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Biochemical Alterations in Liver Function Parameters among Entamoeba Histolytica–Positive Patients at Al-Bayda Medical Center, Libya

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ABSTRACT

Entamoeba histolytica infection remains a major public health problem in endemic regions and may lead to extraintestinal complications, particularly hepatic involvement. While amoebic liver abscess is well recognized, biochemical alterations in liver function among *E. histolytica*–positive patients without clinically evident hepatic disease remain insufficiently characterized. This study aimed to evaluate liver function parameters and their interrelationships among patients diagnosed with *Entamoeba histolytica* at Al-Bayda Medical Center, Libya. A laboratory-based cross-sectional study was conducted in 2025 and included 30 patients with confirmed *E. histolytica* infection. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and direct (conjugated) bilirubin were measured using routine clinical chemistry methods. Data distribution was assessed using the Shapiro–Wilk test. One-sample *t*-tests or Wilcoxon signed-rank tests were applied to compare observed values with reference limits, and Spearman's rank correlation analysis was used to assess associations among biochemical parameters. Elevated aminotransferases were common, particularly AST (56.7%) and ALT (40.0%), indicating frequent hepatocellular stress. In contrast, elevations in ALP (36.7%) and direct bilirubin (30.0%) were less frequent, while total bilirubin elevation was rare (6.7%). Strong positive correlations were observed among ALT, AST, and ALP, whereas total and direct bilirubin were strongly correlated with each other but showed weak associations with liver enzymes. In conclusion, *Entamoeba histolytica* infection was associated predominantly with hepatocellular enzyme alterations, with minimal disruption of bilirubin metabolism. Routine liver function testing may aid in the early detection of hepatic involvement in amoebiasis, even in the absence of overt liver disease.

Introduction

Entamoeba histolytica is a protozoan parasite that causes amoebiasis, a globally distributed infection that remains an important public health concern, particularly in low- and middle-income countries where sanitation and hygiene conditions are suboptimal [1]. While most infections are asymptomatic or limited to the intestinal tract, a proportion of cases progress to extraintestinal disease, most notably amoebic liver abscess, which represents the most severe clinical manifestation and a major cause of morbidity and mortality associated with *E. histolytica* infection [2,3].

Hepatic involvement results from hematogenous dissemination of trophozoites from the colon to the liver via the portal circulation, where parasite-induced cytotoxicity, inflammatory mediators, and host immune responses collectively contribute to hepatocellular injury and tissue destruction [4,5]. Several clinical and experimental studies have reported alterations in liver function parameters during amoebic liver infection; however, the nature and magnitude of these biochemical disturbances may vary according to disease severity, duration of infection, host immune status, and underlying hepatic condition [6,7].

Understanding liver function alterations in patients with *E. histolytica* infection is clinically relevant, as such changes may support diagnosis, guide treatment decisions, and assist in differentiating amoebic liver involvement from other hepatic disorders with overlapping clinical presentations. Despite the recognized burden of amoebiasis in many regions, there is limited local evidence describing biochemical liver abnormalities among affected patients in North Africa, including Libya. This study was therefore conducted to assess alterations in liver function parameters among *E. histolytica*–positive patients attending Al-Bayda Medical Center, to contribute context-specific data to the existing body of knowledge.

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Methods

This analytical cross-sectional study was conducted at Al-Bayda Medical Center, Libya, and included patients who underwent laboratory testing for *Entamoeba histolytica* during the study period. Only cases confirmed as *E. histolytica*-positive by stool microscopy and/or antigen detection were included in the analysis, whereas patients with incomplete records or coexisting hepatic disorders were excluded in order to minimize potential confounding effects on liver function parameters.

Demographic and clinical information, including age, sex, and laboratory results, was obtained from patient records using a standardized data extraction sheet. Liver function parameters evaluated in this study included serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, and direct bilirubin, as routinely measured in the hospital laboratory. All biochemical analyses were performed using automated analyzers following standard operating procedures and manufacturer-recommended quality-control protocols [8].

