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Variation in Two Behavior Hormones Concentration in Patients with Clinical Amoebiasis and their Impact on Activity of Digestive Related Enzymes

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ABSTRACT: Entamoebiasis infection is a globally and locally distributed intestinal protozoan parasite, which is able to harm lower GIT, and may disseminate out of intestine. The aim of the present study was to investigate the possible role of serious amoebiasis infection on cortisol and oxytocin hormones in clinically infected patients and tracing the change in the activity of four enzymes those associated with digestion and processing of nutrients. 169 participants were subjected to the present study; their ages were ranged between18-50 years old. The sampling includes three groups; control group (60 participants) those have no common chronic diseases, the other 109 participant were patients with chronic GIT disturbance, who revised endoscopy unites in Ibn Sina Teaching Hospital and Medical Research Educational Hospital in Nineveh Governorate, Who had GIT disturbance. 60 patients of the 109 proved microscopically to have intestinal amoebiasis. Sampling was performed during the period from September 2025 to February 2026. Blood and Stool sample were collected from the participant added to inspired materials in case of endoscopy. Concentrations of cortisol and oxytocin hormones were estimated in blood. Furthermore, activity of the digestive enzymes; pancreatic amylase and lipase added to the liver enzymes; ALT and FASN were estimated also. Total blood protein, fasting blood glucose and triglycerides concentrations were estimated also. Significant increment at $P \leq 0.05$were recorded in concentration of blood cortisol and decrement in concentration of blood Oxytocin were recorded in Clinical amoebiasis cases. Significant Increment in activities of Pancreatic lipase and amylase were recorded also.

INTRODUCTION

Entamoeba histolytica is globally distributed intestinal parasite, that may cause serious amoebic dysentery to man, added to its ability to cause extra intestinal harmful amoebiasis. Since this parasite transmitted to man fecoorally, bad sanitation and poor hygiene is closely related to its endemicity (Paniker, 2017). Severe clinical outcomes may combined with entamoebiasis progression, including malnutrition, diarrhea, tissue atrophy, and anemia. (Mandal, 2015). Entamoebiasis severity is a matter of parasitic virulence, host resistance and the intestinal condition (Yanagawa and Singh 2023). Immune response to entamoebiasis infection associated with histolytic effect during mucosal and sub mucosal Invasion in cecum and colon (Paniker, 2017). GIT disturbance upon *E. histolytica* attack may develop intestinal malabsorbsion, loose of appetite, thus vitamins and iron deficiency (WHO, 2025).

Entamoebiasis infections may induce changes in some hormones. The relationship between *Entamoeba histolytica* infection and hormonal regulation is a specialized area of study, primarily focusing on how glucocorticoids (cortisol) exacerbate amoebic

virulence and how sex steroids influence host susceptibility. Moonah, *et al.* (2013) discusses how stress-induced corticosteroids suppress the intestinal IgA response and promote invasive disease. Stanley (2003) explains how *E. histolytica* liver abscesses were more common significantly in post-pubertal males, linking it to the hormonal milieu. Haque, *et al.* (2003) describe the physiological changes during pregnancy that increase the risk of invasive amebiasis and the clinical management of pregnant patients. Sánchez-Guillén, *et al.* (2006) demonstrate that elevated host cortisol further accelerates amoebiasis infection promoting deeper intestinal invasion and potential dissemination into the portal circulation. As for enzyme studies, Abdulali, (2026) referred to ALT activity elevation in patients with amoebic liver abscess. Bansal (2014) concluded that *E. histolytica* upregulates its own amylase production (ß-amylase) to degrade intestinal carbohydrate reserves during tissue entry. Kumar, *et al.* (2021) demonstrated that extra-intestinal complications of amebiasis can contiguous spread or severe abdominal distension/peritonitis elevates serum pancreatic amylase and lipase. Castellanos-Castro (2020) studied how *E. histolytica* intercepts host fatty acid sources and alters hepatic/intestinal lipid synthesis enzymes (including host FASN regulation) for membrane construction and cyst wall synthesis.

The studies that correlate between hormonal changes in serious amoebiasis infections and the subsequent alterations in digestive enzymes activity are rare.

Thus, the present work was aimed to investigate the relation between clinical amoebiasis infections and the variation in the concentrations of cortisol and oxytocin hormones, and the possible role of the hormonal changes *VS* parasitic infection on the activity of some digestive related enzymes.

MATERIAL AND METHODS

Study design:

169 stool and blood samples were collected during September 2025 and February 2026, the samples were collected from general hospitals and chosen volunteers those reside either at Right or Left Banks of the Tigris River passerby Nineveh Governorate. The participant ages were ranged between (18-50) years. Before sample collection, demographic and clinical data were obtained from the participants. Data were collected through structured face-to-face interviews with participants using a pre-designed and validated questionnaire. Each Participant was screened for chronic diseases. Gastroenteritis symptoms under seniority of specialist physician. Serious amoebiasis cases were chosen from patients who had chronic gastroenteritis and submitted to colonoscopy. Subsequently, laboratory detecting for *E. histolytica* infections were performed depending on microscopic finding of the parasite in stool and colon inspired materials. Accordingly, the studied cases were categorized to three groups; each group was subdivided into three age groups, as following (Table.1):

-**First group (Control Group**): included 60 volunteer, apparently healthy individuals with negative laboratory finding for *E. histolytica* and have no GIT disturbances, no common chronic diseases (Diabetes, cardiovascular diseases, rhomboidal and adrenal problems).

