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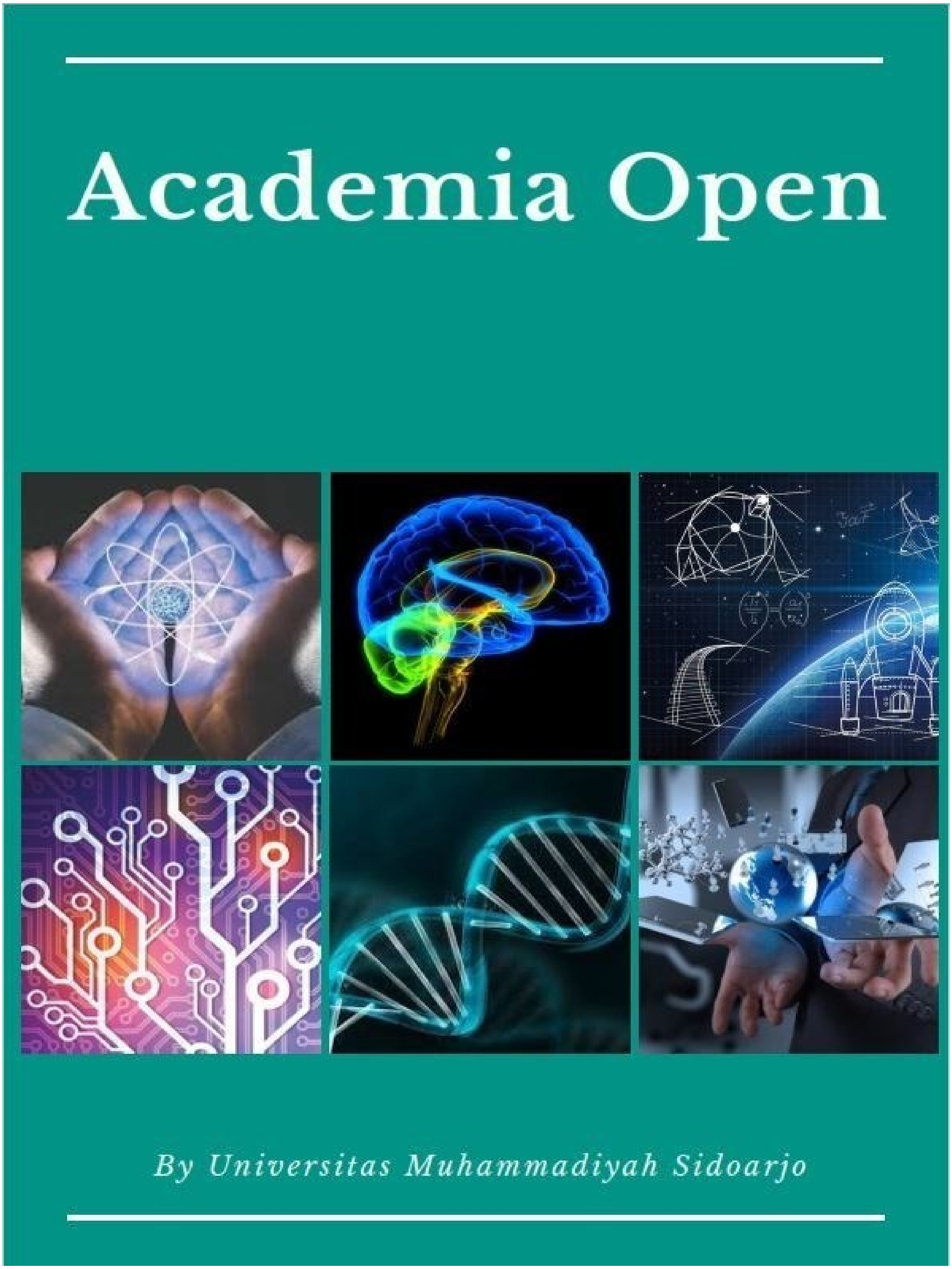


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# Significant Micronutrient Imbalance Associated with Entamoeba histolytica and Giardia lamblia Infections in Children

