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### A Study of Certain Physiological Characteristics of Infected Children with Diarrhea Owing to Entamoeba Histolytica and Related Bacteria in Children of Thi-Qar Province

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#### ABSTRACT

The present study aims to evaluate the physiological characteristics of E. histolytica and common pathogenic bacteria in children under the age of five who are experiencing diarrhoea. The present investigation was carried out at Bint Al-Huda Teaching Hospital for Women and Children and Al-Mousawi Hospital under the jurisdiction of Thi-Qar Province. The present study observed a significant decrease in the count of red blood cells, haemoglobin blood, and hematocrite in patients infected with E. histolytica and pathogenic bacteria, as well as in patients infected with E. histolytica alone, compared to the control groups. However, there was no significant difference within patients for both males and females. The blood parameters of male and female patients with diarrhoea (plate late test) exhibited a statistically significant increase ( $P \ge 0.05$ ) in comparison to the control group. There was no statistically significant difference ( $P \ge 0.05$ ) observed between male and female patients with diarrhoea produced by E. histolytica and pathogenic bacteria, compared to those with diarrhoea caused only by E. histolytica. There were no significant changes  $(P \ge 0.05)$  in any blood parameters (mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration) between male and female patients with diarrhoea and the control group. The findings of the study revealed that there was no statistically significant increase (p < p0.05) in the levels of Aspartate aminotransferase, Alanine aminotransferase, and Alkaline phosphatase in patients infected with E. histolytica. However, in patients infected solely with E. histolytica, there was a significant increase in enzyme levels compared to both the pathogenic and control groups. The present study demonstrated a notable elevation in the levels of urea and creatinine among male and female patients diagnosed with diarrhoea in comparison to the control group. However, no statistically significant difference was observed between male and female patients with diarrhoea caused by E. histolytica and pathogenic bacteria, as compared to those with diarrhoea solely caused by E. histolytica.

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KEYWORDS: Diarrhea, E. histolytica, Pathogenic bacteria, hemoglobin	https://ijpbms.com/

#### INTRODUCTION

Diarrhoea is a prominent contributor to morbidity and mortality in newborns and children globally, particularly in underdeveloped nations. Children under the age of 5 experience around 2.5 million deaths due to gastroenteritis annually [1]. According to [2]. Entamoeba histolytica, the causative agent of amebiasis, is the third leading cause of mortality among parasitic infections, following malaria and schistosomiasis. Dysentery is mostly transmitted by the

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consumption of contaminated food and water, as well as

inadequate sanitation practices. Various bacterial strains,

such as Shigella, Salmonella, Campylobacter, and

Escherichia coli, have been identified as causative agents of

component that can potentially impact the clinical manifestation and ultimate prognosis of E. histolytica infections. E. histolytica can have several effects, including asymptomatic colonisation, amebic dysentery, and invasive extraintestinal infections. The most prevalent form of amebic infection is amebic liver abscess. The primary clinical symptoms of amoebiasis are colitis and liver abscesses. The processes of pathogenesis in E. histolytica include the adhesion, penetration, and subsequent killing of host cells within host tissues. The amoeba's main virulence factors, galactose and N-acetylgalactosamine, play a mediating role in these processes. The parasite releases these enzymes to break the intestinal mucus and epithelial barrier, hence facilitating tissue penetration by the trophozoites. There are two distinct phases in the life cycle of the parasite, namely the infective cyst and the invasive trophozoite. This study aimed to assess the physiological impact of children infected with E. histolytica and pathogenic bacteria, as well as children under the age of five who are infected with Entamoeba histolytica and pathogenic bacteria, in Thi-Oar province. The study included the measurement of various parameters such as CBC (RBCs, WBC count and differential, Hb, HCT, MCV, MCH, MCHC, and PLT) and liver enzymes (ALT, AST, and ALP). Evaluation of Renal Function: Urea and Creatinine Levels.

