis in the intestine but enhance dietary K1 absorption through potentially damaged intestinal mucosa (Giuliano *et al*., 2010). Possible causes of folate deficiency include inadequate intake and malabsorption due to loss of intestinal surface area from active inflammation, resection, or fistulae. Folate deficiency may also be caused by therapeutic agents, such as sulfasalazine and methotrexate, which can inhibit folate absorption. This is consistent with (Goh *et al*. .,2001). Folate depletion increases the depth of the crypts of the intestinal mucosa in the duodenum and jejunum (Klipstein *et al*.,1973), which results in a decreased villi-to-lumen ratio (Howard *et al*.,1974). Folate deficiency causes megablast changes in the nucleus of epithelial cells (Klipstein *et al*.,1973) and reduced crypt mitosis (Howard *et al*.,1974). These changes were more pronounced in the ileum with elongation of the crypts, increase in goblet cells, and decrease in Paneth cells Although gut bacteria can produce some folate, a folate-deficient diet significantly alters microbial diversity in mice (Da Silva *et al*., 2019).

An increased number of bacteria in the small intestine may, directly or indirectly, interfere with many functions of the small intestine resulting in intraluminal bile acid deficiency leading to fat malabsorption, steatorrhea and varying degrees of nonspecific inflammation and mucosal injury leading to Altering the absorption of micronutrients,furthermore, since most bacteria require cobalamin for growth, increased concentrations of bacteria can cause cobalamin deficiency with megaloblastic anemia and possible neurological changes, An overabundance of bacteria in the upper small intestine may produce folic acid, overcoming folate malabsorption due to high pH and poor absorption functions. This is consistent with Lauritano *et al*., 2010. Low levels of folic acid and vitamin B12 have also been largely associated with hyperhomocysteinemia. in the blood of patients (Romagnuolo *et al*., 2021). Inflammatory bowel diseases are inflammatory gastrointestinal disorders that are generally thought to predispose to vitamin B12 deficiency, as the vitamin in the diet must bind to gastric intrinsic factor (a glycoprotein produced by parietal cells in the human stomach (Alpers, 2005) for uptake in the distal ileum (Headstrom *et al*., 2008) . On the other hand, free radicals are chemical species that contain a non-paired electron and can induce oxidative stress (Di Meo *et al*.,2020). One example is nitric oxide, which forms complexes with metal ions, including cobalt (Haurani *et al*., 1989), which is a structural component of vitamin B12 and thus renders it unavailable for bacterial vitamin B12 biosynthesis, Moreover, exposure of vitamin producers, such as *B.fragilis*, to free radicals such as hydrogen peroxide can inhibit their growth, reducing their ability to biosynthesize vitamins (Hossain *et al*.,2022; Rocha *et al*.,1996).

The positive correlation between vitamin k and vitamin B is attributed to the fact that our intestines contain bacteria that produce B vitamins and vitamin K. Many gut bacteria require certain vitamins for their growth. At the same time, helper bacteria create competition among themselves, and deficiency of these vitamins impairs normal cellular metabolism. and stimulate the development of many chronic diseases in humans. Therefore, these vitamins are necessary not only for the host but also for the bacteria that live in the intestine. The dietary supply of these vitamins is necessary to meet the host's daily needs, and this is consistent with (Hossain *et al*.,2022) and since those infected with the amoeba parasite The condition of the tissues are exposed to a depletion of beneficial bacteria in the intestine with the presence of pro-inflammatory interleukins that reduce the presence of these bacteria, so the deficiency of these vitamins together is a natural result under these conditions.

Intestinal bacteria assembling vitamin K, which is a necessary cofactor in the production of prothrombin and other blood clotting factors. Treatment with antibiotics, especially in people with a diet low in vitamin K, can lead to low plasma prothrombin levels and a tendency to bleed as the intestinal bacteria synthesize biotin, vitamin B12, folic acid and thiamine due to their bad effect on the bacterial flora, they may affect the picture bleeding, and they also lead to the accumulation of cholesterol and low-density lipoprotein, and they also affect the level of phosphorus (Hamad, 2019). The reason for the positive correlation between vitamin C and vitamin K may be attributed to the apoptosis-inducing activity of vitamins C and K and their analogues. Vitamin C exhibits both reductive and oxidative activities, depending on the environment in which this vitamin is present. The apoptosis-inducing activity of ascorbate is stimulated by Cu2+, lignin and ion chelator, and is inactivated by catalase, Fe3+, Co2+ and saliva. On the other hand, vitamin K2, which contains a geranylgeranyl group as a side chain, and vitamin K3 induces apoptosis of various

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JCHR (2023) 13(4), 722-730 | ISSN:2251-6727

cultured cells including osteoclasts and osteoblasts, by raising peroxide and superoxide radicals. The synergy between vitamins C and K, and between these vitamins and antiproliferative factors, is consistent with (Fabisiak *et al*.,2017).

Entamoeba histolytica plays a major role in the pathophysiology of the disease as it leads to the production of reactive oxygen species and this can be an effective strategy against the parasite. Ascorbic acid or vitamin C is known to act as an antioxidant at physiological concentrations and as a pro-oxidant at high pharmacological concentrations (Mastrangelo *et al*.,2017). Ascorbate acts as an antioxidant by neutralizing potentially harmful free radicals, and ascorbate acts as a pro-oxidant, in the presence of stimulating metal ions, on the formation of H2O2 and ROS. The antioxidant also had anti-parasitic activity which was demonstrated in a study conducted by the researcher (Puente *et al*., 2018) when *Trypanosoma cruzi* parasites were treated with a combination of vitamin B12 and vitamin C. Intestinal inflammation is associated with an imbalance between Reactive oxygen species and the antioxidant response, which leads to oxidative stress and is a critical cause in the pathophysiological process of some chronic diseases, resulting from an imbalance between pro- and antioxidants, leading to potential cellular damage and dysfunction, shown by many Studies showed that oxidative stress as an important factor in the pathogenesis, progression, and severity of inflammatory bowel diseases showed that the use of preventive drugs to inhibit oxidative stress led to an improvement in the health status of patients, vitamin K showed its ability to reduce intestinal oxidative stress by regulating the expression of pro-oxidant enzymes and antioxidants (Lai *et al*., 2022). As it protects cellular membranes from damage caused by excess free radicals, both vitamins share the same function.

Folate and vitamin B12 are essential micronutrients, both are involved in one-carbon metabolism, including the remethylation of homocysteine to methionine and participation in DNA synthesis (Azimi *et al*., 2018). Their deficiency, therefore, can lead to impairments in one-carbon metabolism. Elevated plasma homocysteine, which can be associated with general health problems such as cardiovascular diseases and cancers, therefore, the positive association between vitamin B9 and vitamin B12 may be attributed to the fact that vitamin B12 deficiency affects the functional performance of folic acid, due to the decrease in methyltransferase enzyme level, that is affected by a decrease in cobalamin (B12) concentration. Vitamin B12 deficiency can lead to low levels of methionine, which in turn disrupts folate metabolism by sequestering an increased proportion of folate as a 5-methyl derivative, which is a poor substrate. to manufacture folylpolyglutamate, which negatively affects tissue retention of folate, and this is consistent with (Shane and Stokstad, 1985).

Conclusion.

Based on the results obtained, it was found that infection with the *Entamoeba histolytica* leads to biochemical changes, especially at the level of vitamins in terms of a decrease in their concentration in the blood of patients.

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