-**Second group (with Serious entamebiasis infections)**: include 30 patients clinically symptomatic patients who subjected to colonoscopy and had abdominal cramp, diarrhea, fever, nausea, loss of appetite, and confirmed to have *E.histolytica* parasite during stool and inspired fluid microscopic examination.

-**Third group** (**Chronic gastroenteritis symptomatic -** negative for entamoebiasis): included 79 patients subjected to colonoscopy and had clinical GIT symptoms- but negative for *E. histolytica* depending on microscopic exam for both stool and colon inspirited materials. In the three groups, sampling from pregnant or breasting women was avoided.

Table.1: Distribution of male and female cases subjected to the study according to age groups and relation, to amoebiasis infection:

Age group	Control participants	Clinical amoebiais cases	Chronic GIT disturbance cases		Total		
	Male	female	male	female	male	female	
18-30	10	10	4	4	13	12	43
31-40	10	10	5	4	13	11	53
41-50	10	10	7	6	15	15	63
Total	30	30	16	14	41	38	**169**

Stool sampling: Samples were collected in clean, dry containers; initially macro examination for the collected samples was performed. This was including physical characteristics of the collected stool focusing on consistency and color.

Collecting Lower GIT Inspiration:

Colon inspired materials were collected from chronic gastroenteritis patients during colonoscopy, Which was applied in three Endoscopy units in Nineveh General hospitals. Specific Cannula Device was used under seniority of professional clinical team. The operation performed under localized anesthesia.

These inspired samples were put in sterilized Plain Tubes. Transferred to Parasitology laboratory In Biology Department, College of Science, University of Mosul.

Microscopic detection of The parasite: Stool samples and colon inspirited materials were examined microscopically to detect trophozoite and cyst stages of *E. histolytica* parasite, using Wet mount preparation with normal saline and iodine stain, added to flotation technique (Leventhal and Cheadle, 2012). Compound Light Microscope (Model:C208-THD9) accompanied with digital camera was used to visualized the examined films.

Blood Sampling and Biochemical analysis:

Approximately 3 mL of venous blood was collected from each participant via venipuncture. The collected blood was placed in a gel- tube to obtain blood serum, which then frozen under -20 °C for subsequent biochemical studies:

Biochemical Studies:

I-Hormonal Study:

A-Cortisol concentration: Serum cortisol concentration was measured between 8:00–9:00 AM using a competitive ELISA kit from ELK Biotechnology. The assay utilizes microtiter plates pre-coated with human cortisol, which competes with cortisol in samples for biotin-conjugated antibodies. After adding Avidin-HRP and TMB substrate, the enzymatic reaction is stopped with sulfuric acid to produce a measurable color change. Absorbance is measured spectrophotometrically at 450 nm. Cortisol levels was quantified using a standard curve (Karen, *et al.* 2018).

B-Oxytocin Concentration: Serum oxytosin estimation was estimated using a competitive inhibition ELISA kit (ELK Biotechnology, China) on the same equipment utilized for cortisol estimation.

The process involves adding samples to Human OT-pre-coated microplates along with biotin-conjugated antibodies, followed by Avidin-HRP incubation and TMB substrate reaction stopped with sulfuric acid. Absorbance is read spectrophotometrically at 450 nm (±10 nm) to determine oxytocin levels (pg/mL) against a standard curve (ELK Biotechnology, 2024).

II-Enzyme studies:

A-Liver enzymes

a-ALT activity: An enzymatic kinetic method on the cobas c 303 analyzer was used to measure ALT activity. ALT catalyzes amino group transfer to produce pyruvate, which converts NADH to NAD+. The rate of decrease in absorbance at 340 nm is directly proportional to ALT activity in U/L (Roche Diagnostics, 2020).

b-FASN activity: Serum FASN concentration was measured using a Sandwich ELISA kit from ELK Biotechnology. The enzyme binds to a specific antibody plate and forms a complex with a biotin-conjugated antibody. Absorbance of the resulting yellow color was measured at 450 nm using a microplate reader, and concentration was calculated via standard curve (Wild, 2013).

B-Pancreatic enzymes:

a-Amylase: Serum alpha-amylase activity was measured using an enzymatic colorimetric method with Roche Diagnostics kits on the cobas c 303 analyzer. Alpha-amylase catalyzes the hydrolysis of EPS-G7, which is further broken down by glucosidase to produce 4-nitrophenol. The rate of increase in absorbance at 405 nm is measured to determine the enzyme's activity (Roche Diagnostics, 2020).

b-Lipase: Serum lipase activity was determined using an enzymatic colorimetric method on the cobas c 303 analyzer. The test relies on a series of enzymatic reactions producing hydrogen peroxide, which forms a colored complex with 4-aminophenazone and TOOS. The rate of increase in light absorbance at 546 nm is directly proportional to the lipase activity in the sample (Roche Diagnostics, 2020).

