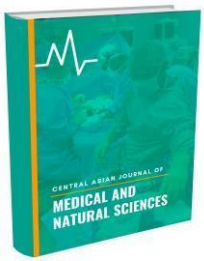

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Article

The Effect of Amoebic Dysentery on Some Physiological Parameters

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Abstract: The study was conducted in Anbar Governorate on patients visiting Fallujah Women's and Children's Hospital and some private laboratories in Fallujah for age groups ranging from (20-1) years and for both sexes. The collection period lasted from July 2023 to December 2023 and amoebic dysentery was diagnosed through microscopic examination of stool samples to investigate the parasite Entamoeba histolytica, and 30positive samples were recorded out of 120 samples and the infection rate reached 25%. The concentration of SOD, CAT, GSH, MDA, G.H and Ghrelin were studied for infected and healthy individuals. The results of the current study showed highly significant differences for CAT for those infected with amoebic dysentery compared to healthy individuals. As for SOD and MDA, they showed a significant difference for those infected compared to healthy individuals. As for G.H and Ghrelin, they did not show any significant differences for those infected compared to healthy individuals. Highly significant differences were shown for SOD for those infected and for both sexes compared to healthy individuals. As for MDA, they showed a significant difference for those infected compared to healthy individuals. As for CAT, GSH, G.H and Ghrelin, they did not show any significant differences for those infected with E.histolytica and for both sexes compared to healthy individuals.

Keywords: Amoebic Dysentery, Some Physiological Parameters, Iraq

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1. Introduction

E. histolytica is an intestinal parasitic protozoan that causes amoebic dysentery in humans, which is one of the leading causes of death and is responsible for 100,000 deaths per year (Wesel *et al*.,2021). This infection leads to severe diarrhea, especially in children under five years of age, and may be accompanied by chronic inflammation, leading to weight loss and delayed growth .This parasite lives and colonizes in the large intestine and may cause symptomatic amoebiasis such as bloody diarrhea, intestinal colic and fever, and may not cause any symptoms (asymptomatic amoebiasis) and is therefore called asymptomatic carriers. Through the studies that were conducted, it was shown that 10% of people infected with the parasite E. histolytica will show symptoms of the disease, while it was shown that approximately 90% may not show symptoms of the disease (asymptomatic), meaning that the disease does not develop in them and they are called carriers of the disease (Ghasemi et al., 2015). This parasite is transmitted from one person to another either through contaminated water or food or through the feces of infected persons containing the infective cyst stage of the parasite (Davhana *et al*.,2020). This parasite has a simple life cycle, consisting of two stages: the feeding stage and the cyst stage. The feeding stage will cause damage to the host, which leads to cell damage,

causing painful cup-shaped ulcers. This causes amoebic dysentery because this stage lives in cavity of large intestine and feeds on the mucous membrane lining the intestine (Petri & Frederick, 2005).

Pathogenicity is related to the parasite and host factors and focuses on the mechanism of action of the parasite mainly which is associated with intestinal amoebiasis (Fotedar et al., 2007). The active stage has amoebapores and many proteolytic enzymes, the most important of which is amoebic cysteine proteinase, which are important virulence factors possessed by the parasite and which tear the intestinal mucosa and the epithelial barrier (Chen et al., 2010). When the feeding stages attack the epithelial cells in the mucosa lining the large intestine as well as with the help of external proteolytic enzymes, amoebic dysentery will occur (Brooks et al., 2004). When the feeding stages adhere to the epithelial layer of the large intestine, infection occurs, as the feeding stages have many virulence factors, the most important of which is the adhesion factor Lectin, which helps the parasite adhere to the host cells, and also contains the enzymes that degrade the external proteins cysteine proteinase, which degrades the cells, as well as the amoebapore, which works to make holes in the surfaces of cells and the enzyme that degrades red blood cells hemoglobinase and the enzyme that degrades fat phospholipase. These factors represent important virulence factors produced by the histolytic amoeba (Shannon et al., 2013). The parasite may settle without invading the tissues and also without showing any symptoms, and in 90% of infections (Tengku and Norhayati, 2011). Amoebic Colitis may occur in 10% of infected individuals, and the infection may also extend outside the intestine to other organs such as the liver, which is the most common, and this occurs in a percentage of Approximately 2% of people infected with the parasite (Khan et al., 2008).