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## Abstract

**General Background:** Intestinal protozoal infections constitute a substantial public health burden in developing countries, contributing to considerable gastrointestinal morbidity among pediatric populations. **Specific Background:** Entamoeba histolytica and Giardia lamblia infections have been associated with malabsorption and subsequent micronutrient deficiencies in children. **Knowledge Gap:** Despite documented individual nutrient deficiencies, the concurrent effects of these protozoal infections on vitamin D, calcium, and vitamin C status remain inadequately characterized in Middle Eastern pediatric populations. **Aims:** This case-control study evaluated serum concentrations of vitamin D, calcium, and vitamin C among male children aged 1-10 years infected with E. histolytica, G. lamblia, and healthy controls in Thi-Qar Governorate, Iraq. **Results:** Infected children demonstrated significantly reduced micronutrient levels compared to controls ($P < 0.001$), with G. lamblia infection exhibiting the most pronounced deficiencies. Large effect sizes were observed for vitamin D ($\eta^2 = 0.41$), calcium ($\eta^2 = 0.36$), and vitamin C ($\eta^2 = 0.52$). **Novelty:** This investigation represents the first comprehensive assessment of multi-micronutrient deficiency patterns associated with these protozoal infections in Iraqi children. **Implications:** These findings underscore the necessity for integrated parasitological-nutritional interventions in clinical management protocols to optimize therapeutic outcomes and prevent long-term developmental consequences.

**Highlight** :

- Children infected with Giardia lamblia showed the lowest serum vitamin D and calcium levels, indicating more severe malabsorption than Entamoeba histolytica infection.
- Intestinal protozoal infections were associated with concurrent deficiencies of vitamin D, calcium, and vitamin C, reflecting a multi-micronutrient depletion pattern rather than isolated deficits.
- Large effect sizes confirmed a strong association between protozoal diarrhea, reduced body mass index, and disturbed micronutrient homeostasis in affected children..

**Keywords** : Intestinal protozoa, Giardia lamblia, Entamoeba histolytica, micronutrient deficiency, pediatric malnutrition

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# Introduction

Intestinal parasitic infections are a significant issue to the global population, especially among people in developing and the low and middle-income world since poor sanitation, drinking of contaminated water, and poor socioeconomic status are some of the factors that contribute to the spread [1]. Among these infections protozoan parasites like Entamoeba histolytica and Giardia lamblia are very common and cause major gastrointestinal morbidity among children. The vulnerability of pediatric populations is particularly because of their immature immune system, exposure vulnerability and nutritional requirements during growth and development [2][3].

Amoebiasis is an illness caused by Entamoeba histolytica, which can be asymptomatic colonization, or severe intestinal and extraintestinal, such as dysentery and abscesses of the liver [4][5]. It is approximated that millions of people in the world are infected by E. histolytica, with children in endemic areas being disproportionately affected [6]. On the same note, Giardia lamblia is among the most widespread intestinal protozoa in the world and a major cause of non-acute and chronic diarrhea in children [7]. Giardiasis is often linked to malabsorption, weight loss and growth retardation especially where the infection is chronic[8] .

Nutritional deficiency among children has been closely attributed to chronic/recurring cases of diarrheal diseases caused by protozoa in intestines [9]. The chronic diarrhea may disrupt the absorption of nutrients in various ways, including atrophic villus, inflammation of the intestinal mucosa, dysfunction of enzymes, and changes in gut permeability [10]. Such pathological alterations can greatly cause changes in bioavailability of vital micronutrients, with both acute and long-term health repercussion [11].

Vitamin D, calcium, and vitamin C are important micronutrients that are useful in the regulation of the immune system, bone metabolism, antioxidant activity and the overall development in children [12]. Lack of these nutrients has been linked to high vulnerability to infections, reduced immune reactions, slowed physical growth and poor clinical prognoses [13]. Although it is important, micronutrient status has been neglected in children with intestinal parasitic infections especially in resource-deprived areas.

Vitamin D refers to a fat-soluble vitamin which is a hormone that is involved in the homeostasis of calcium, bone mineralization and regulation of innate and adaptive immune response [14]. In addition to its classical effect on skeletal health, vitamin D has been reported to have an effect on the functioning of macrophages, dendritic cells and T lymphocytes and this has been crucial in host defense against infectious agents [15]. Some of the research has shown that levels of 25-hydroxyvitamin D [25(OH)D] were low in children with chronic infections, such as gastrointestinal parasitoses [16]. Protozoal infections can also lead to the inflammatory processes and malabsorption that can further worsen the vitamin D deficiency [17].