#### MATERIALS AND METHODS

From November 2018 to April 2019, a well-trained nurse collected five millilitres of venous blood samples from 100 volunteer patients with diarrhoea and 25 healthy children at Bent-Alhuda Technical Hospital for Women and Children. The samples were collected in tubes. Additionally, the clinical history of each patient's parents was obtained and recorded in the attached form. A volume of 3 ml of blood was permitted to undergo coagulation at ambient temperature for a duration of 30 minutes. Subsequently, it was subjected to centrifugation at a speed of 4000 RPM for a period of 5 minutes. To prevent hemolysis, appropriate precautions were implemented. Nevertheless, the hemolysis samples were disregarded. To measure the haematological parameters and ESR, two millilitres of whole blood were added to ETDA-

containing tubes containing serum used to estimate the physiological and immunological markers ALT, AST, ALP, urea, creatinine, and CRP [6].

The current study utilised the statistical package for social science (SPSS) version to analyse the data, and the results were expressed as the mean  $\pm$  standard error (S.E.). The independent sample Chi-square test was employed to assess the statistical significance of differences, with a significance level of P < 0.05.

#### RESULTS

4.7. The Relationship between some Physiological Parameters and the Infection with E. histolytica and Pathogenic Bacteria

#### 4.7.1. Hematological Parameters

#### 4.7.1.1. White Blood cells Count and Differential

From November 2018 to April 2019, a well-trained nurse collected five millilitres of venous blood samples from 100 volunteer patients with diarrhoea and 25 healthy children at Bent-Alhuda Technical Hospital for Women and Children. The samples were collected in tubes. We also obtained and recorded the clinical history of each patient's parents in the attached form. We allowed a volume of 3 ml of blood to undergo coagulation at ambient temperature for 30 minutes. We then centrifuged it at 4000 RPM for 5 minutes. We implemented appropriate precautions to prevent hemolysis. Nevertheless, the hemolysis samples were disregarded. To measure the haematological parameters and ESR, two millilitres of whole blood were added to ETDA-containing tubes containing serum used to estimate the physiological and immunological markers ALT, AST, ALP, urea, creatinine, and CRP[6].

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Table. (1): Total and differential count of WBCs for patients with diarrhea compared with control.

Sex	Parameters	Total WBC	MON%	LYM%	NEU%	BASO%	EOS%
		*10 <sup>3</sup>	M + SD	M + SD	M + SD	M + SD	M + SD
		M + SD					
	Control	6.64 ± 1.21 <sup>a</sup>	$0.21 \pm 0.09$	$1.45 \pm 0.26^{a}$	2.57 ±	$0.33 \pm 0.17$	$0.15\pm0.56$
			a		0.60 <sup>a</sup>	а	а
s	E. histolytica	$10.89 \pm 2.55$	$0.88 \pm 0.44$	$3.59 \pm 1.53^{a}$	$6.41 \pm 3.46$	$0.90\pm0.51$	$1.21\pm0.50$
Males	only	b	b		b	b	b

	E. histolytica	$11.8 \pm 5.49$ <sup>b</sup>	$0.74 \pm 0.34$	$3.83 \pm 1.45^{a}$	$7.19 \pm 2.85$	$0.79 \pm 0.52$	0.88 ± 4.33°
	and p. bacteria						4.35°
	L.S.D.	4.73	0.71	5.49	3.84	0.56	1.06
	Control	6.39 ± 1.42 <sup>a</sup>	<b>0.33</b> ± <b>0.19</b> a	$1.41 \pm 0.38^{a}$	$\begin{array}{c} 2.61 & \pm \\ 0.67^{a} \end{array}$	$0.29\pm0.1^{\rm a}$	$0.34 \pm 0.55$ a
	<i>E. histolytica</i> only	11.96 ± 3.96 <sup>b</sup>	0.98 ± 0.47 b	4.09 ± 1.71 <sup>a</sup>	5.86 ± 2.84	0.82 ± 0.61 <sup>b</sup>	1.13 ± 0.63 b
es	<i>E. histolytica</i> and p. bacteria	12.59 ± 4.03	1.15 ± 0.68 <sup>b</sup>	<b>4.9</b> ± <b>6.67</b> <sup>a</sup>	7.16 ± 3.76 <sup>b</sup>	0.86 ± 0.54 <sup>b</sup>	0.69 ± 0.35°
Females	L.S.D.	5.57	0.64	2.6	3.25	0.52	0.83