Superoxide dismutase (SOD) enzyme: It is a group of important antioxidant mineral enzymes that are present in most living cells and act as a first line of defense against oxidative damage caused by superoxide radicals (O2) by converting these reactive radicals into hydrogen peroxide and oxygen. It plays an important role in restoring cell vitality and reducing the rate of their destruction. Low and diminished SOD activity is associated with oxidative stress. There is evidence that any change in the enzyme may lead to the development of obesity and associated metabolic disorders (Schatzman *et al*,2020).

Catalase enzyme (CAT): Catalase is considered one of the important antioxidant enzymes as it plays an important role in protecting cells from oxidative damage. By catalase, hydrogen peroxide is converted into water and oxygen (Nandi *et al*.,2019).

Glutathione (GSH): is a short peptide consisting of three amino acids: glycine, glutamic acid, and cysteine. Glutathione is a non-protein nitrogenous substance that contains a large amount of sulfur and is characterized by its low molecular weight. Glutathione is found in animal and plant tissues and is also found in most types of bacteria. It is often found in high concentrations and is considered one of the most abundant non-protein cellular thiol compounds. It is considered one of the most important non-enzymatic (endogenous) antioxidants. Glutathione is considered one of the non-protein compounds that contain thiols and the presence of the free thiol group in glutathione provides major protection against severe oxidation states. GSH may play a major role in many drug and biochemical reactions due to these properties (Pour-Aboughadareh *et al*.,2020). Malondialdehyde (MDA): is a highly reactive compound formed by the entrapment of polyunsaturated fatty acids in biological membranes. MDA is one of the most abundant types of aldehydes, resulting from the attack of free radicals on polyunsaturated fatty acids of cell membranes and organ tissues, MDA is an indicator of oxidative stress, which has been linked to a range of health conditions such as inflammation, vascular disease, and heart disease (Gopal et al.,2012)

Growth hormone (GH): It is a peptide protein hormone secreted by the pituitary gland that stimulates growth and stimulates cell reproduction and renewal in humans. Growth hormone is used as a medicine in medicine to treat growth disorders in children and to treat hormone deficiency in adults. It also stimulates the growth of all body tissues, including bones, as it is built and secreted by cells in the anterior pituitary gland called

(Somatotroph), which release between 1-2 milligrams daily. This is important for normal physical growth in children, as its secretion levels gradually increase during childhood and reach their peak during the growth spurt that occurs at puberty. Growth hormone is secreted in batches every three to five hours, as growth hormone is secreted by another hormone called growth hormone-releasing hormone (GHRH), which is secreted from the highest point in the brain, the hypothalamus, which causes the secretion of growth hormone from the pituitary gland (Powers M, 2005). Ghrelin: A 28-amino acid peptide that stimulates hunger. It is produced primarily by the P/D1 cell in the lining of the stomach fundus and the epsilon cell of the pancreas (Mercedes *et al*., 2006). Ghrelin levels increase before meals and decrease after meals. It is considered the antagonist of leptin, which is produced by adipose tissue and which, when present at high levels, induces a feeling of satiety. In some cases of obesity treatment, ghrelin levels are reduced in patients, causing a feeling of satiety before it normally occurs. Ghrelin is a powerful stimulator of growth hormone secretion from the anterior pituitary gland. Ghrelin receptors are G protein-coupled receptors, known as growth hormone secretagogues. Ghrelin binds to a variant of these receptors, GHSR1a splice- which is found in high concentrations in the hypothalamus, pituitary gland (Nazli *et al*., 2014). The study aimed to evaluate the effect of infection with the parasite on some physiological parameters such as (growth hormone, antioxidants SOD and CAT, ghrelin hormone FSH, malondialdehyde MAD, glutathione)