Data were entered into a cleaned database and analyzed using appropriate statistical software. Continuous variables were summarized as means and standard deviations, whereas categorical variables were presented as frequencies and percentages. Comparisons between *E. histolytica*-positive patients and reference values were performed using the one-sample t-test for normally distributed variables, or the non-parametric Wilcoxon signed-rank test when normality assumptions were not met. Associations were considered not statistically significant when $p \geq 0.05$, in accordance with accepted epidemiological reporting practice [9]. The study followed confidentiality standards, and all patient identifiers were removed prior to analysis.

Results

Study Population

A total of 30 patients with confirmed *Entamoeba histolytica* infection were included in the analysis. All participants tested positive for the parasite, and complete liver function profiles were available for every case included in the dataset.

Descriptive Statistics of Liver Function Parameters

Descriptive statistics indicated noticeable variation in liver biochemical markers among *E. histolytica*–positive patients (Table 1). Total bilirubin values ranged from 0.2 to 4.5 mg/dL, with a mean of 0.79 ± 0.76 mg/dL and a median of 0.65 mg/dL, whereas direct bilirubin values exhibited a similar right-skewed pattern, with a mean of 0.32 ± 0.34 mg/dL and a maximum level of 2.0 mg/dL.

Alkaline phosphatase (ALP) concentrations ranged between 75 and 315 U/L, with a mean of 143.97 ± 61.88 U/L and a median of 130 U/L, reflecting marked inter-individual variability. Alanine aminotransferase (ALT) values ranged from 13 to 120 U/L (mean 47.17 ± 24.39 U/L), whereas aspartate aminotransferase (AST) values ranged from 11 to 60 U/L (mean 39.00 ± 12.90 U/L). Skewness analysis demonstrated strong positive skewness for total and direct bilirubin, while ALT and AST values showed distributions approximating normality.

Table 1. Descriptive statistics of liver function parameters among E. histolytica–positive patients (n = 30)

Parameter	Mean ± SD	Median	Min–Max	Skewness
Total Bilirubin (mg/dL)	0.79 ± 0.76	0.65	0.2–4.5	3.94
Direct Bilirubin (mg/dL)	0.32 ± 0.34	0.30	0.1–2.0	3.97
Alkaline Phosphatase (U/L)	143.97 ± 61.88	130.0	75–315	1.28
Alanine Aminotransferase (U/L)	47.17 ± 24.39	46.0	13–120	0.71
Aspartate Aminotransferase (U/L)	39.00 ± 12.90	43.5	11–60	−0.53

Normality Testing and Choice of Statistical Tests

The Shapiro–Wilk test indicated that total bilirubin, direct bilirubin, and ALP significantly deviated from normal distribution ($p < 0.001$ for all), and these variables were therefore analyzed using the Wilcoxon signed-rank test. In contrast, ALT and AST did not show statistically significant deviation from normality (ALT: $p = 0.057$; AST: $p = 0.059$), supporting the use of the one-sample t-test for these parameters.

One-Sample Comparisons with Reference Values

Total bilirubin values were compared with a clinical reference value of 1.2 mg/dL. The Wilcoxon signed-rank test showed a statistically significant difference ($V = 41.5$, $p < 0.001$), indicating that total bilirubin levels were lower than the reference threshold. Direct bilirubin values were compared with a reference value of 0.3 mg/dL and demonstrated no statistically significant difference ($V = 135$, $p = 0.939$). Likewise, ALP values

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did not differ significantly from the reference value of 147 U/L (V = 180.5, p = 0.289). For ALT, comparison with a reference value of 56 U/L yielded a result that was not statistically significant (t = −1.98, df = 29, p = 0.057), with a 95% confidence interval of 38.06–56.27 U/L that included the reference level. AST values were also not statistically significant compared with the reference threshold of 40 U/L (t = −0.42, df = 29, p = 0.674), and the 95% confidence interval (34.18–43.82 U/L) likewise encompassed the reference value.

Categorization of Liver Function Results (Normal vs. Elevated)

Liver function test results among *Entamoeba histolytica*–positive patients were categorized as normal or elevated according to established adult reference ranges to support clinically meaningful interpretation of hepatic involvement. The categorical distribution of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and direct (conjugated) bilirubin is presented in (Figures 1–5), with graphical bars displaying absolute frequencies together with their corresponding percentages.

Elevation of aspartate aminotransferase (AST) represented the most frequently observed biochemical abnormality (Figure 1). More than half of the patients (56.7%, n = 17) exhibited AST values above the upper reference limit, whereas 43.3% (n = 13) maintained levels within the normal range. This predominance of AST elevation is indicative of widespread hepatocellular stress among the studied patients.

