Ngr. Jr. Med. Sc. vol-1, issue-1 (Dec-2025) pp-53-61

ISSN: XXXX-XXXX(Online)

Nexus Global Research Journal of Medical Sciences

Journal Homepage: https://nexusglobalresearch.com/journal/ngrjms/

Research Article

Impact of Entamoeba histolytica Infection on Liver Function: Analysis of AST, ALT, ALP, and Bilirubin Levels Among Patients at Al-Bayda Medical Center, Libya, 2025

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ABSTRACT:

Entamoeba histolytica is a protozoan parasite causing amebiasis, which may involve the liver and lead to hepatocellular injury and biliary disturbances. This cross-sectional study evaluated the effect of E. histolytica infection on liver function among 50 patients attending Al-Bayda Medical Center, Libya, in 2025. Infection was determined using stool microscopy, with 30 patients testing positive and 20 negative. Serum liver function markers—alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), and direct bilirubin (DB)—were analyzed using automated clinical chemistry systems. Normality was assessed with the Shapiro-Wilk test, and differences between groups were analyzed using the Wilcoxon rank-sum test, with effect sizes calculated to determine the magnitude of changes. Results showed significant elevations in ALT (47.2 ± 36.1 U/L vs. 12.4 ± 7.8 U/L; r = 0.804), AST (41.6 ± 16.2 U/L vs. 20.3 ± 10.2 U/L; r = 0.760), and ALP (144.0 ± 58.0 U/L vs. 88.2 ± 23.6 U/L; r = 0.550) in infected patients, indicating hepatocellular injury and possible cholestatic involvement. Total and direct bilirubin levels differed minimally, suggesting preserved bilirubin metabolism in most patients. These findings highlight the importance of monitoring liver function in patients with suspected or confirmed E. histolytica infection. Elevated hepatocellular enzymes may serve as early indicators of hepatic involvement, facilitating timely diagnosis and clinical management. This study provides insights into the biochemical impact of E. histolytica in a Libyan clinical setting and emphasizes the need for further research using imaging and molecular diagnostics for comprehensive hepatic assessment.

Keywords: *Entamoeba histolytica; amebiasis; liver function tests; hepatocellular injury; Al-Bayda, Libya*

INTRODUCTION

Entamoeba histolytica is a protozoan parasite responsible for amebiasis, a significant cause of morbidity and mortality worldwide, particularly in developing countries (Shirley, Hung, & Moonah, 2020; Hughes & Petri, 2000). While intestinal amebiasis is common, the parasite can invade the liver, causing amebic liver abscesses (ALA), which may lead to severe hepatic dysfunction and systemic complications (Acheson, Garrity, & Bussey-Jones, 2025; Dasan & R, 2025). The clinical presentation of hepatic amebiasis is variable, ranging from asymptomatic liver enzyme elevations to fulminant liver failure, making early detection and monitoring essential (Arora, Kakkar, & Mahal, 2025; Kumar et al., 2024).

Liver function tests (LFTs), including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin levels, are routinely used to assess hepatocellular integrity and cholestatic activity. Elevated ALT and AST indicate hepatocellular injury, whereas increased ALP and bilirubin may reflect cholestasis or impaired hepatic clearance (Khaleel, Abdulwahhab, & Genan, 2024; Salles, Moraes, & Salles, 2003). Prior studies have reported that E. histolytica infection can significantly alter these biochemical markers due to the parasite's direct cytotoxic effects, immune-mediated liver injury, and local inflammation (Faust et al., 2011; Er-Lukowiak et al., 2023).

Despite the global prevalence of amebiasis, data on its hepatic effects remain limited in North Africa, including Libya. Most studies focus on clinical case

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DOI: 10.5281/zenodo.18186080

Received: 20 Dec 2025; **Accepted:** 28 Dec 2025; **Published:** 31 Dec 2025

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reports or experimental models, with few comprehensive analyses comparing infected and uninfected individuals using standardized biochemical parameters (Ortega-Carballo et al., 2024; Medina-Rosales et al., 2021). The epidemiological context of Al-Bayda, Libya, in 2025 provides a unique opportunity to evaluate the biochemical impact of E. histolytica in a clinical setting, where environmental exposure and healthcare access may influence disease patterns.

