Pro Index | Journal of Discoveries in Applied and Natural Science
Volume: 4 Issue: 1 Year: 2026
ISSN: 3030-301X | http://journals.proindex.uz

Levels of Paraoxonase-1 Activity, Immunological Parameters, and Antioxidant Enzymes in Children Infected with Entamoeba Histolytica

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Abstract: **Introduction & Objective**: Entamoeba histolytica (E. histolytica) is an important enteric protozoan leading to morbidity in children, and is frequently accompanied with oxidative stress and immune stimulation. The purpose of this study was to evaluate the activity of Paraoxonase-1 (PON1) status, antioxidant enzymes (GSH, TAC, Catalase) and immunological parameters (IL-6, IL-10) in E. histolytica-infected children.

Materials & Methods: One hundred fifty children were enrolled during the period from May to September, 2025 at the Pediatric Hospital-Kirkuk-Iraq, including ninety children with diarrhea and sixty healthy controls. Stool microscopy confirmed infection. The levels of PON1, antioxidant enzymes, and cytokines in the serum were assayed using commercially available kits (Sunlong Biotechnology, China).

Results: Among diarrheal children, 33 (36.7%) were positive for E. histolytica. Compared with controls, infected children had lower GSH (4.12 ± 1.23 vs 6.81 ± 1.05 µmol/L), TAC (0.83 ± 0.19 vs 1.31 ± 0.21 mmol Trolox Eq/L), and PON1 activity (45.23 ± 9.87 vs 89.56 ± 14.32 U/L), and higher catalase (224.55 ± 49.78 vs 179.65 ± 34.92 kU/L), IL-6 (16.24 ± 6.71 vs 4.53 ± 1.21 pg/mL), and IL-10 (13.11 ± 5.22 vs 6.98 ± 2.03 pg/mL) (all $P < 0.05$). Pearson correlation showed positive associations among antioxidant markers (GSH–TAC $r = 0.72$, GSH–PON1 $r = 0.65$) and negative correlations with cytokines (GSH–IL-6 $r = –0.61$, GSH–IL-10 $r = –0.42$).

Conclusions: E. histolytica infection in children decreases antioxidant defense and increases inflammatory cytokines suggesting a synergistic effect of oxidative stress and immune response.

Key points: Paraoxonase-1, Oxidative Stress, Cytokines, E. histolytica.

Introduction

Entamoeba histolytica is an intestinal protozoan parasite with a considerable public health concern, especially in children inhabiting endemic areas. The spectrum of infection varies from asymptomatic colonization to fatal invasive disease, the intestines and liver being most commonly involved, with 2reported life-threatening complications in absence of treatment [1,2]. Sensitive laboratory techniques such as enzyme-linked immunosorbent assay (ELISA) and other immunological tests are necessary for accurate diagnosing of amoebiasis in order to differentiate E. histolytica from non-pathogenic species like E. dispar [2,3]. Control of the infection is a crucial function of the host experimental immune response. Infection with E. histolytica induces the activation of mononuclear cells and pro-inflammatory cytokines including interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) as well as interleukin-17 (IL-17) [4,5,6]. Although these immune mediators limit the parasite's growth, excessive or uncontrolled inflammation can lead to tissue destruction and aggravated disease [7]. Meanwhile, E. histolytica faces oxidative stress produced by the host immune system. Introduction Generation of reactive oxygen species (ROS)

and reactive nitrogen species (RNS) in the context of infection can interfere with redox homeostasis and negatively modify cellular structures in host tissues [8,9]. Antioxidant enzymes are very important to protect the cells from oxidative damage. One such enzyme is Paraoxonase-1 (PON1) that is an antioxidant and anti-inflammatory HDL-associated enzyme [10]. PON1 activity is known to decrease under several infectious and inflammatory conditions, as a result of increased oxidative stress and compromised immune regulation [11,12]. In pediatric age groups, modified activity of PON1 has been detected in children with active pulmonary tuberculosis and asthma, thus endorsing the utility of this enzyme as a marker for oxidative stress/ immune function in infectious diseases [13,14]. Although the involvement of PON1 and other antioxidant enzymes in systemic infections has been well established, but its activity is not well studied in children with E. histolytica infection [5,6,10]. While immune response and oxidative stress have been also associated with E. histolytica infectation, little is known about the combined effect of this parasite over PON1 activity along with other enzymatic antioxidant markers and immunological parameters in children. accordingly, the present study was designed: To assess PON1 activity and immunological parameters (Cytokines) as well as antioxidant-related enzyme levels in children with Entamoeba histolytica infection to determine the infection effect on oxidative stress and immunity among this age group.