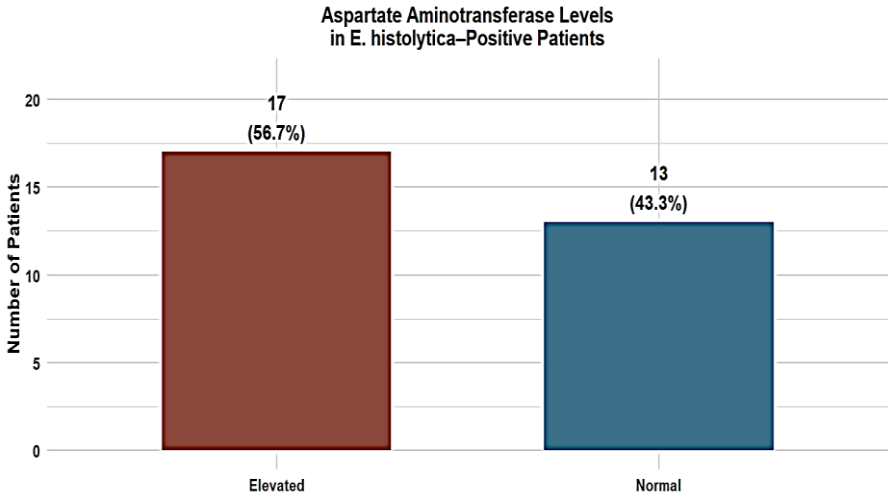

Figure 1. Bar graph showing the frequency distribution of aspartate aminotransferase (AST) levels (normal vs. elevated) in patients positive for Entamoeba histolytica.

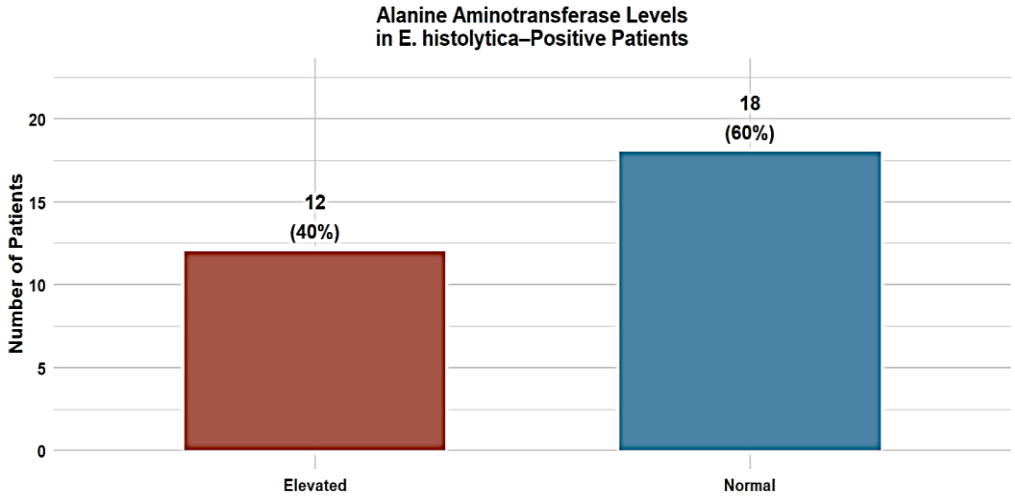

Figure 2. Bar graph illustrating the distribution of alanine aminotransferase (ALT) levels categorized as normal or elevated among Entamoeba histolytica–positive patients. Bars represent frequencies, with percentages displayed beneath the counts.

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Alanine aminotransferase (ALT) abnormalities were likewise common (Figure 2). Elevated ALT values were recorded in 40.0% (n = 12) of patients, whereas 60.0% (n = 18) showed normal concentrations. The concurrent elevation of both ALT and AST in a substantial proportion of individuals supports a biochemical pattern consistent with hepatocellular injury rather than incidental or isolated fluctuations in enzyme activity.