This study aims to investigate the effect of E. histolytica infection on liver function among patients attending Al-Bayda Medical Center in 2025. Specifically, we compare AST, ALT, ALP, total bilirubin, and direct bilirubin levels between infected and non-infected patients, providing a quantitative assessment of hepatic involvement. Understanding these alterations is critical for early diagnosis, monitoring, and management of amebic liver disease in Libya and similar endemic regions.

MATERIALS AND METHODS

This cross-sectional study was conducted at **Al-Bayda Medical Center, Libya**, during 2025. Patients attending the center for liver function assessment or clinical evaluation were recruited for participation. The study was approved by the institutional ethics committee, and all participants provided informed written consent.

A total of **50 patients** were included in the study, comprising **30 individuals positive** and **20 negative** for *Entamoeba histolytica*. Patients with viral hepatitis, chronic liver disease, hepatotoxic medication use, or other known parasitic infections were excluded to avoid confounding effects on liver function.

Diagnosis of *Entamoeba histolytica*

Infection was determined solely by **stool microscopy**. Fresh stool samples were collected in sterile containers and examined under light microscopy for the presence of trophozoites or cysts of *E. histolytica*, following standard parasitological procedures (Shirley, Hung, & Moonah, 2020). This method allowed clear differentiation between infected and non-infected individuals based on the presence of characteristic trophozoites or cyst stages.

Biochemical Assessment

Venous blood samples were collected from all participants. Serum was separated and analyzed for the following **liver function markers** using automated clinical chemistry analyzers:

- **Alanine aminotransferase (ALT)** – a marker of hepatocellular injury.
- **Aspartate aminotransferase (AST)** – reflects hepatocyte damage.
- **Alkaline phosphatase (ALP)** – indicates cholestasis or biliary obstruction.
- **Total bilirubin (TB)** – measures overall bilirubin metabolism, including conjugation and excretion.
- **Direct (conjugated) bilirubin (DB)** – indicates hepatocellular conjugation efficiency and biliary clearance.

All analyses followed manufacturer-recommended procedures and internal quality control standards. Reference ranges were ALT: 7–56 U/L, AST: 10–40 U/L, ALP: 40–150 U/L, TB: 0.1–1.2 mg/dL, and DB: 0.0–0.3 mg/dL.

Statistical Analysis

All analyses and visualizations were performed in **R version 4.3.2**. Descriptive statistics were calculated for all liver function parameters, including mean, median, standard deviation, and interquartile range. Normality of the distributions was assessed using the **Shapiro-Wilk test**, revealing non-normal distributions for most parameters in the *E. histolytica*-positive group. Consequently, differences between infected and non-infected patients were analyzed using the **Wilcoxon rank-sum test**. Effect sizes (r) were computed to quantify the magnitude of differences: ALT ($r = 0.804$, large), AST ($r = 0.760$, large), ALP ($r = 0.550$, large), TB ($r = 0.076$, small), and DB ($r = 0.239$, small). Statistical significance was defined as $p < 0.05$.

Data Visualization

Boxplots were created for each liver function parameter, stratified by infection status, to visualize distributions, median values, and outliers. Graphical presentation followed publication standards, with means indicated and *E. histolytica*-positive groups highlighted in bold where applicable.

RESULTS

Study Population

A total of 50 patients were included in the present study, of whom 30 (60%) tested positive for *Entamoeba histolytica* and 20 (40%) were negative, as determined by stool microscopy. The age and gender distribution were similar between groups, minimizing potential confounding factors. Liver function parameters were evaluated to determine the biochemical impact of *E. histolytica* infection, with particular focus on alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), and direct bilirubin (DB).