Note: the different letters with subscribe (a, b, c, ..... etc) refer to significant difference

#### 4.7.1.2. Other Hematological Parameters

The blood parameters of male and female patients with diarrhoea (RBC, Hb, and HCT) exhibited a statistically significant decrease ( $P \ge 0.05$ ) in comparison to the control group. There was no statistically significant difference ( $P \ge 0.05$ ) observed between male and female patients with

diarrhoea produced by E. histolytica and pathogenic bacteria, compared to those with diarrhoea caused only by E. histolytica. There were no significant changes ( $P \ge 0.05$ ) in other blood parameters (MCV, MCH, and MCHC) between male and female patients with diarrhoea and the control group. Table 2.

Sex	Parameters	RBCs *106 M + SD	PLT * 10 <sup>3</sup> M + SD	Hb gm/dl M+SD	HCT% M+SD	MCV fl M+SD	MCH pg M+SD	MCHC gm/dl M+SD
	Control	$5.14 \pm 0.31_{a}$	$240.0 \pm 33.9$	$12.8\pm0.68^{\ a}$	$39.5 \pm 2.1$ <sup>a</sup>	90.35 ± 3.8 <sup>a</sup>	32.38 ± 1.9 <sup>a</sup>	$\begin{array}{rrr} 31.70 & \pm \\ 0.97^{\ a} \end{array}$
	<i>E. histolytica</i> only	4.30 ± 0.57 b	365.2 ± 104.9 <sup>b</sup>	10.63 ± 1.55 b	31.9 ± 3.65 b	90.77 ± 5.3 a	29.25 ± 3.7 <sup>a</sup>	30.57 ± 3.2 <sup>a</sup>
Males	<i>E. histolytica</i> and P. bacteria	4.13 ± 0.57 b	328.2 ± 94.4 <sup>b</sup>	11.01 ± 1.32 b	$32.6\pm3.8^{\text{ b}}$	89.10 ± 5.5 <sup>a</sup>	30.33 ± 3.1 <sup>a</sup>	30.99 ± 4.7 <sup>a</sup>
Ma	L.S.D.	0.84	125.2	2.16	14.8	2.84	4.63	1.23
	Control	$\begin{array}{c} 4.95 \pm 0.32 \\ a \end{array}$	240.63 ± 33.8 <sup>a</sup>	$12.9 \pm 1.13$ <sup>a</sup>	$39.56 \pm 3.6$	$93.72 \pm 4.1$	30.43 ± 2.8 <sup>a</sup>	32.55 ± 2.3 <sup>a</sup>
	<i>E. histolytica</i> only	4.32 ± 0.49 <sup>b</sup>	405.33 ± 103.0 <sup>b</sup>	$10.44 \pm 1.7^{b}$	31.32 ± 4.15 <sup>b</sup>	90.58 ± 4.2 <sup>a</sup>	29.72 ± 4.9 <sup>a</sup>	30.99 ± 1.98 <sup>a</sup>
Females	<i>E. histolytica</i> and P. bacteria	4.26 ± 0.68 <sup>b</sup>	347.66 ± 152.6 <sup>b</sup>	$10.47 \pm 1.35^{\text{b}}$	32.27 ± 3.3 <sup>b</sup>	89.54 ± 6.9 a	29.18 ± 3.7 <sup>a</sup>	30.77 ± 3.7 <sup>a</sup>
Fen	L.S.D.	0.62	164.69	2.47	13.83	3.14	5.71	1.56

Table. (2): Blood parameters for patients with diarrhea compared with control.

