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THE SILENT IMPACT OF *ENTAMOEBA HISTOLYTICA*: ASSOCIATIONS WITH GROWTH RETARDATION AND MICRONUTRIENT DEPLETION IN CHILDREN

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ABSTRACTS
Entamoeba histolytica (*E. histolytica*) is an intestinal pathogen that causes high morbidity in children from developing nations. *E. histolytica* cause gastrointestinal symptoms, but its presence can also lead to malnutrition and micronutrient deficiencies due to poor absorption of nutrients, intestinal inflammation, and high energy requirements. The purpose of this study was to evaluate the association between *E. histolytica* infection, clinical presentation, anthropometric measures, and serum micronutrients among children. The comparative cross-sectional study was designed in which children were divided into the infected and non-infected groups by examining their stool samples for *E. histolytica*. Socio-demographic parameters and clinical symptoms were recorded by the utilization of questionnaire. GraphPad prism version 8 were used to perform statistical analysis. According to the results, 57.8% children were infected with *E. histolytica* and 42.1% were found as non-infected. Among the infected children, females (55.5%) were highly prevalent to this infection compared to males (44.4%). The infected children particularly 43.4% were highly belong to > 5 – 9 age group and most of them about 59.3% lived in rural areas. Abdominal pain (67.9%), diarrhea (58%) and fever (54.3%) were the most noted clinical symptoms among the infected children. The anthropometric parameters significantly ($P < 0.01$) reduced in all infected children including weight, height and BMI. The serum ferritin (ng/mL) and zinc (µg/dL) levels in infected children were significantly reduced with the p-value of < 0.01 in comparison to non-infected children while dramatic significant reduction noted in vitamin D (ng/mL) levels with p-value of < 0.0001. These all micronutrient deficiencies were significantly correlated with the anthropometric parameters of infected children ($p < 0.0001$). The elevated serum levels of these micronutrients were found in the reduced weight group, whereas decreased levels were detected in the reduced height and reduced BMI groups ($p < 0.01$-0.0001). Micronutrient deficiencies were highly correlated with poor growth in infected children. These findings demonstrate the necessity for integrated nutritional programs, routine micronutrient testing, and effective parasitic control measures to enhance child growth and health.

KEYWORDS: *Entamoeba histolytica,* Micronutrients, Clinical manifestation, Anthropometric parameters, Micronutrient deficiencies

INTRODUCTION

Entamoeba histolytica (*E. histolytica*) is a harmful protozoan that causes amebiasis, responsible for major health problem in number of developing countries [1]. This parasite spreads mainly through the fecal-oral route due to contaminated water, food or poor hygiene. These factors are considered as significant risk factors for the occurrence of infection [2]. Although many infected people are asymptomatic while invasive disease can be able to cause dysentery, amoebic colitis, extra-intestinal manifestations and considerable morbidity in children [3]. According to recent reports, *E. histolytica* remains a major cause of gastrointestinal infections in places with poor sanitation and malnutrition [4].

Children are especially vulnerable to the consequences of intestinal parasitic infections since they need more nutrients for their growth and development [5]. *E. histolytica* have the ability to cause infection and damages the intestinal epithelium via several ways of virulence mechanisms such as adherence to the cells, tissue invasion, cytotoxicity which leads to inflammation responses [6]. The normal structure and function of the gut is influenced by these pathological changes. Due to this reason, the absorption of essential nutrients from food becomes less effective in damaged intestine. As a result, this poor nutrient absorption causes weight loss, inadequate growth and deficiencies of important minerals and vitamins in children. Recent reports showed that childhood undernutrition is highly linked to enteric infections. These infections inflame the intestinal lining, damage cells and mess with the body's digestion and absorption system of nutrients. So, affected kids have a higher risk of stunted growth, malnutrition and other health problems [5].

The relationship is highly interconnected between intestinal infections and malnutrition. The parasitic and intestinal infections are more vulnerable in children with malnutrition and have weak immune system. On the other hand, long-term intestinal infections suppress the absorption and utilization of nutrients effectively in malnourished children [7]. Chronic intestinal inflammation from these infections causes the release of cytokines. These substances decrease appetite, ramp up the body's need for energy and make the body break down muscle and fat. All of this makes nutrition worse [8]. Also, these infections mess with growth related hormones like growth hormone and insulin-like growth factor-1 (IGF-1). This disorder can affect physical growth, height gain and overall development in children [9].