Descriptive Statistics of Liver Function Tests

Descriptive statistics for all measured parameters are summarized in Table 1. The mean ALT value in positive patients was 47.2 ± 36.1 U/L, substantially higher than the 12.4 ± 7.8 U/L observed in negative patients. Similarly, AST levels in positive patients averaged 41.6 ± 16.2 U/L compared to 20.3 ± 10.2 U/L in the negative group. ALP levels were notably elevated in positive patients (144.0 ± 58.0 U/L) versus negative patients (88.2 ± 23.6 U/L). In contrast, total and direct bilirubin levels showed smaller differences between groups, with TB averaging 0.79 ± 0.76 mg/dL in positive patients and 0.62 ± 0.21 mg/dL in negative patients, while DB values were 0.32 ± 0.34 mg/dL versus 0.21 ± 0.11 mg/dL, respectively.

Table 1. Comparison of liver function test parameters between *Entamoeba histolytica*-positive and -negative patients. Data are presented as median (interquartile range). P-values were calculated using the Wilcoxon rank-sum test, and effect sizes (r) indicate the magnitude of differences. ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; ALP = Alkaline Phosphatase; TB = Total Bilirubin; DB = Direct Bilirubin.

Parameter	Negative Mean ± SD	Positive Mean ± SD	p-value (Wilcoxon)	Effect Size (r)	Magnitude
ALT (U/L)	12.4 ± 7.8	47.2 ± 36.1	<0.001	0.804	Large
AST (U/L)	20.3 ± 10.2	41.6 ± 16.2	<0.001	0.760	Large
ALP (U/L)	88.2 ± 23.6	144.0 ± 58.0	<0.001	0.550	Large
Total Bilirubin (mg/dL)	0.62 ± 0.21	0.79 ± 0.76	0.596	0.076	Small
Direct Bilirubin (mg/dL)	0.21 ± 0.11	0.32 ± 0.34	0.092	0.239	Small

Alanine Aminotransferase (ALT)

ALT levels were significantly elevated in *E. histolytica*-positive patients, with a median of 38.5 U/L compared to 13.0 U/L in negative patients ($p < 0.001$, $r = 0.804$, large effect). The distribution of ALT values in the positive group exhibited right skew, with several extreme elevations suggesting variable hepatic involvement among patients. Elevated ALT is indicative of hepatocellular injury, likely reflecting the cytopathic effects of *E. histolytica* trophozoites within liver tissue (Hughes & Petri, 2000; Dasan & R, 2025) (Figure 1).