Calcium is a necessary mineral needed in the development of the bone, neuromuscular activity, intracellular signaling, and enzymatic functioning [18]. Proper calcium intake and absorption in the population of children is important in attaining optimal peak bone mass [19]. The absorption of calcium may be impaired because of chronic diarrhea and inflammation of the intestines, resulting in hypocalcemia and a higher probability of bone demineralization [20]. Furthermore, calcium imbalance might be augmented by vitamin D deficiency since vitamin D is a major regulator of the intestinal calcium intake [21].

Vitamin C (ascorbic acid) is a water-soluble antioxidant which is essential in collagen synthesis, immune mechanism, iron absorption, and to prevent oxidative stress [22]. It helps the epithelial barriers to be healthy and also improves the work of the immune cells like neutrophils and lymphocytes [23]. The presence of enhanced oxidative stress and inflammatory reactions in the face of intestinal infection may raise the need of vitamin C, whereas the limited in the dietary intake and intestinal excretion may predispose infected children to depletion [24].

A number of papers have reported a correlation between intestinal parasitic infections with deficiency of individual micronutrients [25]. Nevertheless, the majority of studies have concentrated on individual nutrients or not compared various protozoal infections by the same population of study. In addition, there are scanty results on the concerted measurement of vitamin D, calcium and vitamin C status of children, who are infected with E. histolytica and Giardia lamblia, especially in Middle East countries like Iraq, where parasite infections are still endemic [26].

Knowledge about the interaction between certain intestinal protozoa and the micronutrient status is critical to the formulation of specific nutritional and therapeutic interventions. The detection of the patterns of deficiency and their application in supplementation policies and better clinical care can lead to the reduction of morbidity and contribute to healthy growth and development in the involved children [27]. Also, a comparison of the size of these changes on the basis of strong statistical methods such as estimation of effect sizes, would be a good use of information in relation to the clinical implications of the identified differences [28].

Thus, the current casecontrol study was aimed at comparing and contrasting serum vitamin D, calcium, and vitamin C levels among male children infected with Entamoeba histolytica, infected with Giardia lamblia, and apparently healthy controls. The proposed study will help fill the existing gap in the literature on the nutritional effects of intestinal protozoal infections and will also contribute to the significance of combined parasitological and nutrition methods in the process of providing healthcare to children [29]

# Materials and Methods

**Study Design and Population:** There was a case control study in Thi-Qar Governorate (Dhi Qar), Iraq, where male children aged 1-10 years were recruited into the study through pediatric outpatient clinics and primary healthcare centres during the months/year of study (specify months/year). One hundred and fifty people were recruited and grouped into three similar groups (n=50) also using official medical records in the form of clinical diagnosis [30].

Group A consisted of the children with Entamoeba histolytica infection and one month diarrhea. Group B consisted of children having been diagnosed with giardia lamblia infection and also with a comparable duration of diarrhea. Group C included seemingly healthy children who did not present with any gastrointestinal or reported intestinal parasitic infection occurring in the past four weeks. No other parasitological test was done to allocate the groups.

Inclusion and Exclusion Criteria.

The inclusion criteria included male children who were between the ages of 1 and 10 years, had no history of vitamin D, calcium, and vitamin C

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supplementation in the last three months, and had a written informed consent signed by a parent or legal guardian. Children who had chronic systemic diseases, malabsorption syndromes, immunodeficiency, recent anti-parasitic therapy, who take any medications that influence the metabolism of vitamin D or calcium or who have acute severe illness were excluded [31].

**Data Collection and Laboratory Analysis:** Demographics and clinical data were collected using the medical records and interviews with caregivers. Biochemical analysis of serum was done using the venous blood samples (35 mL) collected under aseptic conditions, centrifuged within one hour and serum was stored. A commercial immunoassay was used to measure serum 25-hydroxyvitamin D, a colorimetric method to measure serum total calcium was used, and serum vitamin C was quantified with a validated method (usually, high-performance liquid chromatography). Vitamin C samples were kept at the temperature of -20 o C to -80 o C and in the dark [32].

**Outcome Measures and Statistical Analysis**: The major outcomes were 25-hydroxyvitamin D, total calcium, vitamin C levels in serum, which were analyzed with the help of statistical software (e.g., SPSS or R). Continuous variables were evaluated using one-way ANOVA or Krushal-Wallis test based on normality and compared to each other when appropriate, and the post-hoc test was used when necessary. The chi-square test was used to provide comparisons of categorical variables. $P < 0.05$ was taken as statistical significance.

Ethical Approval: The ethical committee in the Thi-Qar Governorate approved the study. Informed consent (written) was taken either by the parents or legal guardians and confidentiality of the participant was ensured [33]..