III-Macromolecules estimation:

A-Fasting blood sugar: Concentration of fasting blood glucose was estimated using Hexokinase colorimetric method, the enzymatic reaction were traced via measuring increment in NADH absorption at 340 nm. The absorbance intensity is directly proportional to glucose concentration in the sample under study. Assessing glucose concentration (mg/dl) was performed using Cobas c 303 analytical unit. (Sacks, *et al*., 2023).

B-Triglycerides: Serum triglycerides were estimated using enzymes-calorimetric method (GPO-PAP), which is based on splitting fats into glycerol and free fatty acids, the enzymatic reactions produce hydrogen peroxide and form a pink-colored complex. The intensity of the color is measured spectroscopically at 500-550nm. Intensity of the pink color is directly proportional to the triglyceride concentration in the sample. Assessing triglyceride concentration (mg/dl) was performed using Cobas c 303 analytical unit. ((Sacks, *et al.,* 2023).).

C-Total protein: Total blood proteins concentration (g/dl) were estimated for categorized participants in the study design, using modified Biuret Method (Roche Diagnostics, 2020). The formed cupper-peptide complex has violet color and could be measured spectroscopically at 546 nm via Cobas pure device, module 402 (Roche Diagnostics, Germany).

Statistical analysis: A one-way analysis of variance (ANOVA) was conducted using a completely randomized design (CRD) to analyze the test results and determine the mean and standard deviation. Duncan's multiple range tests were used for all studied variables to determine the differences between the various groups. Accordingly, the differences in the results were considered statistically significant at a probability level of $P \leq 0.05$. "Statistical analysis was conducted using the Statistical Analysis System (SAS) program to calculate the mean and standard deviation (Leo *et al*.; 2017).

RESULTS AND DISCUSSION

Entamoeba histolytica infection alters key host hepatic, pancreatic, and metabolic biomarkers through tissue invasion, systemic inflammation, and parasite-specific metabolic pathways. In the present work we was included 169 males and females participants, their ages ranged between 18-50 years. They distributed according to their relation with entamoebiasis infection to: I-Control group (30male+30 female); II-Clinical amoebiais cases with acute gastroenteritis (16male+14female); III-Chronic gastroenteritis cases but –ve for amoebiais (41male+38 female). The serious amoebiasis cases were proved via clinical manifestation of amebisis infections (amoebic dysentery), added to microscopic finding of *Entamoeba histolytica* cysts and trophozoites in stool and colon inspired materials (Figure.1). Accordingly, biochemical blood tests were performed for each participant in the three categorized groups.

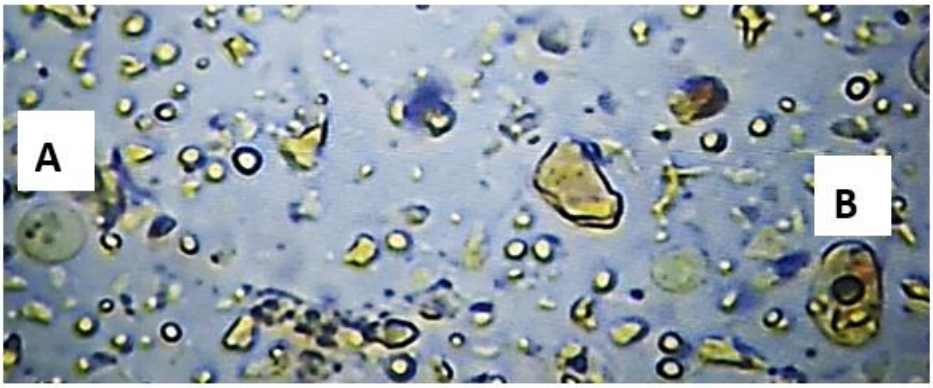

Fig.1: Photomicrograph showing the cyst stage on the left and the trophozoite stage on the right of *E. histolytica* parasite in wet preparation stool sample taken from a patient suffering from amoebic dysentery (400X magnification).

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Biochemical Studies:

I-Hormonal Study:

A-Blood Cortisol level: The study showed a significant increase at P≤0.05 in blood cortisol levels among amoebiasis patients compared to control groups in both sexes in the three age groups (Figure.2 -A&B). Generally, females exhibited higher average cortisol concentrations than males, although this gender variation was not statistically significant. The highest cortisol levels in clinically infected patients were recorded in the 31–40 age groups, with the mean 503.8 µg/dl in males and 527 µg/dl in females. Chronic GIT disturbance patients of the same age group (31–40) also showed significantly elevated cortisol levels (483.2 µg/dl in males and 481.4 µg/dl in females).