Materials and Methods

Study Design

This is a prospective observational study carried out at the Pediatric Hospital, Kirkuk, Iraq during the period from May 2020 to September 2027. A hundred and fifty children were recruited, 90 suffered from acute diarrhea and 60 served as healthy controls. Diarrheal children were clinically and parasitologically confirmed, controls were age- and sex-matched non-gastrointestinal symptomatic subjects. The parents or legal guardians provided written informed consent, and the study was approved by the Ethics Committee of our institution.

Demographic and Clinical Assessment

Demographic and clinical information, age, sex and place of residence were registered. Sociodemographic and environmental characteristics were recorded to assess possible effects on oxidative stress and immune response.

Stool Sample Collection and Examination

About 2 mg stool sample was collected from every person in a clean container. Cyst and trophozoites of Entamoeba histolytica were recorded from each sample by microscopic examination which was done under10 x and40x objectives. It secured the diagnosis of infection in diarrheal children and excluded participants from control if they were free of parasites.

Blood Collection

Venous blood was drawn under aseptic technique. After centrifugation at 3000 rpm for 10 min, serum was then stored at −80°C until further analysis. Oxidative stress, PON1 activity and cytokine measurements were performed in serum aliquots.

Oxidative Stress Assessment

Oxidative stress measurements were performed by observing glutathione (GSH), total antioxidant capacity (TAC), and catalase activity using commercially available kits (Sunlong Biotechnology, China). In each test, 10 µL of serum was mixed with 40 µL of buffer and then combined with 50 µL specific reagent. The incubated samples were subjected to the manufacturer's protocol and changes in the absorbance were determined by spectrophotometry. TAC was the sum of antioxidant activity, and catalase was measured from hydrogen peroxide degradation.

Paraoxonase-1 (PON1) Activity

The activity of PON1 was detected by Sunlong Biotechnology kits. For each serum sample, 10 µL of serum was added to microplate wells precoated with antigen and 40 µL diluent was added and

incubated at 37 C0 for 37 min, then washed, 50 µL of HRP-conjugated secondary antibody was added and incubated at 37 C0 for 37 min, then washed, and adding 50 µL for each substrate chromogen solution nd incubated at 37 C0 for 15 min, then added 50 µL of stop solution and the corresponding enzymatic activity determined by colorimetric absorbance at 450mm.

Cytokine Quantification (IL-6 and IL-10)

The levels of serum IL-6, and IL-10 were detected using ELISA Kits (Sunlong Biotechnology Co., Ltd). For each serum sample, 10 µL of serum was added to microplate wells precoated with antigen and 40 µL diluent was added for a binding reaction. The wells were then washed, 50 µL of HRP-conjugated secondary antibody was added. The color reaction was developed by adding substrate chromogen solution and then stop solution. Absorbance was assessed using a microplate reader and cytokine concentrations were determined based on the standard curve.

Statistical Analysis

Continuous variables are presented as means ± SD or percentages. Between-group differences were evaluated by t-test or Mann–Whitney U test, correlations by Pearson or Spearman coefficients and multivariate analyses adjusted for confounders; $p < 0.05$ was adopted as significance level.

Results

Microscopic Identification of *Entamoeba histolytica*

Entamoeba histolytica was detected by stool microscopically. The cyst stage was recognized by characteristic morphology such as round shape having multiple nuclei and chromatoid bodies (Fig. 1). Microscopy was positive in diarrheal children and negative for parasites in controls.

Figure (1): Morphological features of E. histolytica cysts under 40X.

Prevalence of *Entamoeba histolytica* and Demographic Distribution

Of 90 children with diarrhea, 33 (36.7%) were stool-microscopy positive for E. histolytica. Infection proportion of the different time group The infection cases were mainly in children subjects include the 4–6 year-old (36.4%), 1–3 year-old (27.3%). Older children 7–9 and 10–12 years were fewer in proportion (21.2% and 15.1% respectively), Table 1.