Legend as table (1)

#### 4.7.2. Liver Enzymes

The liver enzymes (ALT, AST, and ALP) of male and female patients with diarrhoea exhibited a statistically significant rise ( $P \ge 0.05$ ) in comparison to the control group. There was no statistically significant increase ( $P \ge 0.05$ ) observed in male patients with ALT who had diarrhoea produced by both E. histolytica and pathogenic bacteria, compared to patients with diarrhoea caused solely by E. histolytica. Patients infected

With E. histolytica plus pathogenic bacteria showed a nonsignificant decrease ( $p \ge 0.05$ ) in AST and ALP levels when compared to male patients infected with E. histolytica alone. After being infected with E. histolytica and pathogenic bacteria, ALT, AST, and ALP levels went up in female patients but not significantly ( $p \ge 0.05$ ) more than in female patients who were only infected with E. histolytica. Table 3.

r		1		
Sex	Parameters Infectious group	ALT UI/ml Mean + SD	AST UI/ml Mean + SD	ALP UI/ml Mean + SD
	Control	$12.5\pm2.90^{\text{ a}}$	$10.92 \pm 2.23$ <sup>a</sup>	$79.0 \pm 11.09^{\text{ a}}$
	E.histolytica	$24.66 \pm 5.23^{\text{ b}}$	$30.93 \pm 10.52$ <sup>b</sup>	133.26 ± 28.09 <b>b</b>
le	<i>E.histolytica</i> and P. Bacteria	28.02 ± 6.84 <sup>b</sup>	$30.06 \pm 7.38$ b	123.26 ± 24.67 b
Male	LSD	12.16	20.0	45.26
	Control	$13.09\pm2.60^{\text{ a}}$	11.27 ± 2.22 <b>a</b>	$71.81 \pm 5.81^{\text{ a}}$
	E.histolytica	$23.41 \pm 5.99^{\text{ b}}$	$26.41 \pm 5.42^{\text{ b}}$	123.75 ± 17.77 <sup>в</sup>
Female	<i>E.histolytica</i> and P. Bacteria	28.40 ± 7.31 <sup>b</sup>	28.96 ± 7.62 b	132.22 ± 28.21 b
Fen	LSD	10.32	15.14	51.93

Legend as table (1)

#### 4.7.3. Kidney Function Tests

A notable elevation ( $P \ge 0.05$ ) was observed in the levels of urea and creatinine among male and female patients diagnosed with diarrhoea in comparison to the control group.

There was no statistically significant difference (P  $\geq 0.05$ ) observed between male and female patients with diarrhoea produced by E. histolytica and pathogenic bacteria, compared to those with diarrhoea caused only by E. histolytica. Table 4.

Table (1): Kidney function test for	patients with diarrhea compared with control
Table (4). Klulley function test for	patients with that the compared with control

Sex	Parameters Infectious group	Urea mg/dl Mean + SD	Creatinine mg/dl Mean + SD
	Control	$18.3 \pm 1.9$ <sup>a</sup>	$0.46 \pm 0.24$ <sup>a</sup>
Male	E.histolytica	29.4 ± 3.7 <sup>b</sup>	$1.3\pm0.74$ b
	E.histolytica and P. Bacteria	28.7 ± 5.49 <sup>b</sup>	$1.29\pm0.64~^{\textbf{b}}$
	LSD	11.11	0.85
Female	Control	17.36 ± 1.5 <sup>a</sup>	$0.45 \pm 0.23$ <sup>a</sup>
	E.histolytica	$29.25\pm5.89^{\text{ b}}$	$1.1\pm0.46~^{b}$
	E.histolytica and P. Bacteria	30.4 ± 5.24 <sup>b</sup>	$1.2 \pm 0.72$ b
Fen	LSD	10.32	15.14