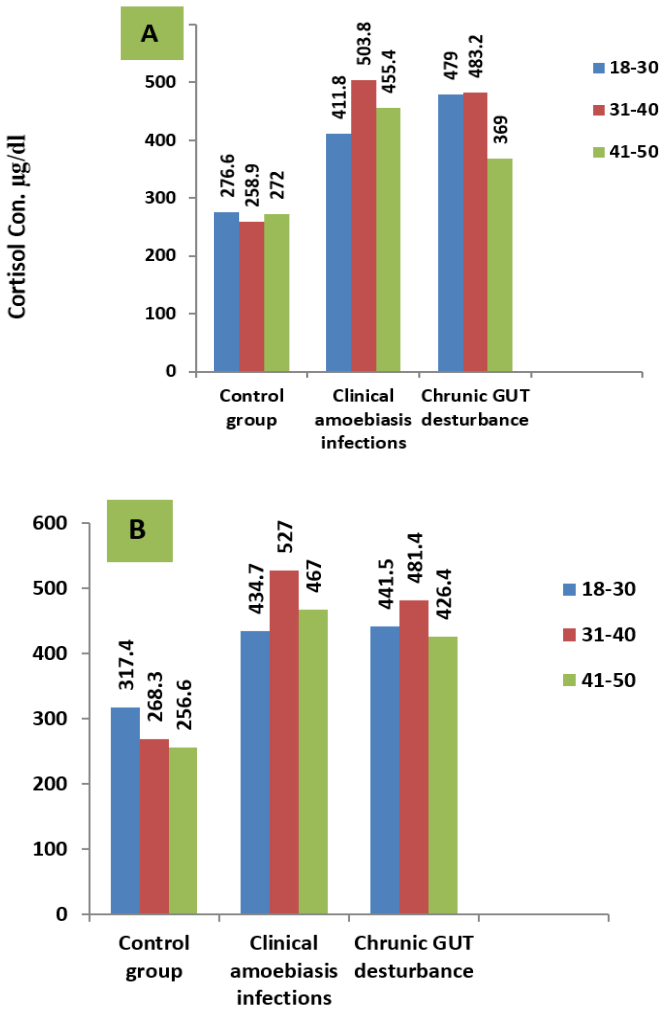

Figure. 2: Variation in cortisol concentrations (µg/dl) in the three aged groups in male (A) and female (B) participants according to their relation to amoebiasis infection. Each value represents mean of (N) participants.

The findings of the present study aligned with those of Al-Nuaimi (2021), which demonstrated that *Entamoeba gingivalis* infection induces a significant increase in salivary cortisol, confirming that parasitic infections extend beyond localized clinical effects to cause systemic hormonal imbalances in the host. In contrast, the results diverged from Fingan *et al.* (2025) regarding intestinal parasites and toxoplasmosis in male patients with chronic liver diseases in Baghdad, where the researcher found no significant correlation between parasitic infection and cortisol levels. The significant rise in cortisol levels indicates the body's physiological response to stress caused by parasitic infections such as *E. histolytica*, with older age groups showing the highest concentrations due to naturally increasing baseline levels with age progression. These findings align with Banu *et al.* (2005), who noted that physical and psychological stress stimulates the adrenal gland, causing immunosuppression that aids infection persistence and intestinal damage. Similarly, Shirbazou *et al.* (2011) observed significantly elevated cortisol levels accompanied

by increased anxiety in toxoplasmosis patients. They confirmed that parasitic infection acts as a strong activator for the hypothalamic-pituitary-adrenal axis, driving cortisol secretion as a defense mechanism toward bio stress of tissue.

B-Oxytocin Concentration: As illustrated in (Figure.3-A&B), clinical amoebiasis infections significantly reduced oxytocin levels at P≤0.05 in both sexes across the three age groups compared to control group. Males generally exhibited slightly higher baseline oxytocin levels than females, particularly in the 41–50 age group. Infected females recorded oxytocin concentrations of 2.9, 2.4, and 2.5 pg./mL in the 18–30, 31–40, and 41–50 age groups, respectively. Chronic GIT disturbance patients caused the further drops in the 41–50 age bracket, reaching 1.6 pg./mL in males and 1.4 pg./mL in females.

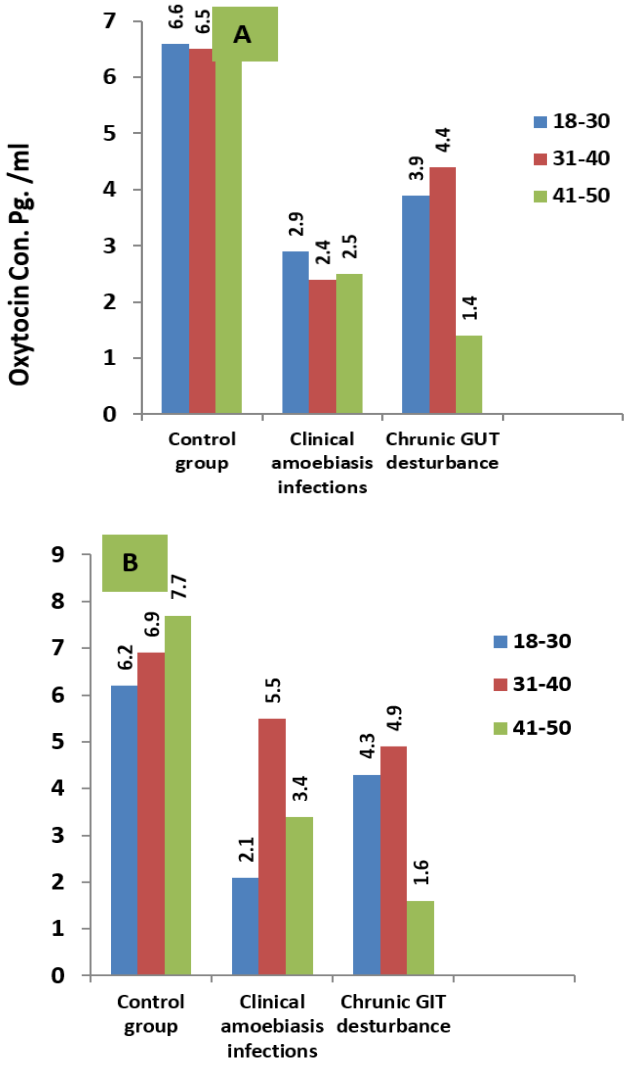

Figure.3 : Variation in oxytocin concentrations (pg/ml) in the three aged groups in both male (A) and female (B) participants according to their relation to amoebiasis infection. Each value represents mean of (N) participants.