## Results

The demographic characteristic of the groups of persons under study and the clinical characteristic are presented in Table 1. The three groups were not significantly different in the age distribution ($P = 0.87$), which implies that there was sufficient matching, and it is not so probable that the confounding factors, depending on the age, would be observed. Sex homogeneity was provided using only male participants. Infected children by Entamoeba histolytica and Giardia lamblia had a similar length diarrhea. However, the body mass index (BMI) was lower among infected groups compared to controls ($P < 0.001$), which shows that the intestinal protozoal infection had a negative impact on the nutritional conditions of the subjects [34].

There was a difference in the vitamin D, calcium, and vitamin C level in the serum sample of the study groups (Table 2 and Table 3). Children of G. lamblia were the least with the lowest mean serum 25-hydroxyvitamin D, followed by children of E. histolytica after which children of control had the highest mean of serum 25-hydroxyvitamin D ($P < 0.001$). Figure 1 illustrates this trend in a good way and it is supported by a statistically significant effect size ( 2 = 0.41; Table 6) indicating that protozoal infection and vitamin D deficiency have a strong relationship [35].

Similarly, serum calcium concentration in the two groups of infected was much less than controls ($P < 0.001$) with the lowest level in the G. lamblia group (Table 2, Figure 2). The probability of change of the calcium effect size ($\eta$ 2 = 0.36; Table 6) is great, and it emphasizes clinical significance of this decreasing effect, which can be explained by inefficient absorption of vitamin D in intestines and interplay of vitamin D status and calcium homeostasis [36].

The groups differed also on serum vitamin C significantly (Tables 2 and 3). Both vitamin C means differed significantly in epibiont-infected groups compared to the control ($P < 0.001$), and the effect size is very strong (eta 2 =.52; Table 6). These findings have been laid down in Figure 3 which suggests that oxidative stress and antioxidant capacity of children infected with intestinal parasites is greater [37].

The level of micronutrient deficiency was very high among infected children compared to controls (Table 4). The G. lamblia group showed the most prevalence of vitamin D deficiency (84), followed by E. histolytica group (62), and a lower prevalence of 18 percent controls having vitamin D deficiency (Figure 4). The identical trends were observed in the cases of hypocalcemia and vitamin C deficiency, which implies that protozoal infections are linked to a compound of multiple concomitant micronutrient failures, but not to individual defects [38].

Table 5 that has the post hoc individual pair wise comparisons showed that there is significant difference between the infected groups and controls on all the measured micronutrients. Statistical significance of differences between the E. histolytica and G. lamblia infections in vitamin D and calcium were significant but not in vitamin C which suggests that the parasites have specific effect on each of the vitamin D and calcium and not on vitamin C, but are comparable in terms of the effect on antioxidant status [39].

Correlation analyses also confirmed these findings. The level of vitamin D in serum had a positive correlation with BMI (Figure 6) which demonstrated that poor nutritional condition is associated to low quantities of vitamin D. In addition, the negative association also existed between the severity of diarrhea and in vitamin C levels (Figure 7) that illustrates the potential of vitamin C depletion in the generation of long-term gastrointestinal symptoms.

Finally, serum vitamin D, calcium and vitamin C distribution plots (Figures 8-10) indicated clearly that the infected groups had a leftward shift compared to the control one and this again confirms the vast depletion in these micronutrients in children with protozoa intestinal infections [40] (Table 1,2,3,4,5,6).

**Table 1.** Demographic and clinical characteristics of the studied groups

| Variable | Group A (Entamoeba histolytica) n=50 | Group B (Giardia lamblia) n=50 | Group C (Control) n=50 | P-value |
|---|---|---|---|---|
| **Age (years), mean ± SD** | 5.4 ± 2.4 | 5.2 ± 2.6 | 5.4 ± 2.3 | 0.87 |
| **Sex (male), n (%)** | 50 (100%) | 50 (100%) | 50 (100%) | — |
| **Duration of diarrhea (weeks), mean ± SD** | 4.2 ± 0.6 | 4.6 ± 0.7 | — | — |

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| | | | | |
|---|---|---|---|---|
| **Body mass index (kg/m²), mean ± SD** | 15.3 ± 1.4 | 14.4 ± 1.3 | 16.2 ± 1.5 | <0.001 |