Legend as table (1)

#### DISCUSSION

5.1. The Relationship between some Physiological Parameters and the Infection with *E.histolytica* and Pathogenic Bacteria

#### 5.1.1. Hematological Parameters

The present investigation revealed that the patient infected with E. histolytica, a pathogenic bacterium, had a high count of total white blood cells (WBC). Notably, there were substantial differences observed between the patient and the control group, but no significant differences were found within the patient population. In Karbala [7]. Observed a notable rise in the total white blood cell (WBC) count among patients infected with E. histolytica. Similarly [8] conducted a study in Al Haweeja/Kirkuk, Iraq, examining 207 patients infected with G. lamblia, E. histolytica, and pathogenic bacteria. The findings revealed a positive correlation between E. histolytica infection and total white blood cell count in these patients. [9] Conducted a study in Gaza and observed that E. histolytica had no significant impact on the overall white blood cell count, except for the percentage of EOS. According to [10], in vivo models provide evidence suggesting that cell-mediated pathways play a role in the development of immunity against invasive amoebiasis. The

study findings indicated a statistically significant rise (P  $\geq$ 0.05) in the differential count of white blood cells (WBCs) among male and female patients with diarrhoea in comparison to the control group, with the exception of lymphocytes. There were no statistically significant changes  $(P \ge 0.05)$  in the differential count of white blood cells (WBCs) between male and female patients who were infected with both E. histolytica and pathogenic bacteria, as compared to individuals who were solely infected with E. histolytica. According to [7], the findings indicated a statistically significant elevation in eosinophil counts (P≥ 0.05) among male and female patients diagnosed with diarrhoea caused by both E. histolytica and pathogenic bacteria, in comparison to patients with diarrhoea solely caused by E. histolytica.[11], examined 27 patients infected with E. histolytica and recorded the differential WBC has a direct relationship with the infection of the parasite as well as lack or delay taking appropriate treatment, while [12], study immune response of Amoebiasis and immune evasion by Entamoeba histolytica and record, E. histolytica infection alters the microbiota composition and induced disposes was characterised by fewer Bacteroides, Clostridia, Lactobacillus, Campylobacter, and Eubacterium species, and increased Bifidobacterium species, and this leads to increase levels of EOS and MON and there is no rise in the level of the other components of white blood cells. The host's immune responses, encompassing both innate and adaptive mechanisms, exhibited considerable strength against the invasive E. histolytica. However, the parasite managed to devising techniques persist bv to evade the immunesystem[13].

The present study observed a significant decrease in the counts of red blood cells (RBC), haemoglobin (Hb), and hematocrit (HCT) in patients infected with E. histolytica and pathogenic bacteria. This decrease was observed only in patients infected with E. histolytica, compared to the control group. There were no differences within patients for both males and females. Additionally, the count of platelet-rich plasma (PLT) increased significantly in both patients, and there were significant differences between patients and the control group. However, there were no differences in the levels of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), or mean corpuscular haemoglobin (MCH) between the patients and the control group. In their study, investigated a cohort of 81 patients who were infected with E. histolytica and Helminthes. The researchers observed a decline in the rate of erythrocytes and haemoglobin levels among the patients infected with the parasite. However, no significant increase in platelet levels was observed. Additionally, no changes were found in blood cell volume or haemoglobin concentration within the cells. In a study conducted by [9] in Al Haweeja/Kirkuk, Iraq, a total of 207 patients who were infected with G. Lamblia, E. histolytica, and harmful bacteria were evaluated. The findings of the

study indicated that the presence of amoeba parasites did not have any significant impact on blood parameters. The potential explanation for the observed disparity could be attributed to the researcher's analysis of individuals whose parasite load did not reach a level that would induce malabsorption or result in significant blood loss impacting iron levels. Several studies have indicated a significant correlation between the elevated occurrence of intestinal pathogens, particularly parasites, and the development of anaemia and a notable increase in leukocyte levels. This association can be attributed to the bleeding induced by the infiltration of E. histolytica [14].