Entamoeba histolytica damages the intestinal mucosa, reducing localized oxytocin production by epithelial cells alongside pituitary secretion (Danhof *et al.*, 2023).Infection-induced psychological stress elevates cortisol levels, which directly antagonizes oxytocin and lowers its concentration in the blood (Welch *et al.*, 2014). Reduced oxytocin disrupts gastrointestinal motility, impairs local intestinal resistance against inflammation, and causes nutrient malabsorption (Welch *et al.*, 2014).This drop signals host hormonal imbalance and loss of oxytocin's protective role due to parasite-induced biological stress (Sippel *et al.*, 2017). Beside, parasitic infections alter the host environment and trigger immune responses that directly impact the endocrine system (Romano *et al.*, 2015). Observed oxytocin and cortisol shifts represent a physiological stress response to parasitic invasion (Romano *et al.*, 2015). Host sex and age modulate infection outcomes, making females and older age groups more susceptible to endocrine disruptions (Romano *et al.*, 2015).

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II-Enzyme studies:

A-Liver enzymes activity:

a-ALT: As seen in (Table.2), Clinical E. histolytica infection significantly increased serum ALT levels at P≤0.05 in both sexes and all age groups compared to control group.The highest ALT activities recorded in infected females aged 41–50 (36.2 ± 14.9 U/L) and infected males aged 31–40 (34.8±9.7U/L). Despite significant increases—including Chronic GIT disturbance females aged 41–50 (33.6±12.6 U/L). Worthy to know that all recorded ALT levels remained within clinically normal ranges according to (Timbrell, 2024).

Table.2 Variation in ALT activity U/L in the blood of the three age groups, for both males and females involved in the study, according to their relation with clinical amoebiasis infection:

Age group	Control group (Mean ± SD)		Clinical amoebiasis infections (Mean ± SD)		Chronic GIT disturbance (Mean ± SD)	
	Male	Female	Male	Female	Male	Female
18-30	21.2^{c} ±4.7 N=10	17.1^{cd} ±3.5 N=10	32.8^{ab}±9.3 N=4	33.8^{ab}±8.5 N=4	26.2^{c}±6.8 N=13	24.6^{c}±4.8 N=15
31-40	23.3^{c}±5.1 N=10	17.9^{cd}±3.6 (14-24) N=10	34.8^{ab}±9.7 (21-45) N=5	30.4^{b}±8.1 (17-38) N=4	30.8^{b}±9.2 (27-43) N=13	28.2^{b}±13.7 N=11
41-50	21.6^{c}±4.9 N=10	18.8^{cd}±3.8 N=10	34.4^{ab}±8.4 N=7	36.2^{a}±14.9 N=6	31^{b}±7.2 N=15	33.6^{ab}±12.6 N=15
Total	27	17.9	34	33.5	29.3	22.8

*Each value represent mean of N participants± slandered deviation. Different litters referred to significant different between the values at P≤0.05, according to Duncan's multiple range test.

Normal serum ALT levels observed in clinical amebiasis align with findings from Purnasari *et al.* (2024) and Al-Haboobi (2014). Although within normal limits, mild ALT elevations signal early hepatic cellular stress caused by parasite metabolic wastes entering the portal circulation (Moriles *et al.,* 2024). Slight ALT increases (30–100 U/L) serve as critical biomarkers for early-stage tissue damage (Helsper *et al.,* 2012).Virulent *E. histolytica* strains release cysteine protease-5 and amoebapores that degrade intestinal tight junctions, facilitating hepatic penetration via the portal vein (Medina-Rosales *et al*., 2021).Furthermore, chronic infections accelerates liver free-radical generation, leading to persistent hepatic tissue strain (Desure *et al.,* 2021). On the other hands, amebiasis induces severe systemic oxidative stress, marked by elevated malon aldehyde accumulation and depleted glutathione (GSH) antioxidant defenses (Al-Kakey, 2006).Parasitic stress mechanisms mirror other *Entamoeba* species, such as E. gingivalis, which trigger oxidative damage and deplete host antioxidants (Nuaimi *et al*., 2022).

b-FASN concentration: Clinical amebiasis caused a slight significant increase at P≤0.05 in blood FASN levels, averaging 8.76 ng./mL in females and 6.7 ng/mL in males (Table. 3). The highest FASN concentrations recorded in infected patients aged 18–30, reaching 10.3 ±1.2 ng/ml in females and 7.5 ±0.5 ng/mL in males. Patients with Chronic GIT disturbance those aged 18–30 also showed significant differences, averaging 8.1 ng./mL in females and 6.9 ±0.6 ng/mL in males.