Micronutrient deficiencies are a big health problem linked to intestinal parasitic infections in children [10]. These infections make it harder for the body to take in and use essential nutrients, raising the chances of nutritional deficiencies. Iron deficiency shown by low serum ferritin levels, is super common around the world and it hurts cognitive development, weakens immunity and leaves kids feeling tired [11]. Zinc is key for immune strength, healing wounds, growing cells and general development. Likewise, vitamin D is crucial for healthy bones and for controlling immune responses [12]. According to reports, children with *E. histolytica* and other intestinal protozoan parasites usually have less ferritin, zinc, and vitamins in their systems than kids without these infections. This means that parasitic infections could be a major cause of hidden micronutrient deficiencies and might really hurt kids' growth and overall health [13].

Recent studies showed that *E. histolytica* can change kids' immune responses. Infected children usually have more interleukins and other inflammatory stuff in their bodies. This shows the immune system is working hard to fight off the parasites [14]. Although, people recognizing how intestinal parasites affect kids' nutrition, we still lack data particularly about how *E. histolytica* infections impact kids' growth and certain health markers in developing countries [4]. However, to understand these infections, the evaluation of both anthropometric and biochemical indicators are offer important insight into the overall health impact of *E. histolytica* infection in pediatric populations. Thus, the present study designed to evaluate the association of *E. histolytica* infection with nutritional status and selected biochemical parameters such as serum ferritin, vitamin D and zinc levels among children. Understanding these interconnections can be helpful to sustain the progress of integrated intervention strategies like nutritional rehabilitation, infection control and improved child health outcomes

MATERIALS AND METHODS

Study Setting and Study Population

This hospital-based comparative cross-sectional study evaluated the link between Amebiasis and nutritional and biochemical factors in children. The study was carried out in pediatric outpatient departments and diagnostic lab of healthcare facility. The duration of study period was for 6 months from 5-January to 30-June 2025. A total of 140 participants were included in this study. A consecutive non-probability sampling technique aligned with STARD guidelines was utilized to collect data [15]. So, we enrolled every eligible patient who showed up during the study period to achieve the target sample size. This method helps minimize selection bias, making sure the group represents how the disease actually presents in real life [16].

Inclusion and Exclusion Criteria

In inclusion criteria, children with age group 2–12 years were allowed to participate in this study. All the participated children confirmed the *E. histolytica* infection with no chronic systemic illness. Consent from parents/guardians was taken prior to data collection. In exclusion criteria, children under age 2 and older than 12 years along with chronic diseases such as hepatic, renal and metabolic disorders were not included in this study. The children who took antiparasitic or antibiotic medication within 2 weeks before the confirmation of *E. histolytica* were excluded from the study.

Socio-demographic and case history parameters of the study

To collect the demographic parameters, a structured questionnaire was administered to parents or guardians. The demographic parameters were consisted of age, gender (male and female), residential status (urban and rural) and water facility (poor and good). A thorough history taking was done including personal symptoms (abdominal pain, diarrhea, vomiting and fever) along with past, current, diet and family history.

Laboratory Investigations

Stool sample collection

Five to eight grams of each participant's fresh stool sample was collected in a sterile screw-capped container. The samples were sent to the laboratory within two hours through the cold chain technique (2-8°C). After receiving the samples, they were processed under microscopic examination and ELISA antigen detection.

Light Microscopy

In microspy, stool specimens were processed by both direct wet mount preparations (saline and Lugol's iodine) and the formalin-ethyl acetate (FEA) concentration technique, which enhances cyst and trophozoite recovery through sedimentation [10]. Slides were examined at 10x and 40x objective magnification by two independent trained parasitologists. Discrepant interpretations were adjudicated by a senior expert. Any Entamoeba morphotype was recorded as microscopy-positive, acknowledging the inherent inability of light microscopy to distinguish E. histolytica from E. dispar or E. moshkovskii.