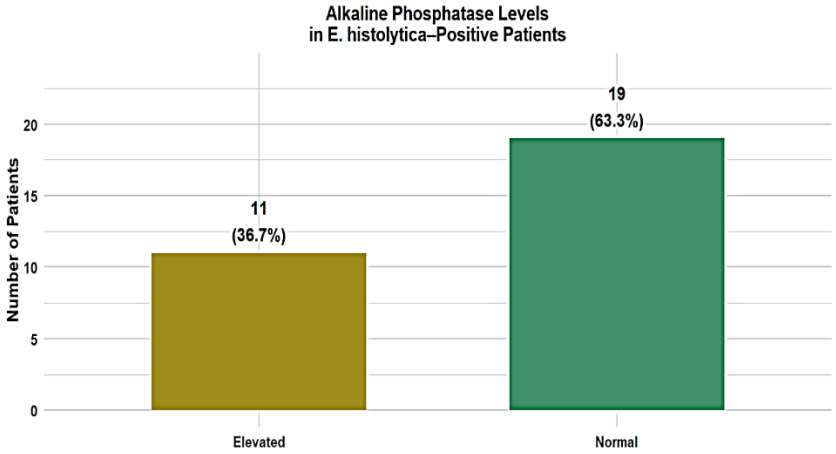

Figure 3. Bar graph depicting alkaline phosphatase (ALP) levels classified as normal or elevated among E. histolytica–positive patients, highlighting the proportion with possible cholestatic involvement.

In contrast, abnormalities in alkaline phosphatase (ALP) were less frequent (Figure 3). Elevated ALP levels were observed in 36.7% (n = 11) of patients, whereas the majority (63.3%, n = 19) remained within the normal reference interval. This distribution suggests that cholestatic or biliary involvement occurred in a subset of cases but did not constitute the predominant biochemical feature associated with E. histolytica infection in this cohort.

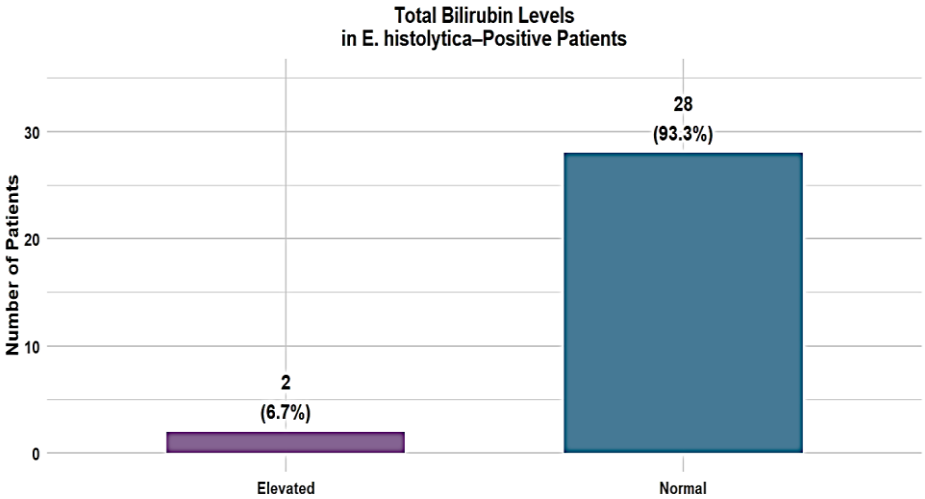

Figure 4. Bar graph representing total bilirubin levels (normal vs. elevated) in Entamoeba histolytica–positive patients, demonstrating the low prevalence of hyperbilirubinemia.

Bilirubin parameters demonstrated relatively limited disturbance. As illustrated in (Figure 4), total bilirubin levels were largely preserved, with elevation identified in only 6.7% (n = 2) of patients, whereas 93.3% (n = 28) remained within the normal range.