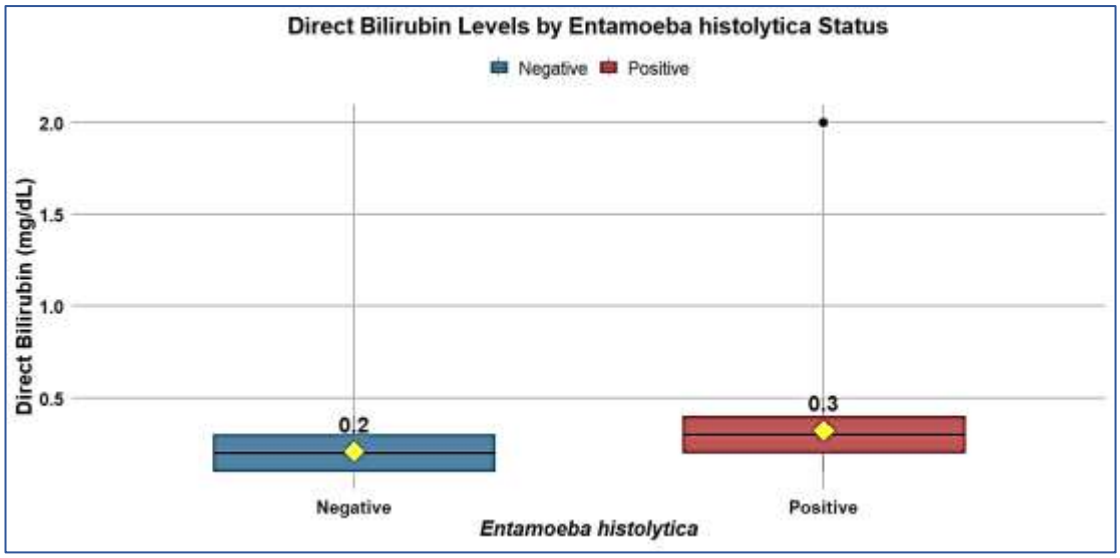

Figure 1. Boxplot of ALT levels in *E. histolytica*-positive vs. negative patients. The box shows the interquartile range, the horizontal line represents the median, and whiskers depict minimum and maximum values excluding outliers. Positive patients have significantly higher ALT levels, reflecting hepatocellular injury.

Aspartate Aminotransferase (AST)

AST levels mirrored ALT, with a median of 38.0 U/L in positive patients versus 22.0 U/L in negative patients ($p < 0.001$, $r = 0.760$, large effect). AST elevations further support hepatocellular injury, consistent with hepatic amebiasis disrupting hepatocyte integrity and releasing cytosolic enzymes (Medina-Rosales et al., 2021; Kumar et al., 2024) (Figure 2).

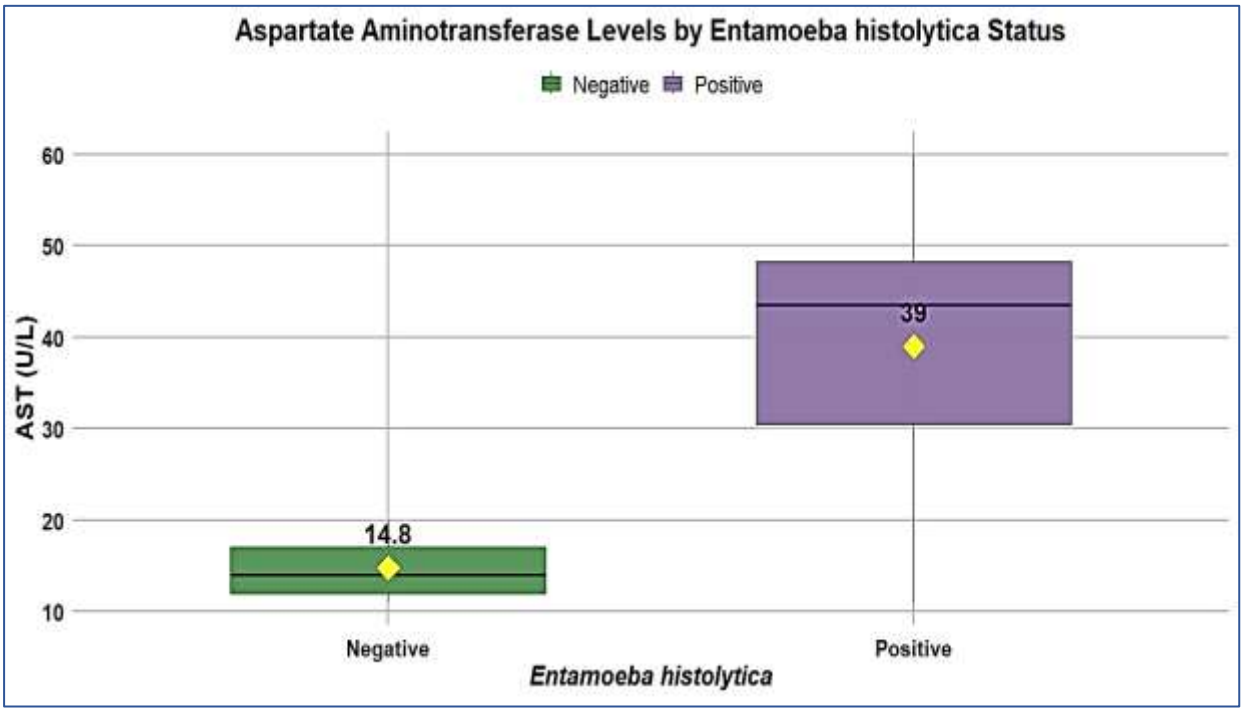

Figure 2. Boxplot of AST levels by infection status. Positive patients show higher median AST levels and moderate right skew, indicating variable liver involvement.