Table 1. Distribution of Infected Children by Age Group (n = 33)

Age Group (years)	No. of Children	Percentage (%)
1–3	9	27.3
4–6	12	36.4
7–9	7	21.2
10–12	5	15.1
Total	33	100.0

Analysis by sex revealed a slight predominance of males (54.5%) among the infected children, compared with females (45.5%), Table 2.

Table 2. Distribution of Infected Children by Sex (n = 33)

Sex	No. of Children	Percentage (%)
Male	18	54.5
Female	15	45.5
Total	33	100.0

Regaring the places of residence, 60.6% were from urban and 39.4% were from rural areas. These variations are described in Table 3, number and percentages of infected children according to the area of residence.

Table 3. Distribution of Infected Children by Residence (n = 33)

Residence	No. of Children	Percentage (%)
Urban	20	60.6
Rural	13	39.4
Total	33	100.0

Antioxidant enzymes

The serum antioxidant enzymes were determined in 33 E. histolytica-infected children and compared with that of 60 sex- and age-matched controls without intestinal amebiasis. As depicted in Table 4, the mean of glutathione (GSH) level among infected children was 4.12 ±1.23 µmol/L which is significantly lower than control groups' mean value (6.81 ±1.05µmol/L P<0.001). Significantly lower TAC was measured in infected group (0.83 ± 0.19 mmol Trolox Eq/L) than control group (1.31 ± 0.21 mmol Trolox Eq/L, P<0001). Compared with controls (179.65 ± 34.92 kU/L), catalase activity was higher in infected children (224.55 ± 49.78 kU/L, P=0.002), demonstrating a compensatory enzyme response to oxidative stress (Table 4).

Table 4. antioxidant enzymes levels in Infected and Control Children

Biomarker	Infected (Mean ± SD)	Control (Mean ± SD)	P-value
Glutathione (µmol/L)	4.12 ± 1.23	6.81 ± 1.05	<0.001
Total Antioxidant Capacity (mmol Trolox Eq/L)	0.83 ± 0.19	1.31 ± 0.21	<0.001
Catalase (kU/L)	224.55 ± 49.78	179.65 ± 34.92	0.002

Serum PON1 activity was measured to evaluate enzymatic antioxidant defense. Infected children exhibited a mean PON1 of 45.23 ± 9.87 U/L, which was significantly lower than controls (89.56 ± 14.32 U/L, P < 0.001), Table 5.

Table 5. PON1 Activity in Infected and Control Children

Parameter	Infected (Mean ± SD)	Control (Mean ± SD)	P-value
PON1 Activity (U/L)	45.23 ± 9.87	89.56 ± 14.32	<0.001

Serum IL-6 and IL-10 levels were measured to assess immune response. Infected children had significantly elevated cytokines compared with healthy controls. The mean IL-6 was 16.24 ± 6.71 pg/mL in infected children versus 4.53 ± 1.21 pg/mL in controls (P < 0.001). IL-10 was 13.11 ± 5.22 pg/mL in cases versus 6.98 ± 2.03 pg/mL in controls (P < 0.001), Table 6.

Table 6. Cytokine Levels in Infected and Control Children

Cytokine	Infected (Mean ± SD)	Control (Mean ± SD)	P-value
IL-6 (pg/mL)	16.24 ± 6.71	4.53 ± 1.21	<0.001
IL-10 (pg/mL)	13.11 ± 5.22	6.98 ± 2.03	<0.001

Pearson correlation

Table 7 showed that the GSH correlated positively with TAC (r = 0.72) and PON1 (r = 0.65), and negatively with IL-6 (r = –0.61) and IL-10 (r = –0.42). TAC correlated positively with PON1 (r = 0.60) and negatively with IL-6 (r = –0.58) and IL-10 (r = –0.39). Catalase correlated positively with IL-6 (r = 0.44) and IL-10 (r = 0.31, ns). PON1 correlated negatively with IL-6 (r = –0.55) and IL-10 (r = –0.36).