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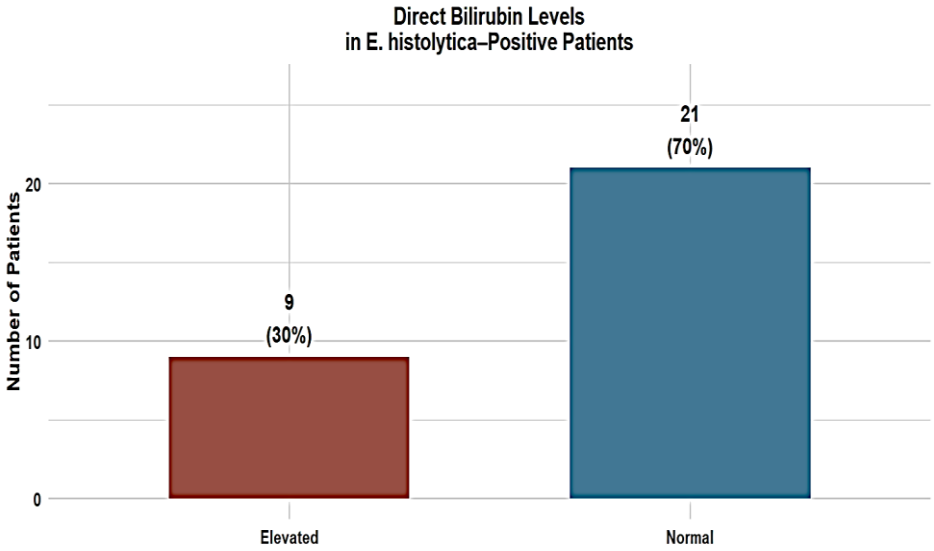

Figure 5. Bar graph illustrating the distribution of direct (conjugated) bilirubin levels categorized as normal or elevated among Entamoeba histolytica–positive patients.

Direct (conjugated) bilirubin levels were elevated in 30.0% (n = 9) of patients, while the majority (70.0%, n = 21) demonstrated normal values (Figure 5). These findings indicate that clinically relevant hyperbilirubinemia was uncommon despite the presence of enzyme abnormalities. Overall, the categorical analysis indicates that *E. histolytica* infection in this study population was primarily associated with elevations in aminotransferases—particularly AST—whereas bilirubin parameters were largely preserved. This biochemical profile is consistent with a predominantly hepatocellular pattern of hepatic involvement rather than a cholestatic process.

Correlation Analysis of Liver Function Parameters

Spearman's rank correlation analysis was performed to assess the interrelationships among liver function parameters in patients positive for *Entamoeba histolytica*, as several variables demonstrated non-normal distribution. The correlation matrix and corresponding significance levels are illustrated graphically in (Figure 6).

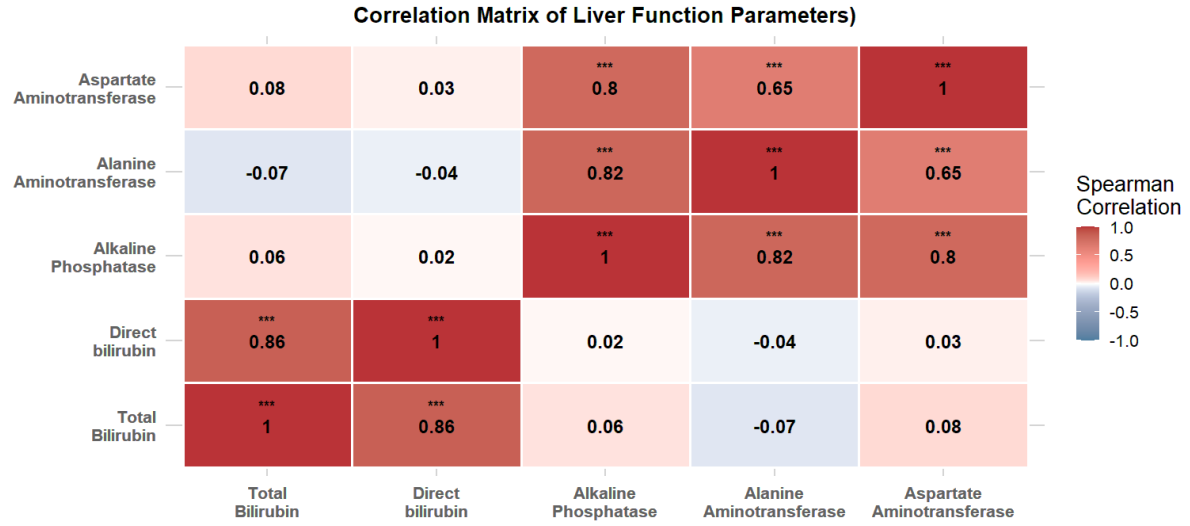

Figure 6. Spearman correlation matrix of liver function parameters (ALT, AST, ALP, total bilirubin, and direct bilirubin) in Entamoeba histolytica–positive patients.