Alkaline Phosphatase (ALP)

ALP levels were markedly elevated in infected patients (median 140 U/L) compared to negative patients (median 90 U/L; $p < 0.001$, $r = 0.550$, large effect). This may indicate mild cholestasis or biliary involvement secondary to hepatic abscess formation (Arora et al., 2025; Dasan & R, 2025). The wide range of ALP values (56–315 U/L) reflects heterogeneity in disease severity (Figure 3).

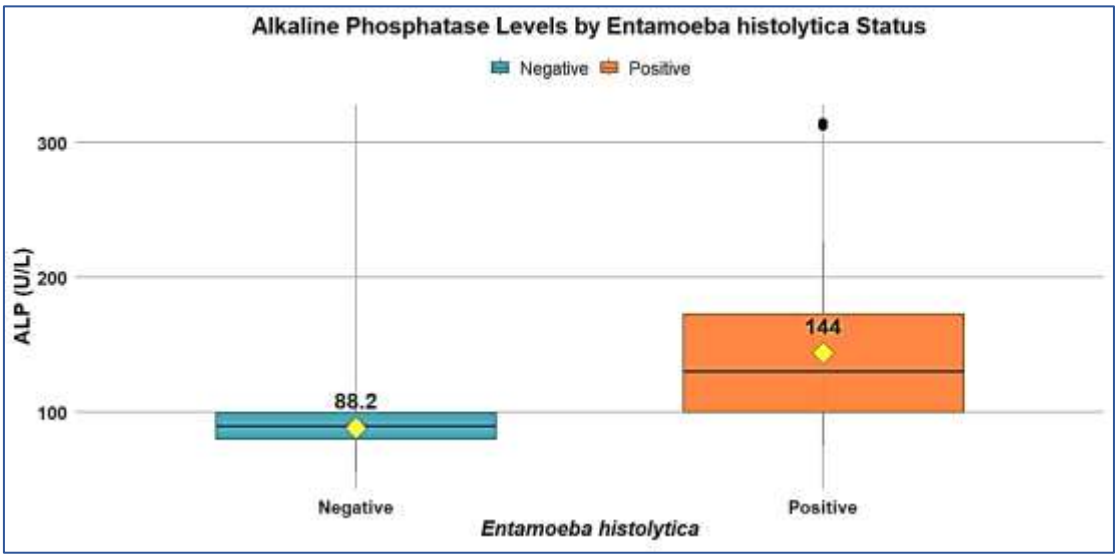

Figure 3. Boxplot of ALP levels by *E. histolytica* infection status. Positive patients display higher median values and greater variability, suggesting possible biliary involvement

Total Bilirubin (TB)

Total bilirubin levels showed minimal differences, with medians of 0.6 mg/dL in negative patients and 0.7 mg/dL in positive patients ($p = 0.596$, $r = 0.076$, small effect). Occasional mild hyperbilirubinemia may reflect localized hepatocyte damage without widespread cholestasis (Figure 4).