Table. 3: Variation in FASN concentration ng/ml in the blood of the three age groups, for both males and females involved in the study, according to their relation with clinical amoebiasis infection:

Age group	Control group (Mean ± SD)		Clinical amoebiasis infections (Mean ± SD)		Chronic GIT disturbance (Mean ± SD)	
	Male	Female	Male	Female	Male	Female
18-30	4.5^{b}±0.99 N=4	3.0^{b}±0.3 N=4	7.5^{a} ±0.5 N=10	10.3^{a}±1.2 N=10	6.9^{a}±0.6 N=13	8.1^{a}±0.7 N=15
31-40	3.8^{b}±0.7 N=5	2.9^{b}±0.7 N=4	6.7^{a}±0.2 N=10	8.7^{a}±1.14 N=10	5.5^{a}±0.5 N=13	4.4^{b}±0.8 N=11
41-50	3.2^{b}±0.9 N=7	3.8^{b}±1.1 N=6	5.8^{ab}±0.3 N=10	7.3^{a}±0.5 N=10	2.4^{b}±0.9 N=15	3.8^{b}±1.1 N=15
Total	3.8	3.2	6.7	8.76	4.9	5.4

*Each value represent mean of N participants± slandered deviation. Different litters referred to significant different between the values at P≤0.05, according to Duncan's multiple range test.

Fatty acid synthase (FASN) converts excess glucose into saturated fatty acids, typically maintaining low baseline levels in circulation. Chronic GI disturbance and invasive infections destroy mucosal epithelial cells, causing intracellular FASN to leak directly into blood stream. On the other hands, stress-induced cortisol elevation increases glucose levels, prompting hepatic up regulation and release of FASN to manage metabolic stress (Rashid *et al.,* 1997). Furthermore, E. histolytica lacks complete lipid synthesis pathways, relying on host short-chain fatty acids harvested from gut microbiota (Castellanos-Castro, 2020). E. histolytica possesses a structurally distinct parasite-specific FASN used to elongate host-derived fatty acids (Mi-ichi *et al.*, 2024).Circulating FASN elevations reflect cellular breakdown, metabolic stress, and immune-driven tissue remodeling during acute intestinal injury (Softic *et al.*, 2016). In the present study, despite observed increases, serum FASN concentrations in clinical amebiasis patients remained within medically normal limits depending on (Fazli *et al.*, 2021).

B-Pancreatic enzymes activity:

a-Amylase: The present study showed a significant increase (P≤0.05) in serum pancreatic amylase activity in patients with clinical amebiasis and non-parasitic intestinal symptoms compared to healthy controls (Table.4). Among amebiasis patients, the highest activity was observed in females aged 18–30 (139.4±48.7 U/L), followed by males of the same age group (122.4±41.3 U/L). In patients with chronic GIT disturbance, the highest activity was in males aged 31–40 (136±55.9 U/L), while the minimum elevation across both groups occurred in males aged 41–50.

Table.4: Variation in blood Pancreatic amylase activity U/L in the three age groups, for both males and females involved in the study, according to their relation with clinical amoebiasis infection:

Age group	Control group (Mean ± SD)		Clinical amoebiasis infections (Mean ± SD)		Chronic GIT disturbance (Mean ± SD)	
	Male	Female	Male	Female	Male	Female
18-30	80.8^{d} ±25.1 N=10	85^{de}±10.3 N=10	122.4^{b}±41.3 N=4	139.4^{a}±38.7 N=4	121.6^{b}±45.8 N=13	130.2^{a}±67.7 N=15
31-40	79.4^{e}±28.2	82.8^{e}±11.9	119^{b}±50.5	95^{c}±9.1	136^{a}±55.9	130.4^{a}±71.3

	(45-105) N=10	(68-99) N=10	(73-200) N=5	(86-109) N=4	(75-206) N=13	N=11
41-50	73.4^{e}±21.9 N=10	85de±12.8 N=10	94.2^{d}±15.3 N=7	103.4^{c}±35.5 N=6	101^{c}±26.8 N=15	127.8^{ab}±47.9 N=15
Total	77.9	84.3	112.9	112.6	119.5	129.5

*Each value represent mean of N participants± slandered deviation. Different litters referred to significant different between the values at P≤0.05, according to Duncan's multiple range test.

These results align with Khafif (2019), who reported increased intestinal parasite-induced amylase activity and a positive correlation with infection severity. Purnasari *et al.* (2024) noted that amoebic dysentery, particularly with extra-intestinal extension, triggers pancreatic inflammation and acute rises in serum amylase and lipase. This elevation serves as a biochemical marker of tissue damage or functional irritation caused by parasitic toxins or migration. Conversely, Slevani (2025) observed a significant 38% decrease in serum alpha-amylase activity among Giardia-infected patients compared to healthy controls.

b-Lipase: Table.5 shows a significant increase (P≤0.05) in pancreatic lipase activity in male and female infected with clinical amebiasis (averaging 45.1 and 42.3 U/L) and patients with chronic GIT disturbance (averaging 36.9 and 35.5 U/L) compared to control groups. The highest lipase levels recorded in clinical amebiasis patients aged 41–50, reaching 48.6±4.1 U/L in males and 46.4±9.3 U/L in .