ELISA antigen detection
Stool antigen detection was done by TechLab E. histolytica II antigen test kit from TechLab Inc. (USA). This test detects the presence of E. histolytica antigen in stool samples. It is based on an immunochromatographic technique specific for Gal/GalNAc galactose-inhibitable adherence lectin. Detection limit was determined using optical density at 450nm wavelength with microplate reader. Test results were interpreted as either positive or negative depending on the cut-off value determined by manufacturer. Positive, negative and blank controls were included in all plates. All doubtful samples were tested in duplicates [10].

Assessment of Nutritional Status
The children stood on the ground barefoot with the back against the vertical backboard with their buttocks and shoulders touching the board. The same individual took the average of three measurements of height and weight separately. Anthropometric measurements were noted with the utilization of standard protocols. The hight was measured by the utilization of stadiometer (cm), weight was measured by using with the help of calibrated digital weighing scale (kg). The body index was calculated as weight (kg)/height (m^2).

Biochemical Estimation
Blood samples were taken in the sterile tube to determine the ferritin, zinc and Vitamin D levels in the serum. For this purpose, Cobas e-series immunoassay analysers and Elecsys reagent kits from Roche were utilized [17].

Statistical Analysis
GraphPad prism version 8 was utilized to analyze all the data in a statistical way. The experiments were carried out in triplicates (n = 3). Data were presented as mean ± standard error of the mean (SEM). One-way and two-way analysis of variance (ANOVA) followed by appropriate post hoc tests (e.g., Tukey's multiple comparisons test) were used to determine statistical significance. Statistical significance was defined as the P value < 0.05.

RESULTS
The number of participants involved in this study was 140, comprising 81(57.8%) infected with *E. hitolytica* and 59 (42.1%) non-infected patients. In gender distribution category, females were more prevalent (55.5%) in comparison to males (44.4%) in the group of infected children. Likewise, the prevalence of females and males in non-infected children was found as 61.0% and 38.9%, respectively. Based on age distribution, most infected children belonged to the age group greater than 5-8 years (43.2%) followed by 2-5 years (29.6%) and 8-12 years (27.1%). Among non-infected children, 42.3% children were belong to the age group 5-8 years while moderate presence 35.5% was found in 8-12 years age category and least presence of infected children 22.0% was observed in 2-5 years age category. Rural residents constituted a higher percentage (59.3%) of infected and 66.1% of non-infected children in comparison to urban residents. All the demographic parameters of the study presented in Table 1.

Table 1. Socio-demographic Parameters of the Participants (n =140)

Demographic Parameters	Infected (N=81)	Non-Infected (N=59)
Gender		
Male	36 (44.4%)	23 (38.9%)
Female	45 (55.5%)	36 (61.0%)
Age		
> 2 – 5	24 (29.6%)	13 (22.0%)
> 5 – 8	35 (43.2%)	25 (42.3%)
> 8 – 12	22 (27.1%)	21 (35.5%)
Residential Background		
Urban Areas	33 (40.7%)	20 (33.8%)
Rural Areas	48 (59.3%)	39 (66.1%)

Clinical Manifestations of the Study Participants
The clinical manifestations were more prevalent in the infected children than in the non-infected children (Figure 1). Abdominal pain was recorded in about 67.9% of the infected children while only 32.2% had abdominal pain in non-infected people (Figure 1A). As shown in Figure 1B, diarrhea occurred in almost 58% among the infected children in comparison to non-infected children contain only 23.7% positive toward diarrhea. Furthermore, vomiting was common in about 53.8%

of the infected children and 37% was recorded in the non-infected children (Figure 1C). Fever was highly recorded in infected children category (54.3%) while low percentage 32.5% of non-infected children had fever (Figure 1D). Overall, the higher negative percentage of all clinical manifestations was found in non-infected category of children.