2. Materials and Methods

1**- Collect stool samples**: 90 stool samples were collected from patients visiting Fallujah Women's and Children's Hospital and some private laboratories in Fallujah in Anbar Governorate. The collection period lasted from July 2023 until December 2023. The study included examining samples from both males and females. The stool samples were examined microscopically, where an amount of stool was taken the size of a matchstick head from several places using a clean wooden stick, and the taken amount was mixed with the physiological saline solution on the slide, after which the slide cover was placed and then examined with a light microscope with an oil lens under a magnification of 100X to confirm the presence of the parasite (Singh et al, 2009).

2: **Collect Blood samples**: 5 ml of blood was drawn from patients suffering from diarrhea and proven to be infected with *E.histolytica*, as well as from uninfected individuals in the control groups. The blood was placed in test tubes free of anticoagulant and left for approximately 15 minutes at 37 degrees Celsius. After that, the serum was obtained by centrifuging at a speed of (3000 rpm) for 5 minutes, and stored at (-20) degrees Celsius in new, clean plastic tubes until physiological tests were performed.

3: Estimation of concentrations of physiological tests

3-1: **Estimation of the activity of the enzyme superoxide dismutase SOD**: The activity of the enzyme superoxide dismutase was estimated using the modified photochemical Nitroblue Tetrazolum (NBT) method. This method included the use of sodium cyanide as an inhibitor of the peroxidase enzyme. This method depends on estimating the activity of the enzyme SOD indirectly through the appearance of a change in the optical density of formazin formed by the reduction of O2 to the dye nitrobluetetrazolium (NBT), which in turn is generated by irradiation of the serum of the boil (since the decrease in the optical density of formazin indicates an increase in the activity of the enzyme SOD) (Brown & Goldstein, 1983).

3-2**: Estimation of catalase enzyme activity CAT:** The principle of the method involves the reaction of ammonium meta vanadate with hydrogen peroxide under acidic conditions, reducing vanadium (V) to (III). Hydrogen peroxide is a strong oxidant that leads to the formation of the red-orange peroxovanadium complex, which absorbs at 452 nm (Brown & Goldstein, 1983).

3-3: **Estimation of (GSH) in Serum**: Glutathione was measured in serum using the Ellman reagent method (Brown & Goldstein, 1983).

3-4**: Estimation of malondialdehyde (MDA) concentration.:** The modified Thiobarbituric acid (TBA) reaction method used by researchers Shah and Guidet was used to measure

MAD, which represents one of the final products of the peroxidation process of fat, and its level is an indicator of this process, as the measurement depends on the reaction between fat peroxides, especially malondialdehyde, with TBA in a medium that depends on the acidic pH function.

3-5: **Estimation of growth hormone (GH) concentration:** Growth hormone analysis was performed in the Japanese Tosoh device (Brown & Goldstein,1983).

3-6: **Estimation of ghrelin hormone (FSH) concentration**: Ghrelin hormone analysis was performed in the Japanese Tosoh device (Brown & Goldstein,1983).

4 **Statistical analysis:** The results were statistically analyzed using the statistical program Ver. Minitab 17 according to the t-test (and ANOVA) F test). The arithmetic means were compared to determine the significant differences using Duncan's multiple range test at a probability level of 0.05. (Morgan and Case, 2013).

3. Results and Discussion

Stool samples were collected from patients visiting Fallujah Women's and Children's Hospital and some private laboratories in Fallujah in Anbar Governorate for age groups ranging from (20-1) years and for both sexes. The collection period continued from July 2023 until December 2023. *E.histolytica* was diagnosed through microscopic examination of stool samples in 30 samples out of 120 samples, with a percentage of 25%. The results of this study agreed with what was reached by (Jinkeez *et al*., 2021) inKirkuk, where the infection rate reached 23.57%, while the results of our current study exceeded the results of both (Kadir et al.,2018) in Tikrit, where the infection rate reached 9.3% .