Color gradients reflect the strength and direction of the correlations, whereas the numeric values denote the respective correlation coefficients. Statistically significant associations were observed primarily among liver enzyme markers and between total and direct bilirubin values. A very strong positive correlation was identified between total bilirubin and direct (conjugated) bilirubin ($\rho = 0.86$, $p < 0.001$), indicating that

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increases in total bilirubin were largely attributable to corresponding increases in its conjugated fraction. This pattern reflects a preserved physiological linkage between bilirubin components and suggests consistency in bilirubin metabolic processing among the study participants.

Among liver enzymes, strong and statistically significant positive correlations were detected. Alkaline phosphatase (ALP) demonstrated a strong correlation with alanine aminotransferase (ALT) ($\rho = 0.82$, $p < 0.001$) and with aspartate aminotransferase (AST) ($\rho = 0.80$, $p < 0.001$). In addition, ALT showed a moderately strong correlation with AST ($\rho = 0.65$, $p < 0.001$). These findings indicate that elevations in hepatocellular and enzyme-related markers tended to occur concurrently, suggesting a coordinated biochemical response consistent with hepatic stress associated with *E. histolytica* infection.

In contrast, bilirubin parameters demonstrated weak and non-significant correlations with aminotransferases and ALP. Total bilirubin showed negligible correlation with ALT ($\rho = -0.07$, $p = 0.72$), AST ($\rho = 0.08$, $p = 0.67$), and ALP ($\rho = 0.06$, $p = 0.76$). Similarly, direct bilirubin exhibited no statistically significant correlation with ALT ($\rho = -0.04$, $p = 0.82$), AST ($\rho = 0.03$, $p = 0.88$), or ALP ($\rho = 0.02$, $p = 0.94$). These observations suggest that bilirubin alterations occurred largely independent of enzyme changes within the study cohort. Overall, the correlation analysis reveals two distinct biochemical patterns: a cluster characterized by closely interrelated hepatocellular and cholestatic enzymes (ALT, AST, and ALP), and a second cluster involving bilirubin parameters that were strongly associated with each other but largely unrelated to enzyme activity. This profile supports the presence of predominantly hepatocellular involvement, without widespread impairment of bilirubin excretory function in *E. histolytica*–positive patients.

Discussion

The present study provides a detailed biochemical characterization of liver function parameters among patients positive for *Entamoeba histolytica* at Al-Bayda Medical Center, Libya, during 2025. Through the combined application of descriptive statistics, inferential comparison with reference thresholds, categorical classification, and correlation analysis, the study contributes context-specific insight into the pattern and extent of hepatic involvement associated with amoebic infection among patients without clinically confirmed liver abscess. These findings align with previous reports indicating that biochemical alterations may occur even in the absence of overt hepatic complications, reflecting the complex interaction between host immunity, parasite virulence, and hepatic tissue response [1,2].

A principal finding of this study is the relatively high frequency of aminotransferase elevation, particularly AST, which was elevated in more than half of the examined patients, whereas ALT elevation was observed in 40% of cases. This biochemical pattern is consistent with hepatocellular stress or injury and corresponds with findings from earlier clinical and experimental investigations reporting enzyme disturbances in invasive amoebiasis and subclinical hepatic involvement [3,4]. The predominance of AST elevation over ALT may reflect broader tissue-level disturbance, since AST is distributed in hepatic as well as extrahepatic tissues, including muscle and erythrocytes. Experimental models have demonstrated that *E. histolytica* trophozoites can induce hepatocyte cytolysis, inflammatory mediator release, and immune-mediated cellular damage, all of which may contribute to enzyme leakage into circulation even in the absence of radiological abscess formation [5,6].

Despite the presence of abnormal aminotransferase values in a notable subset of patients, the inferential comparison with population reference limits did not demonstrate statistically significant increases at the group level. This suggests that hepatic biochemical alterations were heterogeneous rather than uniformly distributed across all infected individuals, an observation similarly reported in previous studies where mean enzyme values remained within reference ranges despite clinically meaningful abnormalities in selected patients [7,8]. Such variability may be influenced by duration of infection, host immune status, nutritional factors, and underlying hepatic conditions.