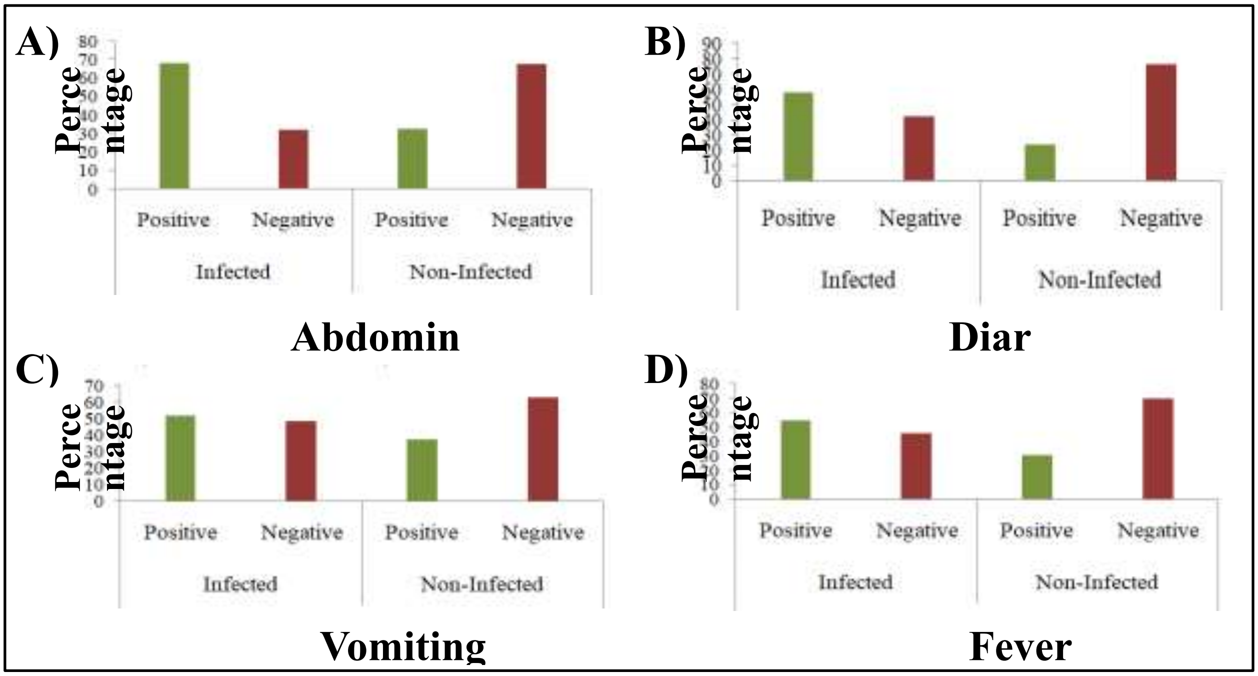

Figure 1. Clinical manifestation distribution among the infected and Non-infected children: A) Abdominal pain, B) Diarrhea, C) Vomiting and D) Fever

Anthropometric Parameters of the Study Participants

Based on the results obtained from the anthropometric parameters, the weight (kg), height (cm) and BMI (kg/m^2) was recorded as 29.23 ± 1.2 kg, 118.6 ± 2.1 cm and 15.08 ± 0.9 kg/m^2 in infected children while 43.2 ± 2.1 kg, 145 ± 2.1 cm and 16.3 ± 0.6 kg/m^2 were observed in non-infected children. According to comparative analysis as indicated in Figure 2, the weight and height significantly ($P < 0.01$) reduced in infected children in comparison to non-infected children. Moreover, there was no considerable difference in BMI between the two groups (Non-infected and Infected) ($p>0.05$).