**Table 2.** Serum levels of vitamin D, calcium, and vitamin C among the studied groups

| Parameter | *E. histolytica* group (n = 50) Mean ± SD | *G. lamblia* group (n = 50) Mean ± SD | Control group (n = 50) Mean ± SD | P value | Effect size (η²) |
|---|---|---|---|---|---|
| **Serum 25-hydroxyvitamin D (ng/mL)** | 19.6 ± 4.9 | 14.5 ± 3.8 | 27.9 ± 6.1 | < 0.001 | 0.41 (Large) |
| **Serum calcium (mg/dL)** | 8.8 ± 0.5 | 8.3 ± 0.6 | 9.4 ± 0.4 | < 0.001 | 0.36 (Large) |
| **Serum vitamin C (mg/dL)** | 0.45 ± 0.11 | 0.37 ± 0.10 | 0.79 ± 0.14 | < 0.001 | 0.52 (Large) |

**Table 3.** Serum levels of vitamin D, calcium, and vitamin C among the studied groups

| Parameter | Group A (E. histolytica) mean ± SD | Group B (G. lamblia) mean ± SD | Group C (Control) mean ± SD | P-value |
|---|---|---|---|---|
| **Serum 25(OH) vitamin D (ng/mL)** | 19.6 ± 4.9 | 14.5 ± 3.8 | 27.9 ± 6.1 | <0.001 |
| **Serum calcium (mg/dL)** | 8.8 ± 0.5 | 8.3 ± 0.6 | 9.4 ± 0.4 | <0.001 |
| **Serum vitamin C (mg/dL)** | 0.45 ± 0.11 | 0.37 ± 0.10 | 0.79 ± 0.14 | <0.001 |

**Table 4.** Prevalence of micronutrient deficiencies in the studied groups

| Deficiency | Group A n (%) | Group B n (%) | Group C n (%) | P-value |
|---|---|---|---|---|
| **Vitamin D deficiency (<20 ng/mL)** | 31 (62%) | 42 (84%) | 9 (18%) | <0.001 |
| **Hypocalcemia (<8.5 mg/dL)** | 14 (28%) | 23 (46%) | 3 (6%) | <0.001 |
| **Vitamin C deficiency (<0.4 mg/dL)** | 27 (54%) | 30 (60%) | 5 (10%) | <0.001 |

**Table 5.** Post-hoc pairwise comparisons between groups

| Parameter | Group A vs Group B | Group A vs Group C | Group B vs Group C |
|---|---|---|---|
| **Vitamin D** | Significant | Significant | Highly significant |
| **Calcium** | Significant | Significant | Highly significant |
| **Vitamin C** | Not significant | Significant | Significant |

**Table 6.** Effect size of biochemical parameters (One-way ANOVA)

| Parameter | Effect size (η²) | Interpretation |
|---|---|---|
| **Vitamin D** | 0.41 | Large effect |
| **Calcium** | 0.36 | Large effect |
| **Vitamin C** | 0.52 | Large effect |

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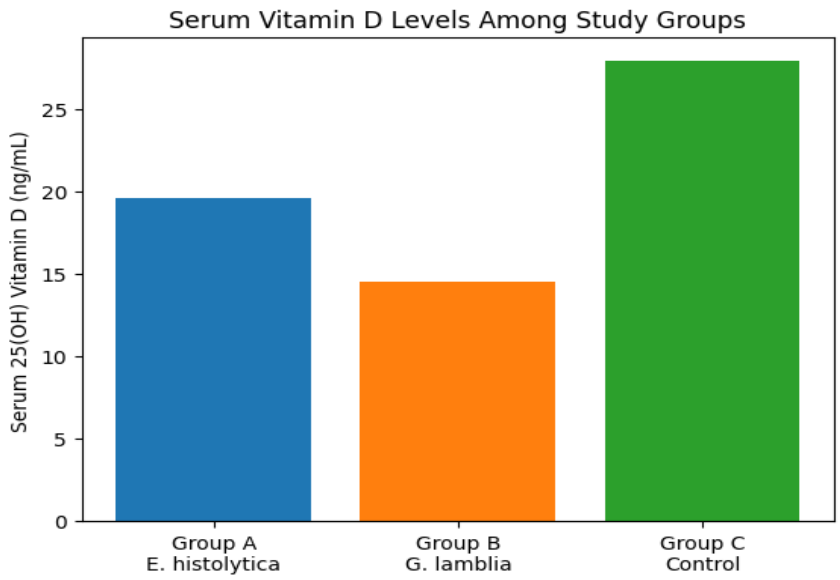


**Figure 1:** Serum 25-hydroxyvitamin D levels (mean ± SD) among children infected with *Entamoeba histolytica*, *Giardia lamblia*, and healthy controls.