Table.5: Variation in blood pancreatic lipase activity U/L in the three age groups, for both males and females involved in the study, according to their relation with clinical amoebiasis infection:

Age group	**Control group (Mean ± SD)**		**Clinical amoebiasis infections (Mean ± SD)**		**Chronic GIT disturbance (Mean ± SD)**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
18-30	22.2^{d} ±2.8 N=10	21.6^{d}±3.6 N=10	41.4^{b}±11.7 N=4	38.4^{b}±9.2 N=4	33.8^{c}±3.3 N=13	30.6^{c}±9.2 N=15
31-40	23^{d}±3.2 N=10	22^{d}±2.6 N=10	45.2^{a}±5.5 N=5	42.2^{b}±4.7 N=4	36.5^{b}±3.6 N=13	37.2^{b}±7.8 N=11
41-50	23.8^{d}±4.4 N=10	25.8^{d}±4.2 N=10	48.6^{a}±4.1 N=7	46.4^{a}±9.3 N=6	40.4^{b}±3.8 N=15	38.8^{b}±13.6 N=15
Total	23	23.1	45.1	42.3	36.9	35.5

*Each value represent mean of N participants± slandered deviation. Different litters referred to significant different between the values at P≤0.05, according to Duncan's multiple range test.

Current findings align with Khafif (2019) and Purnasari *et al.* (2024), those demonstrating that elevated lipase activity during intestinal protozoan infections directly correlated with parasite virulence. Increased lipase could be considered as a biomarker for mucosal injury and malabsorption caused when *E. histolytica*'s destruct tissues and then cause wall ulcerations. Results contrast with Slevany (2025), as *Giardia* induces secretory insufficiency, whereas invasive amebiasis inflicts severe mucosal structural damage. Infection-induced stress alters hormonal balance elevating cortisol and depressing oxytocin further driving host metabolic disturbances and physiological stress (Welch *et al.*, 2014; Sippel *et al.*, 2017).

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III-Macromolecules estimation:

A-Fasting blood sugar concentration: Clinical amoebiasis infection fount to cause a significant increase at P≤0.05 in fasting blood glucose compared to controls and carrier groups (As illustrated in table. 6), With total average of 110.3 mg/dL in males and 108.1 mg/dL in females. Hyperglycemia was most pronounced in the 31–40 and 41–50 age groups, driven by elevated metabolic and biological stress during active infection. Maximum fasting glucose levels recorded in the 41–50 age group, reaching 128 ±11.5mg/dL in infected males and 117 ±15.7mg/dL in infected females.

Table.6: Variation in fasting blood glucose concentration mg/dl in the three age groups, for both males and females involved in the study, according to their relation with clinical amoebiasis infection:

Age group	Control group (Mean ± SD)		Clinical amoebiasis infections (Mean ± SD)		Chronic GIT disturbance (Mean ± SD)	
	Male	Female	Male	Female	Male	Female
18-30	89.8^{c} ±11.6 N=10	84.6^{d}±12.8 N=10	90.7^{c}±12.5 N=4	102.2^{ab}±9.3 N=4	90.4^{c}±7.9 N=13	97.6^{b}±17.1 N=15
31-40	90.8^{c}±10.8 N=10	87^{cd}±11.4 N=10	112^{a}±14.2 N=5	105^{ab}±16.8 N=4	93^{c}±12.9 N=13	97^{b}±14.7 N=11
41-50	87^{c}±9.1 N=10	90^{c}±11.3 N=10	128^{a}±11.5 N=7	117^{a}±15.7 N=6	128^{a}±16.8 N=15	107^{ab}±22.2 N=15
Total	89.2	87.3	110.3	108.1	103.8	100.5

*Each value represent mean of N participants± slandered deviation. Different litters referred to significant different between the values at P≤0.05, according to Duncan's multiple range test.

The current findings align with Yahya *et al.* (2018), those demonstrating that intestinal parasites, specifically *E. histolytica*, caused a significant rise in fasting blood glucose by directly affecting metabolic hormones and adipokines like leptin. Furthermore, elevated blood glucose levels in amoebiasis patients stem from parasite-induced psychological stress, which leads to increased cortisol and decreased oxytocin concentrations.As noted by Young *et al.* (2021), reduced oxytocin impairs glucose tolerance while elevated cortisol drives insulin resistance, collectively inducing hyperglycemia.

B-Triglycerides: Clinical amoebiasis infection found to cause a significant increase at P≤0.05 in serum triglyceride levels across in all age groups and both sexes compared to control groups (Table.7). Maximum triglyceride concentrations reached 172 ±35.4mg/dL in infected males aged 41–50 and 167 ± 40.9 mg/dL in infected females aged 31–40. Patients with chronic GIT disturbance also showed significant triglyceride elevations, peaking in the 41–50 age group at 112 ±27.6mg/dL for males and 117 ±26.6 mg/dL for females.

Table.7: Variation in blood Triglycerides concentration mg/dL in the three age groups, for both males and females involved in the study, according to their relation with clinical amoebiasis infection:

Age group	Control group (Mean ± SD)		Clinical amoebiasis infections (Mean ± SD)		Chronic GIT disturbance (Mean ± SD)	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
18-30	84^{d} ±23.4 N=10	83^{cb}±12.4 N=10	151^{b}±31.8 N=4	158^{ab}±30.1 N=4	97^{c}±17.1 N=13	98 ^{c}±18.8 N=15
31-40	86^{d}±24.1	87^{cd}±11,3	160^{a}±29.7	167^{a}±20.9	94^{c}±9.9	97^{c}±24.9

	N=10	N=10	N=5	N=4	N=13	N=11
41-50	82^{d}±19.8 N=10	93^{cd}±20.6 N=10	172^{a}±25.4 N=7	166^{a}±21.1 N=6	112^{c}±27.6 N=15	113^{c}±26.6 N=15
Total	84	87.7	161	163.7	101	102.7

*Each value represent mean of N participants± slandered deviation. Different litters referred to significant different between the values at P≤0.05, according to Duncan's multiple range test.