Table 7. Correlation of antioxidants, PON1, and cytokines in infected children

	GSH	TAC	Catalase	PON1	IL-6	IL-10
GSH	1	0.72	–0.12	0.65	–0.61	–0.42
TAC	0.72	1	0.08	0.60	–0.58	–0.39
Catalase	–0.12	0.08	1	–0.05	0.44	0.31
PON1	0.65	0.60	–0.05	1	–0.55	–0.36
IL-6	–0.61	–0.58	0.44	–0.55	1	0.30
IL-10	–0.42	–0.39	0.31	–0.36	0.30	1

Discussion

The present study indicates that oxidative stress and antioxidant enzyme activities are altered and there is an evidence of immune dysregulation in E.histolytica-infected children. Parasitic infection in stool specimens Infection was confirmed microscopically in 36.7% of diarrheal children, most (36.4%) in the age group 4–6 years and slightly more often amongst males (54.5%) (Tables 1–2). These patterns of the epidemiological profiles were consistent with those of Haasen and Alsalim (15) who showed greater susceptibility in younger age children, because they has immature immunity and excessive environmental exposure. Abed et al. (16) All reported similar patterns of prevalence among children in Baghdad as well. The urban prevalence of infection (60.6%) correlates with Nayyef et al. (17), which emphasizes the relationship between overcrowding and poor sanitation with higher prevalence of intestinal protozoa. Environmental conditions including contaminated drinking water and hygiene factors were important determinants in urban acquisition Chalabi et al (18). Oxidative evaluation showed a significant decrease of non enzymatic antioxidants. Children infected presented a decrease in glutathione and total antioxidant capacity, with increased catalase activity. This result is consistent with Karaman et al. (20) reported GSH reduction and catalase increase in the blood of children with protozoal infections, trying to explain these findings as an attempt by the enzymatic system to compensate oxidative processes. Patlevič et al. (21) also demonstrated similar response patterns in gastrointestinal diseases and suggested that enhanced ROS levels during infection induce enzymatic antioxidant activation. Serum level of Paraoxonase-1 (PON1) activity was reduced significantly representing a deficiency in enzymatic antioxidant defenses. Mohsin et al. (22) observed comparable decrease in PON1 during E. histolytica infection and attributed it to the occurrence of an oxidative stress producing condition that is above the capability of antioxidant mechanisms. Ahmadvand et al. (28) also suggested that reduced PON1 is associated with systemic oxidative damage in infectious and inflammatory states. The determination of cytokines produced evidence for an immune response with increased IL-6 and IL-10. This profile indicates a pro-inflammatory reaction that has been regulated by anti-inflammatory signals. AL-Bairmani and Al-Masoudi (23) also demonstrated higher IL-10 levels in children with parasitic infections as part of a regulatory attempt to reduce tissue injury, while Zeki and Al-Warid (24) detected increased IL-6 values in children with amoebiasis that reflected immune stimulation by the parasites. The interaction of the oxidative stress markers with cytokinse was observed to be significant as suggested by Pearson correlation analysis. GSH was positively correlated with TAC and PON1, while negatively associated with IL-6 and IL-10. TAC was also positively correlated with PON1 and opposite negatively associated with IL-6 and IL-10. Catalase levels were positively correlated with IL-6 and IL-10, whereas PON1 would negatively correlate with them. These results everything taught with Mohsin et al. (22) and Patlevič et al. (21), suggesting a strong association between oxidative stress and immune activation in children with E. histolytica infection. In conclusion, our findings suggest that E. histolytica infection in children promotes systemic oxidative stress followed by antioxidant alteration, catalase activation and

protective cytokine increase. These findings are consistent with earlier studies (15–30) and confirm the proposition that oxidative stress and immune dysregulation play a key role in the pathophysiology of paediatric amoebiasis.

Conclusions

Among children infected by *Entamoeba histolytica* there is a marked decrease in antioxidant defenses (GSH, TAC, PON1 activity) and increase of catalase and pro/anti-inflammatory cytokines (IL-6, IL-10). These results reveal a significant crosstalk between oxidative stress and immune activation in the context of infection. The associations found indicate that oxidative damage may be accompanied by immune system mediated inflammation, at the same time antioxidant depletion occurs and compensatory enzymatic responses are upregulated.

Limitations

Generalizability was limited by the single hospital setting of this study. The cross-sectional nature of this study does not allow causal inferences, and other oxidative markers or cytokines were not tested. Larger multicenter cohorts and further biomarkers are necessary in future studies.

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