#### 5.1.2. Liver Enzymes

The findings of the present study indicate a significant increase in the levels of ALT, AST, and ALP in patients infected with both pathogenic and non-pathogenic E. histolytica. The results demonstrate a significant rise in enzyme levels when comparing both groups of patients to the control group. However, no significant differences were observed within patients or between males and females. In a study conducted by [15] in France, patients with E. histolytica were studied. The researchers observed that during the initial days of infection, E. histolytica did not have any impact on liver enzymes. However, as the infection advanced without treatment, the parasite began to damage the liver enzymes. In a study conducted by [16], a total of 262 patients who were infected with both E. histolytica and the hepatitis B virus were evaluated. The researchers observed a notable elevation in the levels of ALT, AST, and ALP in patients infected with both E. histolytica and B viruses, as compared to those infected only with the hepatitis B virus. The research conducted by [17] involved a sample of 207 patients who were infected with E. histolytica. The study revealed that all levels of liver enzymes were elevated and exhibited abnormal values. In contrast, [18] conducted a study in rural Germany, focusing on patients with liver abscesses. They employed immunohistochemistry and quantitative flow cytometry techniques to investigate the relationship between E. histolytica infection and liver enzymes. The findings of their study indicated that there was no significant correlation observed between E. histolytica infection and liver enzyme levels. The variability in liver enzyme levels across different studies can be attributed to various factors. One possible explanation is that some studies have focused on patients with diarrhoea within three days of its onset, which allows the parasite to quickly spread to other parts of the intestines and reach the liver. Additionally, the level of health awareness and the stage of the disease at the time of sample collection may also play a role. For instance, [15] included patients infected with E. histolytica during the initial stage of infection.

#### 5.1.3. Kidney Function Tests

The present investigation revealed a notable elevation in the levels of Urea and Creatinine among male and female

individuals diagnosed with diarrhoea in comparison to the control group. However, no statistically significant distinction was observed between male and female patients with diarrhoea caused by E. histolytica and pathogenic bacteria, as compared to those with diarrhoea solely caused by E. histolytica. The present study vielded comparable findings to the study conducted by [19] in India. They investigated patients infected with E. histolytica associated with Proteus volgaris and observed a significant increase in urea and creatinine levels compared to the control group. Similarly, [19] examined patients with E. histolytica and found that it had an impact on urea levels but no effect on creatinine levels during the initial days of infection. However, as the infection advances without treatment, the parasite becomes affected. In a prior study conducted by[20], individuals with extraintestinal illness were investigated and all renal function was shown to be significantly abnormal. The current study, in line with previous research, suggests that the increased renal function tests may be caused by a parasite infection in the liver, which affects the urea cycle. Additionally, the study found a negative correlation between liver abscess and the enzyme arginase, which regulates the urea cycle.

#### CONCLUSION

Harmful bacteria such as Salmonella, Shigella, Proteus, and Pseudomonas infected the majority of individuals who contracted E. histolytica. Males are more likely to contract an infection from E. histolytica alone or in combination with other pathogenic bacteria than females, and the incidence is higher in rural areas than in urban ones.

The presence of E. histolytica alone or in combination with pathogenic bacteria has been observed to have an impact on some immunological parameters, leading to elevated levels of F-calprotectin, CRP, and ESR in comparison to the control group.

The presence of E. histolytica alone or in combination with pathogenic bacteria has been observed to have an impact on many physiological parameters in patients, such as haematological parameters, liver enzymes, and kidney function, when compared to a control group. As a result, patients who are infected with both E. histolytica and pathogenic bacteria are more affected by their immune systems and bodies than patients who are only infected with E. histolytica. This indicates that the pathogenic bacteria enhanced the pathogenicity of the parasite.

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