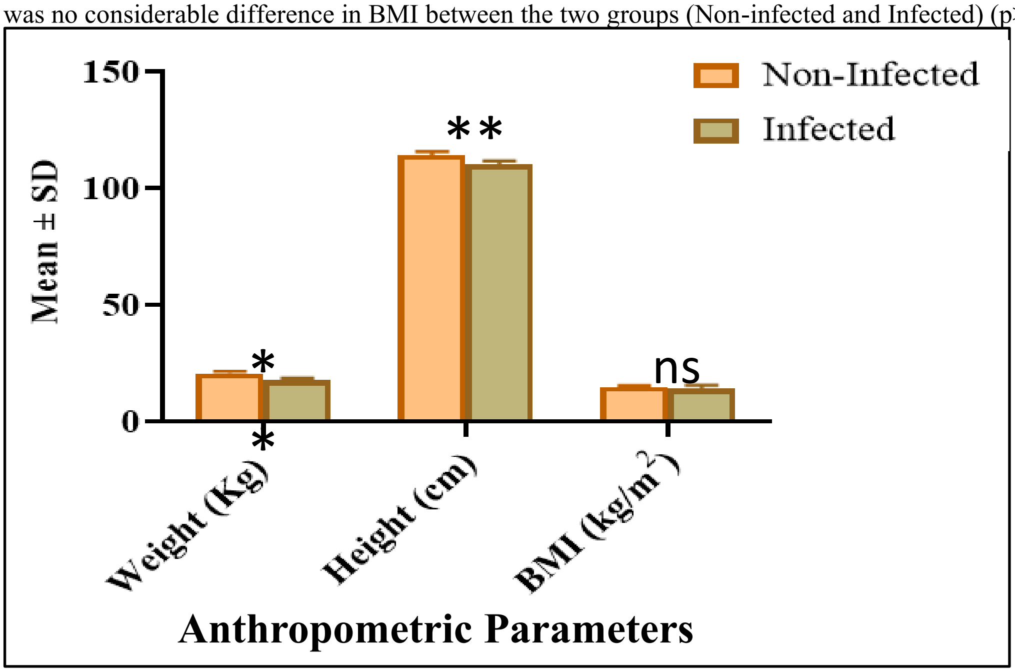

Figure 2. Comparative analysis of the anthropometric parameters of the study participants. Two-way ANOVA followed by Sidak's multiple comparisons test statistically tested the differences between means.; Significant (p <0.01), ns; Non-significant (p>0.05).**

Biochemical Parameters of the Study Participants

Major variations were noted in the values of serum ferritin, zinc, and vitamin D between the infected and non-infected subjects. Non-infected children had high serum ferritin, zinc and vitamin D values (11.83 ± 1.5, 66.87 ± 1.5 and 16.53 ± 1.2 respectively) while the infected children was found with low levels of serum ferritin, zinc and vitamin D (6.3 ± 0.5, 61.85 ± 1.6 and 7.7 ± 1.1 respectively). Comparative evaluation in Figure 3 showed that serum ferritin (ng/mL) and zinc (µg/dL) levels in infected children were significantly reduced with the p-value of < 0.01 in comparison to non-infected children while dramatic significant reduction noted in vitamin D (ng/mL) levels with p-value of < 0.0001.

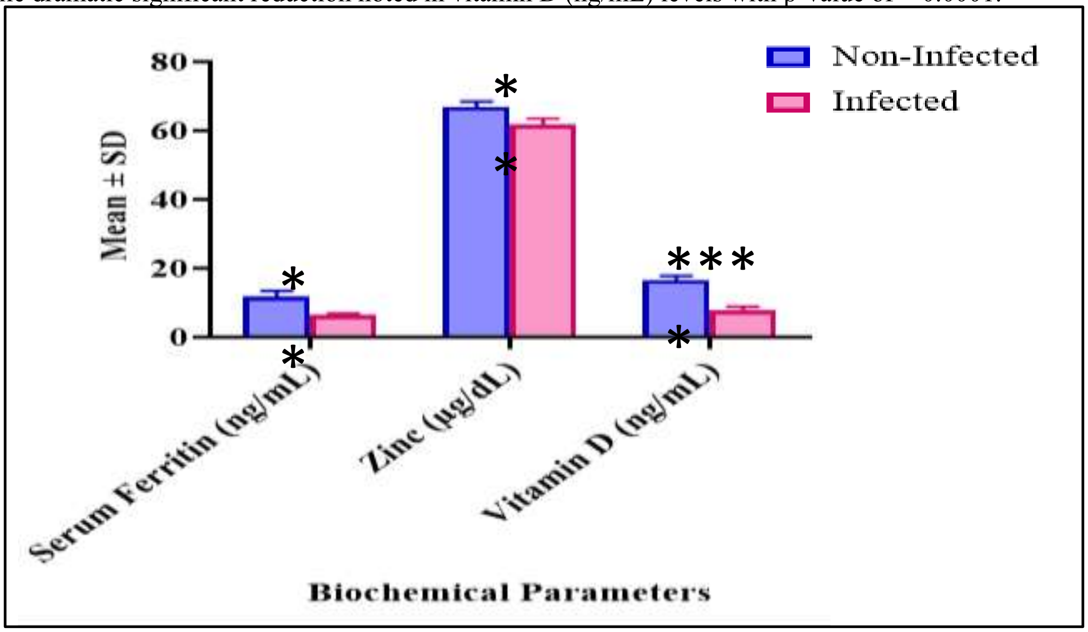

Figure 3. Comparative analysis of the biochemical parameters of the study participants. Two-way ANOVA followed by Sidak's multiple comparisons test statistically tested the differences between means.; Significant (p <0.01), ****; Significant (p <0.0001).**