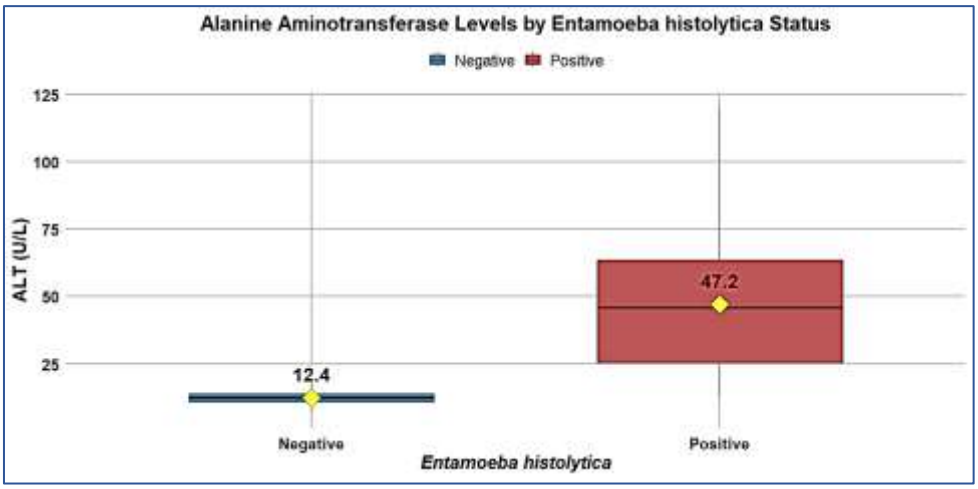

Figure 4. Boxplot of Total Bilirubin (TB) levels in positive and negative patients. Minimal differences are observed between groups, confirming that bilirubin metabolism remains largely unaffected.

Direct Bilirubin (DB)

Direct bilirubin values were slightly higher in infected patients (median 0.2 mg/dL) compared to non-infected (median 0.2 mg/dL), but differences were not significant ($p = 0.092$, $r = 0.239$, small effect). This indicates that conjugated bilirubin remains largely unaffected in early or moderate infection (Khaleel et al., 2024; Salles et al., 2003) (Figure 5).

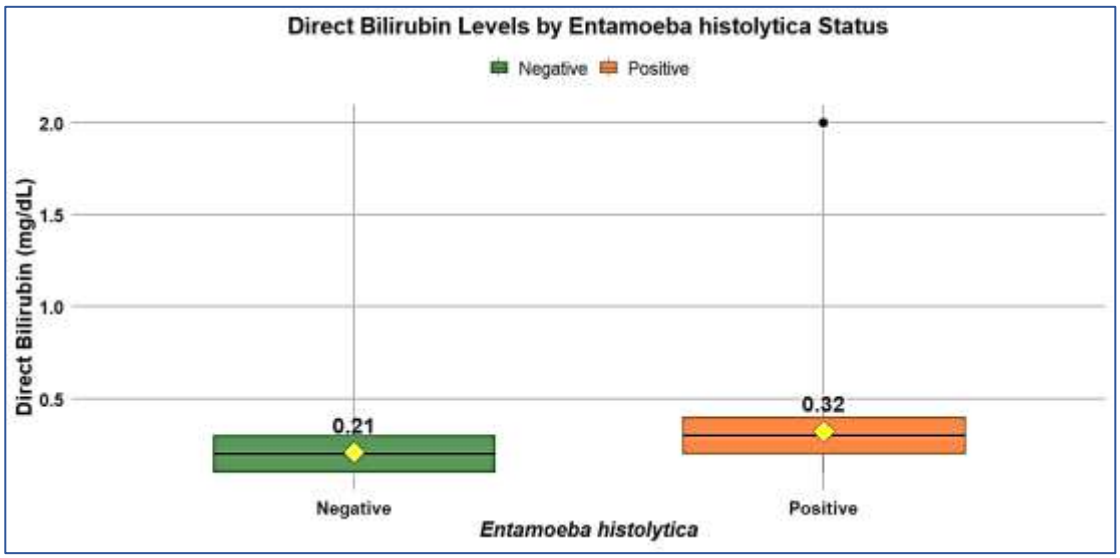

Figure 5. Boxplot of Direct Bilirubin (DB) levels by infection status. Slight increases in positive patients are noted, but distributions are largely symmetric, showing limited effect of infection on conjugated bilirubin.