The difference in the infection rate of this parasite in this study and the previous and mentioned studies is due to the difference in population density, personal hygiene, geographical location, age groups on which the study was conducted, the number of samples examined, as well as the level of sanitation, climatic conditions, and living conditions, while the similarity in the infection rate is due to the similarity in terms of environmental conditions and the cultural, social, and health level (Kurt et al.,2008).

Concentration of SOD, CAT, GSH, MDA, G.H, and Ghrelin for infected and healthy people according to the general analysis.

The results of the current study showed highly significant differences As for CAT for those infected with *E.histolytica* compared to healthy individuals, as for SOD and MDA, there was a significant difference for those infected with *E.histolytica* compared to healthy individuals, as for G.H and Ghrelin, there were no significant differences for those infected with *E.histolytica* compared to healthy individuals as in Table (1).

Many studies and researches have been recorded on the occurrence of oxidative stress, including Demirci and his group (2003) in patients with giardiasis, as well as Sim and his group (2005) in patients with amoebic dysentery. Infection with the histolytic amoeba parasite was directly associated with a sharp increase in free radicals through a significant increase in SOD, CAT, and MDA in people infected with the parasite compared to uninfected people, which confirms the importance of the role of parasites in causing oxidative stress. Some studies have also recorded an increase in the level of malondialdehyde in patients infected with Toxoplasma gondii (Yazar et al., 2003), patients infected with *E.histolytica* (Sim et al., 2005), and patients infected with giardiasis (Demirci et al., 2003). In addition, infection with parasites that cause immune, physiological, and biochemical changes have a major role in increasing malondialdehyde, which is an end product of the oxidation process. The same applies to SOD), and it also agreed with (Seif & Al-Mohmmed, 2021), as the researchers noted levels of Antioxidants were high in untreated patients, and the results of this study were consistent with (Al-Hassan& Al-Mayali,2020) where they recorded high levels in patients with leishmaniasis compared to healthy controls. When infected with amoebiasis, acute inflammation may occur as free radicals are released as a result of the host's immune response, as due to infection with the parasite as well as due to the widespread inflammation it causes inside the body of the living organism, especially the digestive system, it may stimulate the body's immune system to respond directly to the parasitic invasion, as this is done by releasing oxidants from immune cells to eliminate the pathogen. As for glutathione (GSH), the results of our

study differed from (Kaya et al., 2007) when studying Toxoplasma gondii, and our current study recorded high levels of glutathione in patients infected with the parasite compared to those not infected. Glutathione is the main component of the thiol group and is also the main scavenger of free radicals. Glutathione plays an important role in the antioxidant defense system as well as in catalytic and regulatory protein interactions, and in the transfer of electrons and may maintain the correct structure of proteins (Madhikarmi & Murthy, 2011). Glutathione is also an important component of the antioxidant system inside the body for the mechanism of protection inside cells against internal and external indicators that lead to oxidative stress. Glutathione is an antioxidant of internal origin and works against oxidants such as some drugs and pathological infections. As for the growth hormone G.H, the results of our current study agreed with what was reached by (Patricia *et al*.,2022).

The results of the current study showed that there were high significant differences for SOD for those infected with *E.histolytica* for both sexes compared to healthy people, as for MDA, it showed a significant difference for those infected with *E.histolytica* for both sexes compared to healthy people, as for CAT, GSH, G.H, Ghrelin, no significant differences were shown for those infected with *E.histolytica* for both sexes compared to healthy people as in Table (2).