Comparative Association of Biochemical Parameters with the Anthropometric Parameters

The significant association of biochemical profile (micronutrients) was observed with anthropometric parameters (weight (kg), height (cm) and BMI (kg/m^2) among the infected children presented in Figure 4. Figure 4A showed that serum levels of ferritin among all three groups consisted of reduced height, weight and BMI of infected children varied significantly. The ferritin concentration was found significantly higher in the category of weight (8.3 ± 0.7 ng/mL) while moderate levels (5.8 ± 0.3 ng/mL) was found in BMI. The least ferritin levels were observed in height category (4.8 ± 0.2 ng/mL). The comparisons evaluation showed a highly significant difference between weight and height (P < 0.001) and between weight and BMI (P < 0.01), whereas the difference between reduced height and BMI was not significant (p > 0.05). In the figure 4B, the zinc levels were found significantly higher in reduced weight category (60.33 ± 1.5 µg/dL) followed by height (54.37 ± 1.6 µg/dL) and reduced BMI category (50.7 ± 1.7 µg/dL). Highly significant differences were observed between weight and BMI category with the p-value of < 0.0001 while moderate significant difference was found in the category between weight and height with the p-value < 0.001 while non-significant association was found between the height and BMI with the p-value > 0.05. The figure 4C showed the higher vitamin D levels in the category of weight (10.9 ± 1.4 ng/mL) followed by height (7.5 ± 0.8 ng/mL) and BMI (6.3 ± 0.5 ng/mL). In this comparative analysis, the higher significant differences was found between the category of weight and BMI with p-value < 0.001 and moderate significant with the p-value < 0.01 between weight and height while non-significant differences (>0.05) was seen in between the height and weight category. Overall, the all biochemical parameters were found low in children with reduced height and BMI among the infected children.