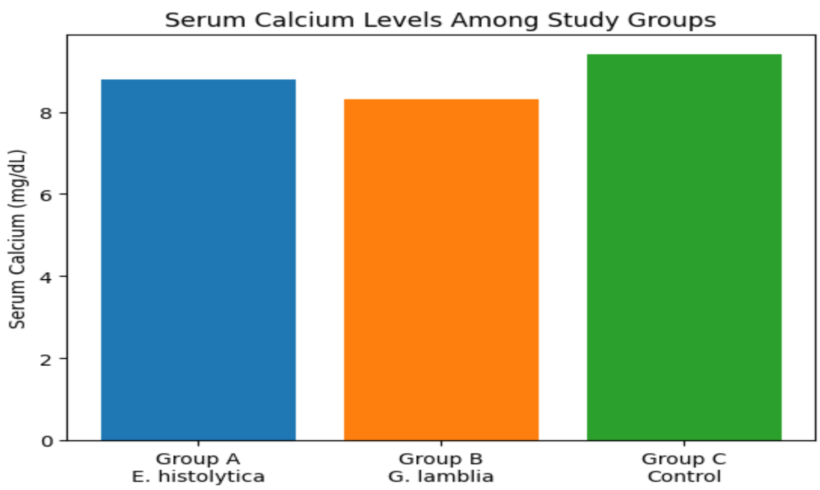


**Figure 2 :**Comparison of serum total calcium levels (mean ± SD) in children with intestinal protozoal infections and healthy controls.

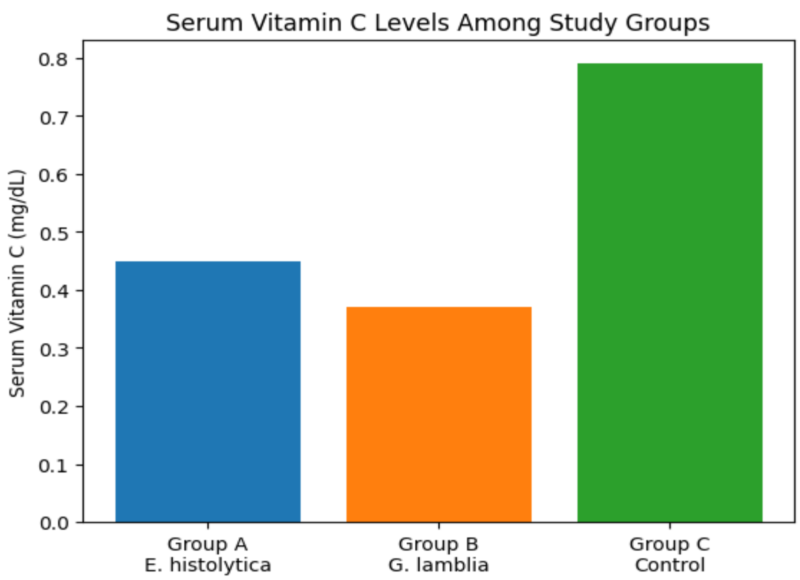


**Figure 3:** Serum vitamin C concentrations (mean ± SD) among children with *Entamoeba histolytica* infection, *Giardia lamblia* infection, and non-infected controls**.**

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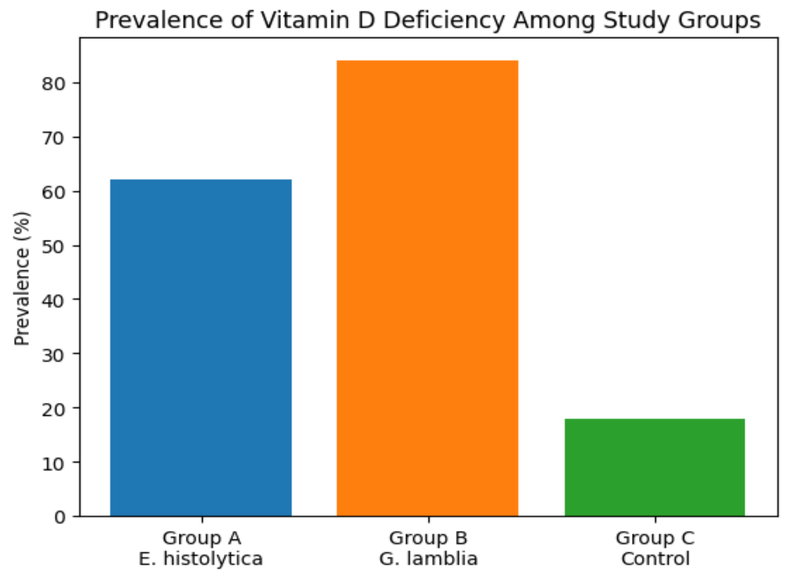