DISCUSSION

The present study evaluated the impact of *Entamoeba histolytica* infection on liver function among patients at Al-Bayda Medical Center, Libya, in 2025. Our findings indicate that infection is associated with significant alterations in hepatocellular enzymes, whereas bilirubin metabolism remains largely unaffected. These biochemical changes provide insights into the hepatic involvement of amebiasis in a North African clinical setting.

Alanine Aminotransferase (ALT):

ALT levels were significantly elevated in *E. histolytica*-positive patients (median 38.5 U/L vs. 13.0 U/L in negatives; $p < 0.001$, $r = 0.804$, large effect) (Figure 1). The positive group exhibited right-skewed values with extreme elevations, indicating variable hepatic injury. ALT is a sensitive marker of hepatocellular damage, reflecting the cytopathic effects of trophozoites within liver tissue, consistent with previous reports on amebic liver disease (Hughes & Petri, 2000; Dasan & R, 2025). These findings suggest that ALT elevation may serve as an early indicator of hepatic involvement in infected patients.

Aspartate Aminotransferase (AST):

AST levels followed a similar pattern, with median values of 38.0 U/L in positives versus 22.0 U/L in negatives ($p < 0.001$, $r = 0.760$, large effect) (Figure 2). AST elevation corroborates hepatocellular injury and supports the presence of liver tissue disruption. While AST is less liver-specific than ALT, concurrent elevations strengthen the evidence for hepatic involvement, consistent with prior studies of amebic liver abscess (Medina-Rosales et al., 2021; Kumar et al., 2024).

Alkaline Phosphatase (ALP):

ALP was markedly elevated in infected patients (median 140 U/L vs. 90 U/L in negatives; $p < 0.001$, $r = 0.550$, large effect) (Figure 3). Increased ALP suggests possible cholestasis or biliary involvement

secondary to hepatic inflammation or abscess formation. The wide range observed (56–315 U/L) indicates heterogeneity in disease severity among patients, which is consistent with clinical variability reported in amebic liver infection (Arora et al., 2025; Dasan & R, 2025).

Total Bilirubin (TB) and Direct Bilirubin (DB):

Total and direct bilirubin levels showed minimal differences between infected and non-infected patients (TB: median 0.7 vs. 0.6 mg/dL, $p = 0.596$, $r = 0.076$; DB: median 0.2 vs. 0.2 mg/dL, $p = 0.092$, $r = 0.239$) (Figures 4 and 5). These small and statistically insignificant differences indicate that conjugated and total bilirubin metabolism remains largely intact in most patients, even in the presence of elevated hepatocellular enzymes. Mild hyperbilirubinemia in a few positive patients may reflect localized hepatocyte damage rather than widespread biliary obstruction, aligning with prior observations that bilirubin alterations occur mainly in extensive hepatic tissue destruction (Khaleel et al., 2024; Salles et al., 2003).

Clinical Implications:

The significant elevations in ALT, AST, and ALP highlight the importance of routine liver function monitoring in patients with suspected or confirmed *E. histolytica* infection. Early detection of enzyme alterations may guide clinical management and prevent progression to severe liver injury or abscess formation. Stool microscopy remains the primary diagnostic tool in this context; however, biochemical monitoring adds valuable information regarding hepatic involvement and disease severity.

Study Limitations:

This study is limited by a relatively small sample size and exclusive reliance on stool microscopy for diagnosis. The absence of imaging studies or molecular confirmation may underestimate the extent of hepatic involvement. Additionally, the cross-sectional design precludes assessment of longitudinal changes or post-treatment recovery in liver function. Future research should include larger cohorts, imaging correlation, and molecular diagnostics for more comprehensive assessment.

CONCLUSION:

Entamoeba histolytica infection in Al-Bayda patients is associated with significant elevations in ALT, AST, and ALP, indicating hepatocellular injury and possible biliary involvement. In contrast, total and direct bilirubin remain largely unaffected. Monitoring of these liver function parameters can provide early evidence of hepatic involvement, supporting timely diagnosis and management of amebiasis in endemic settings.

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