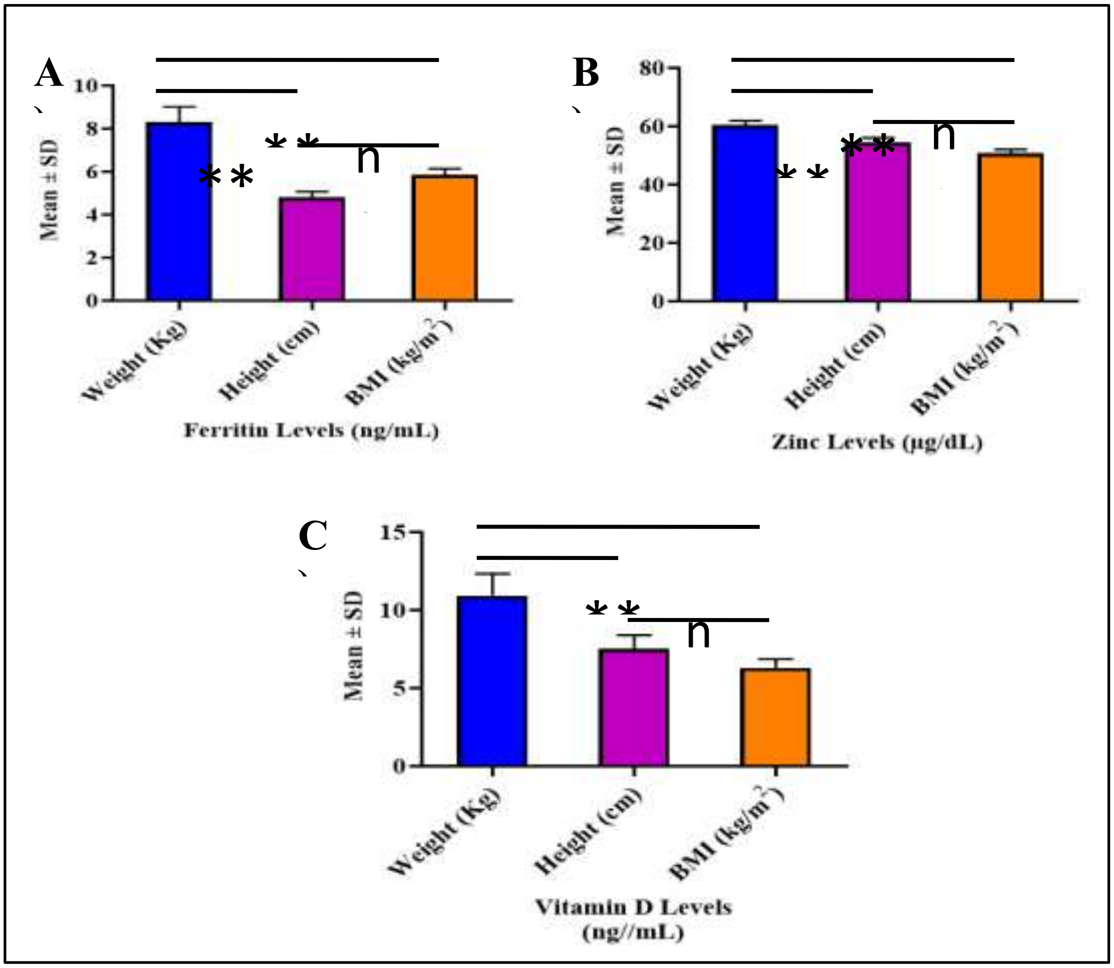

Figure 4. Comparative association of biochemical parameters (Ferritin, Zinc and Vitamin D) with the anthropometric parameters (Weight, Height and BMI) of infected children:

A) Association of serum ferritin among the anthropometric parameters, B) Association of zinc levels among the anthropometric parameters and C) Association of vitamin D levels among the anthropometric parameters. Two-way ANOVA followed by Turkey's multiple comparisons test statistically tested the differences between means.***; Significant (p <0.001), **; Significant (p <0.01), *; Significant (p <0.05), ns; not-significant.

DISCUSSION

Owing to their morbid characteristics and significant implications for health, parasitic infection constitutes the main reason behind sickness and ill health across the globe [18]. The deficiency of micro-nutrients is one of several health problems found in underdeveloped countries that gets complicated by infections and poor nutrition among children because of their high demand [19]. Poor intake of micro-nutrients and barriers against their proper absorption, due to parasite infection, ultimately led to the absence of necessary trace elements [20].

Therefore, the cross-sectional study was aimed to analyze the influence of intestinal parasitic infection on the anthropometric measurements and micronutrient levels. Our study was conducted on 140 cases whose age groups were between 2 and 12 years of both genders and lived in rural and urban areas. According to the results of this study, out of 140 cases, 57.8% children were infected with the presence of *E. histolytica.* The possibility that Pakistani's children are subjected to the parasitic infections might be due to the consumption of dirty vegetables, poor water quality and poor hygiene [21].

The socio-demographic parameters showed that females were more prevalent (55.5%) in comparison to males (44.4%). According to previous report, it was found that females had a higher rate of infection with intestinal parasites than males [22]. In the age category, 43.2% were found infected in 5-8 years of age while contrary to this, a report showed that higher frequency of intestinal infection among those aged > 11 years [23]. In this study, the children belonged to rural areas were found to be more prevalent of the parasitic infection (59.3%) than urban areas (40.7%). These results are in accordance with previous report that parasitic infections were highly associated to rural areas [24].

In the clinical manifestations, the higher frequency of gastrointestinal manifestations (abdominal pain 67.9%, diarrhea 58% and vomiting 53.8%) and fever (53.8%) were found in infected children. The higher incidence of gastrointestinal signs in infected children may be influenced by the pathogenic effects of intestinal parasitosis especially *E. histolytica*, which causes ulcerations of the intestinal mucosa, resulting in diarrhea and abdominal pain. Such disorders might contribute to poor nutrition and stunted development [25].