For the percentage of SOD and CAT, higher results were recorded for males compared to females, and were consistent with many studies, including (Al-Ani, 2018; Al-Warid *et al*., 2017),where they recorded a high infection rate for males compared to In females, the reason may be that males are more present outside the home than females, such as playing in the street and mixing with other people. As for malondialdehyde, the results of the current study agreed with (Ismail &Al-Kakey, 2006), where the results showed that the level of MDA was higher in males and females, respectively, compared to the level of healthy males and females. As for glutathione, it agreed with (Ismail & Al-Kakey, 2006), where the level of glutathione decreased in males compared to females. As for growth hormone and ghrelin hormone, growth hormone decreased in males compared to females, while ghrelin hormone did not record any significant differences.

Table (1) The average physiological parameters of patients and healthy people according to the general analysis

Group	Ghrelin **Mean ± S.D**	G.H **Mean ± S.D**	MDA **Mean ± S.D**	GSH **Mean ± S.D**	CAT **Mean ± S.D**	SOD **Mean ± S.D**
Patients	**2.257±0.927**	**11.25±1.89**	**117577±696.10**	**10.716±0.233**	**0.0488±0.0346**	**4.370±1.600**
control	**2.261±0.767**	**11.51±2.90**	**77724±261.67**	**10.647±0.627**	**0.0250±0.0165**	**0.112±0.170**
P-Value	**^{ns}0.988**	**^{ns}0.775**	**0.010***	**^{ns}0.722**	**0.005****	**0.0006***

*Indicates significant differences at 0.05 probability level
**Indicates highly significant differences at 0.05 probability level
ns indicates no significant differences at 0.05 probability level

Table (2) Average physiological parameters of patients and healthy individuals by gender

Group	Ghrelin	G.H	MDA	GSH	CAT	SOD
Patients **male**	**2.475a±0.423**	**10.952a±1.970**	**117852a±82.831**	**10.7054a±0.2886**	**0.05070a±0.04590**	**4.471a±1.8720**
Patient female						

	2.066a±0.819	**11.506a±1.846**	**117336a±58.496**	**11.506a±1.846**	**0.04707a±0.02201**	**4.275a±1.3800**
Control male	**2.287a±0.757**	**11.450a±3.330**	**86703b±99.160**	**11.450a±3.330**	**0.02816a±0.01909**	**0.075b±0.0581**
Control female	**2.224a±0.869**	**11.600a±2.540**	**79153b±96.980**	**11.600a±2.540**	**0.02061a±0.01253**	**0.035b±0.0194**
P-Value	**0.664 ns**	**0.901ns**	**0.045***	**0.899 ns**	**0.179ns**	**0.0008****

*Similar letters indicate no significant differences at 0.05 probability level
*Different letters indicate significant differences at 0.05 probability level.

4. Conclusion

This study evaluated the prevalence of Entamoeba histolytica infection and its impact on various biochemical indicators among patients at Fallujah Women's and Children's Hospital and private laboratories in Fallujah from July 2023 to December 2023. Of the 120 stool samples analyzed, 30 samples (25%) were positive for E. histolytica. This prevalence is consistent with Jinkeez et al. (2021) in Kirkuk, which reported a 23.57% infection rate, but higher than Kadir et al. (2018) in Tikrit, where the infection rate was 9.3%. Variations in infection rates can be attributed to factors such as population density, personal hygiene, geographic location, age groups studied, sample size, sanitation levels, climatic conditions, and living standards.

The study found significant differences in biochemical indicators such as SOD, CAT, and MDA between infected and healthy individuals, while G.H and Ghrelin levels showed no significant differences. Specifically, increased levels of SOD, CAT, and MDA indicate that E. histolytica infection is associated with oxidative stress. Contrary to previous studies, glutathione levels were higher in infected patients, suggesting its role in the antioxidant defense system despite the infection. The study also observed higher levels of SOD and CAT in males compared to females, while MDA levels were elevated in both genders. These findings underscore the need for further research to explore the underlying mechanisms of parasitic infections and their biochemical impacts, as well as gender differences in response to such infections.

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