In contrast to aminotransferase alterations, bilirubin disturbances were relatively limited in this cohort. Only a very small proportion of patients exhibited elevated total bilirubin, and although direct bilirubin elevation occurred more frequently, overall bilirubin values did not significantly deviate from reference thresholds. This pattern suggests preservation of bilirubin excretory mechanisms and the absence of widespread cholestatic or obstructive dysfunction. These findings differ from patterns documented in complicated amoebic liver abscess, where marked hyperbilirubinemia may result from biliary compression, necrotic tissue destruction, or secondary inflammatory obstruction [9,10].

Alkaline phosphatase elevation was observed in approximately one-third of patients; however, group-level analysis demonstrated no statistically significant deviation from the reference value. This indicates that cholestatic involvement may occur in a subset of infected individuals but does not represent the dominant biochemical feature associated with *E. histolytica* infection in this clinical context. Similar mixed biochemical

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patterns have been reported in studies differentiating hepatocellular-predominant from cholestatic-predominant hepatic responses in parasitic infections [11].

Correlation analysis revealed two distinct biochemical trends. Strong positive associations were observed among ALT, AST, and ALP, indicating that elevations in hepatocellular and enzyme-related markers tended to occur concurrently. This coordinated biochemical response is compatible with hepatic inflammatory stress and immune activation triggered by amoebic invasion, a mechanism supported by immunopathological and experimental evidence demonstrating cytokine-mediated hepatocyte injury and oxidative stress-related enzyme leakage [5,12]. Conversely, total and direct bilirubin exhibited a very strong mutual correlation but showed weak and non-significant associations with aminotransferases and ALP. This dissociation suggests that bilirubin metabolism followed a largely independent trajectory relative to enzyme alterations, reinforcing the interpretation that hepatic involvement in this cohort was predominantly hepatocellular rather than cholestatic or obstructive in nature.

From a clinical perspective, the observed biochemical profile underscores the importance of routine liver function assessment in patients diagnosed with intestinal amoebiasis, even in the absence of clinically apparent hepatic disease. Mild or moderate elevations in aminotransferases may represent early indicators of hepatic stress and warrant follow-up monitoring, particularly in resource-limited endemic settings where access to imaging and advanced diagnostics is restricted. The heterogeneity in biochemical responses also reflects the multifactorial nature of host–parasite interactions, including parasite genotype variation, host immune profile, and environmental determinants reported in prior studies [1,6].

This study has several limitations that should be acknowledged. The relatively small sample size may limit statistical power and restrict generalizability. Diagnosis was based on routine microscopy, which does not differentiate *E. histolytica* from morphologically identical non-pathogenic species such as *E. dispar*. Additionally, imaging studies and inflammatory biomarkers were not available, preventing direct correlation between biochemical alterations and structural hepatic changes. Future research should employ molecular diagnostic confirmation, incorporate larger multicenter cohorts, and adopt longitudinal follow-up designs to clarify the temporal evolution of liver function changes and their prognostic implications in amoebiasis [2,9]. Integrating biochemical, radiological, and immunological markers may further elucidate the underlying mechanisms of hepatic involvement in *E. histolytica* infection.

Conclusion

In summary, the findings of this study indicate that *Entamoeba histolytica* infection among patients attending Al-Bayda Medical Center was primarily associated with biochemical alterations reflecting hepatocellular involvement, particularly through elevations in aminotransferase enzymes, with AST being more frequently elevated than ALT. In contrast, bilirubin parameters and cholestatic markers were largely preserved, and population-level comparisons did not demonstrate statistically significant deviation from reference limits, suggesting that hepatic involvement was generally mild and not indicative of advanced cholestatic dysfunction. The overall biochemical profile observed in this cohort is therefore consistent with a predominantly hepatocellular pattern rather than a cholestatic process, and it highlights the potential value of routine liver function testing in patients with confirmed *E. histolytica* infection, even in the absence of clinically evident liver disease. These results contribute regionally relevant evidence to the understanding of hepatic alterations in amoebiasis and support the need for further research integrating biochemical findings with clinical, radiological, and immunological assessments to clarify their diagnostic and prognostic significance.

Conflict of interest. Nil

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