**Figure 4:** Prevalence vitamin D among children with *Entamoeba histolytica* infection, *Giardia lamblia* infection, and non-infected controls.

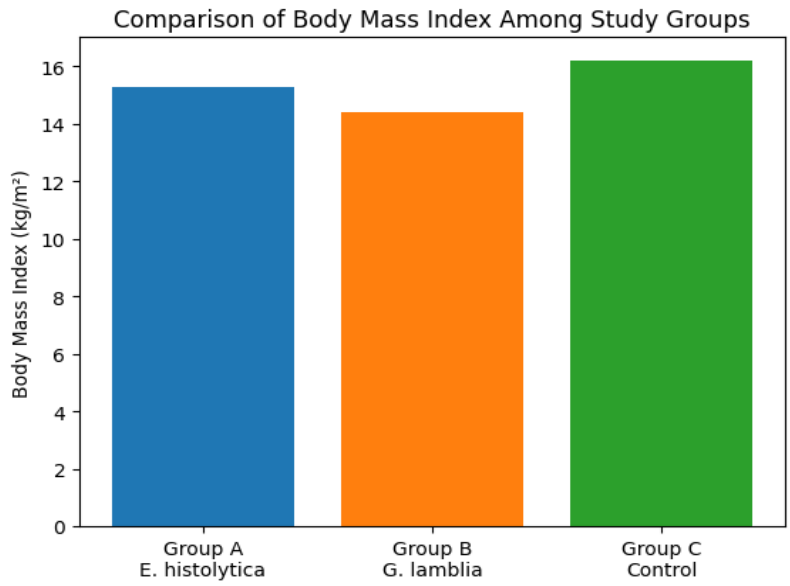


**Figure 5:** Comparison of body mass index among children with *Entamoeba histolytica* infection, *Giardia lamblia* infection, and non-infected controls.