In this study, infected children had significantly lower anthropometric values included weight, height and BMI. This finding is consistent with other studies that found an extremely high correlation between intestinal parasite infection and stunting, being underweight and physical impairment. According to a systematic review, the chances of developing stunting among infected children were more than two times higher than those for uninfected children. Moreover, *E. histolytica* infection was associated with low linear growth and poor nutritional status among pre-school children [26]

In this present study, the non-infected subjects presented with high levels of serum ferritin, zinc, and vitamin D (11.83±1.5, 66.87±1.5, and 16.53±1.2 respectively), whereas the infected group had low levels of serum ferritin, zinc, and vitamin D (6.3±0.5, 61.85±1.6, and 7.7±1.1 respectively). The levels of serum ferritin were significantly lower in patients who were infected in this current study. This has also been previously established through studies revealing that infection by *E. histolytica* is linked with low ferritin levels [27]. Low levels of iron reserves caused by intestinal inflammation, reduced food consumption, intestinal blood loss and poor nutrient absorption may result in iron deficiency. Low levels of serum ferritin have also been observed in children suffering from *E. histolytica* infection in Iraq [28]. The finding on the decreased level of serum zinc in the infected group is in accordance with previous findings [10]. Zinc deficiency can be due to poor absorption through the intestines, increased loss through diarrhea, and increased demand for the mineral during the infectious period. Being that zinc has an important part in immune response, growth, and tissue repair, then deficiency in it may further lead to poor immune status and growth disorders [29].

In addition, it is pertinent to discuss the effect of infection on the vitamin D status in infected children. In addition to its well-known function in calcium metabolism and bone growth, vitamin D acts as a crucial immunomodulator hormone. Vitamin D receptors are present on many immune system cells such as macrophages, dendritic cells and lymphocytes. Low vitamin D levels have been related to defective immune responses and increased risk of infections [30]. The intestinal inflammation resulting from parasitic infection can cause reduced absorption of lipophilic vitamins like vitamin D, leading to low blood levels of vitamin D in infected children [31]. Moreover, deficiency of vitamin D during childhood has been correlated with deficient bone mineralization and impaired growth, hence the shorter heights seen in infected children.

Current results are also supported by the concept of the infection-malnutrition cycle. According to the World Health Organization (WHO), malnutrition and infection are two factors that have a synergetic effect on one another, and they both aggravate the conditions associated with each other [32]. Multiple intestinal infections could affect appetite, nutritional needs, and nutritional absorption, leading to under nutrition. On the other hand, a child suffering from malnutrition is predisposed to infectious diseases due to poor immune function. Thus, the cycle continues, becoming a serious public health concern for developing countries experiencing endemic parasites [33].

As far as public health is concerned, such results suggest that the inclusion of nutritional interventions in the prevention and management of parasitic diseases is essential. Nutritional screening, treatment against intestinal parasites, proper nutrition, and micronutrient supplementation could assist in the mitigation of growth retardation and deficiency of micronutrients in children. In addition, the provision of sanitation facilities, potable water supply, health education, and better personal hygiene habits are also important preventive strategies that can contribute to lowering the risk of parasitic infections and the adverse effects on the health of children.

LIMITATIONS OF THE STUDY

The study was carried out in a certain geographic location using a small population of children, limiting the ability of these findings to be generalized to a wider population. The differences that exist among different geographic locations regarding factors such as socioeconomic levels, dietary intake, environment, health care, sanitation, and parasitic infection could lead to variations in the nutritional and growth status of individuals in different locations.

CONCLUSION

This study showed that children infected with *E. histolytica* revealed a higher prevalence of gastrointestinal symptoms and fever, considerably lower anthropometric measurements (weight, height and BMI) along with reduced serum ferritin, zinc, and vitamin D levels in comparison to non-infected children. These results recommend a significant association between *E. histolytica* infection, impaired nutritional status and micronutrient deficiencies. The observed reductions in growth parameters and essential micronutrients highlight the potential impact of parasitic infection on child health and development. Early detection and proper management of *E. histolytica* infection, along with nutrition evaluation and appropriate supplementations of micronutrients, may reduce health risks. Consequently, additional longitudinal studies on a larger scale are needed in order to understand the causal relationship between parasitic infection, stunting and nutrient deficiencies to help develop an intervention program for affected children.

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AUTHORS' CONTRIBUTIONS:

Ali Abuzar Raza contributed to the study conception, methodology, data collection, analysis, and manuscript preparation. **Iqra Saleem, Sumera Malik, and Sajida Zafar** contributed to data collection, interpretation of findings, and critical revision of the manuscript. **Syed Zeeshan Haider Naqvi** contributed to study supervision, interpretation of results, manuscript review, and final approval of the manuscript. All authors read and approved the final manuscript.

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