Entamoebiasis infection induces severe biological stress, triggering cortisol release and activating peripheral lipolysis in host adipose tissue (Welch *et al*., 2014).Stress-driven lipolysis releases free fatty acids that flood the liver via portal circulation, promoting hepatic triglyceride synthesis (Rashid *et al*., 1997).Infection-induced mucosal inflammation stimulates inflammatory cytokines like TNF-alpha, which suppress lipoprotein lipase (LPL) and impair serum triglyceride clearance (Hoekstra *et al*., 2022).Concurrently, oxytocin depletion removes its regulating effect on lipid metabolism, worsening lipid accumulation and circulating hypertriglyceridemia (Sippel *et al*., 2017).Chronic GIT disorders also elevate serum triglycerides through general psychological and physiological stress-induced lipid mobilization (Young *et al*., 2021). Hypertriglyceridemia serves as an indirect metabolic biomarker reflecting host systemic stress, mucosal tissue injury, and disrupted endocrine homeostasis (Romano *et al*., 2015). Ultimately, parasitic exploitation of host lipids highlights the metabolic burden imposed by invasive trophozoite activity within the intestinal environment (Castellanos-Castro, 2020).

C-Total protein: In table. 8, clinical *amoebiasis* infection seamed to cause a slight reduction in total serum protein levels in all age groups, averaging 6.03 g/dL in males and 5.9 g/dL in females. The lowest protein concentration among females occurred in the 18–30 age group at 5.7±0.3 g/dL. Among infected males, the lowest total protein level was recorded in the 41–50 age group at 5.9±0.2 g/dL.

Table.8: Variation in total blood proteins concentration gm/dL in the three age groups, for both males and females involved in the study, according to their relation with clinical amoebiasis infection:

Age group	**Control group (Mean ± SD)**		**Clinical amoebiasis infections (Mean ± SD)**		**Chronic GIT disturbance (Mean ± SD)**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
18-30	7.1^{ab} ±0.9 N=10	7.6^{a}±0.5 N=10	6.2^{b}±0.1 N=4	5.7^{b}±0.3 N=4	7.1^{ab}±1.1 N=13	7.2^{ab}±0.9 N=15
31-40	7.3^{ab}±0.8 N=10	6.9^{ab}±0.4 N=10	6.0^{b}±0.1 N=5	5.8^{b}±0.2 N=4	7.3^{ab}±0.9 N=13	6.8^{ab}±0.9 N=11
41-50	7.6^{a}±0.7 N=10	7.1^{ab}±0.9 N=10	5.9^{b}±0.2 N=7	6.1^{b}±0.1 N=6	6.9^{ab}±1.1 N=15	6.8^{ab}±0.7 N=15
Total	7.33	7.2	6.03	5.9	7.1	8.13

*Each value represent mean of N participants± slandered deviation. Different litters referred to significant different between the values at P≤0.05, according to Duncan's multiple range test.

Entamoeba histolytica employs metabolic strategies that drain host nutritional resources, contributing directly to decrease total serum protein concentration. Parasite invasion damages intestinal mucosa, allowing metabolic wastes and inflammatory substances to reach the liver via portal circulation (Medina-Rosales *et al*., 2021). Impaired hepatic function reduces the liver's synthesis capacity for major blood proteins like albumin and globulins (Hoekstra *et al*., 2022).Furthermore, psychological stress raises cortisol levels while significantly decreasing oxytocin concentrations in chronic GIT disturbing patients. Elevated

cortisol combined with reduced oxytocin shifts metabolic balance toward increased protein catabolism and altered plasma volume dynamics (Young *et al.,* 2021).Hormonal imbalances suppress hepatic production of anti-inflammatory proteins, lowering overall circulating protein levels. Beside, intestinal mucosal disruption and systemic metabolic stress synergistically drive the observed hypoproteinemia during clinical amebiasis (Medina-Rosales *et al*., 2021).

CONCLUSIONS

In conclusion, clinical *E. histolytica* infection and chronic intestinal disorders trigger significant neuroendocrine dysregulation evidenced by elevated cortisol and reduced oxytocin levels across all age groups due to disease-associated psychological stress. This hormonal imbalance correlates with notable metabolic and enzymatic alterations, including increased pancreatic amylase and lipase activities, slight elevations in FASN and ALT levels indicative of subtle hepatic and pancreatic involvement, and marked hyperglycemia alongside hypertriglyceridemia, particularly in older age groups (31–50 years). Additionally, a mild reduction in total serum protein highlights the broader systemic impact of amoebiasis on host nutrition and metabolic homeostasis. Collectively, these findings demonstrate that intestinal amoebiasis exerts profound direct and indirect pathophysiological effects on host endocrine, enzymatic, and metabolic profiles.

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