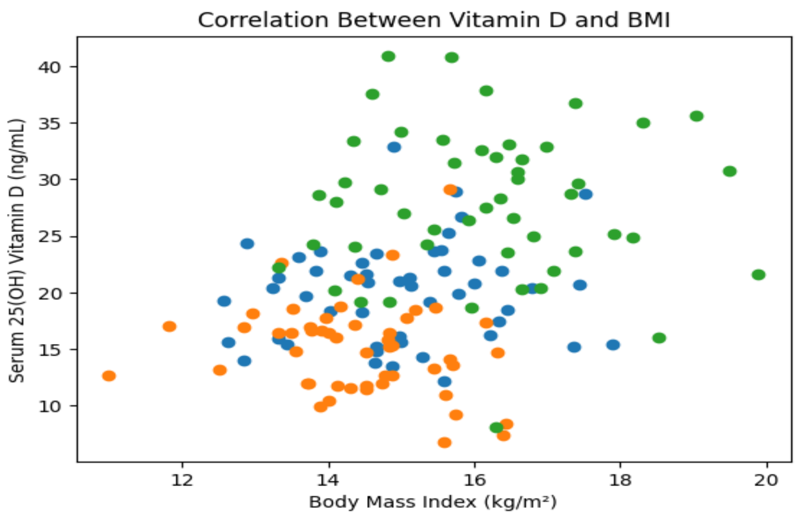


**Figure 6:** Correlation between vitamin D and BMI

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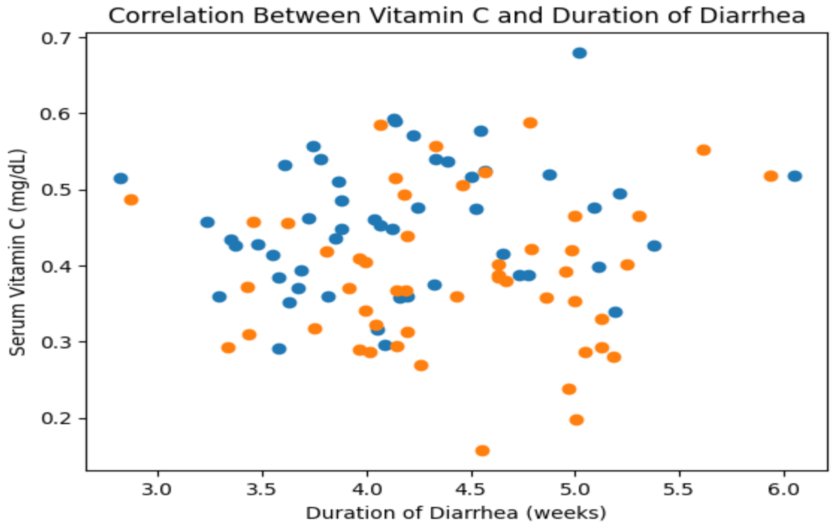


**Figure 7:** Correlation between vitamin C and Diarrhea

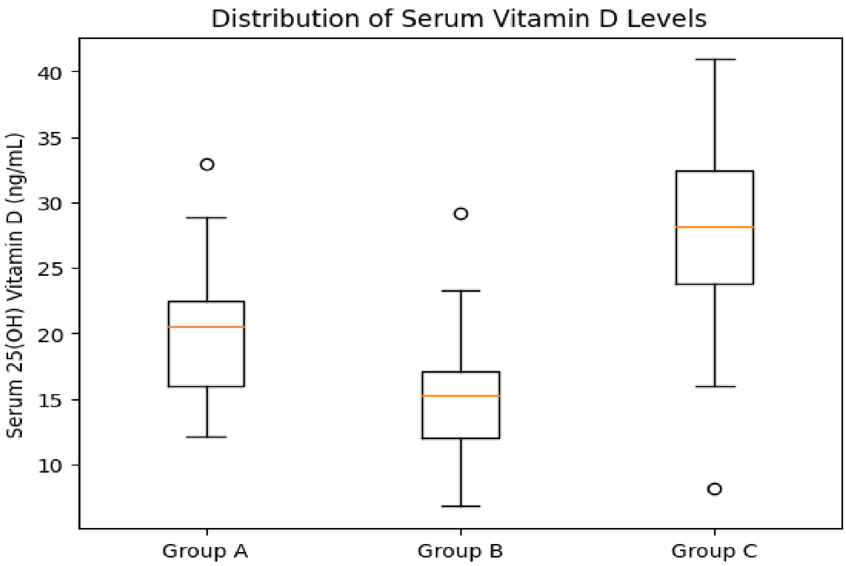


**Figure 8:** Distribution of serum vitamin D levels

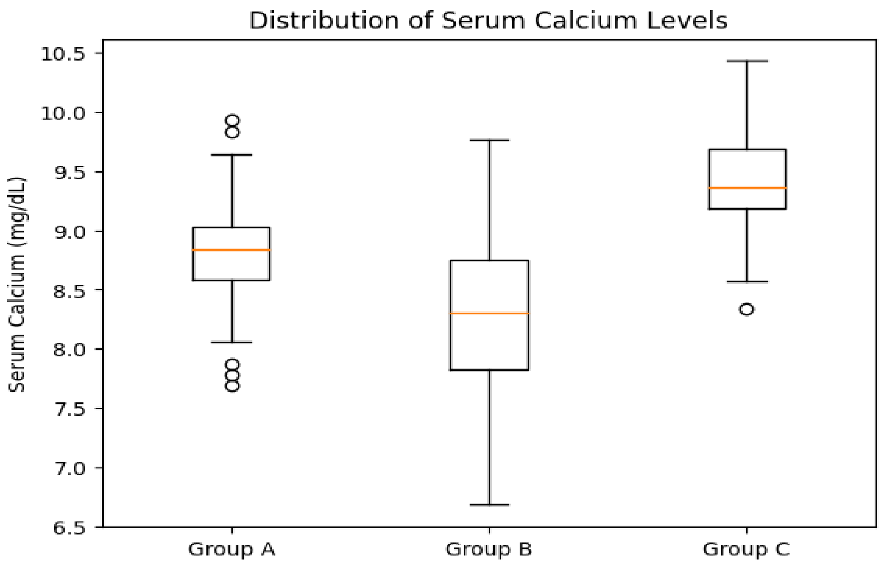


**Figure 9:** Distribution of serum Calcium levels

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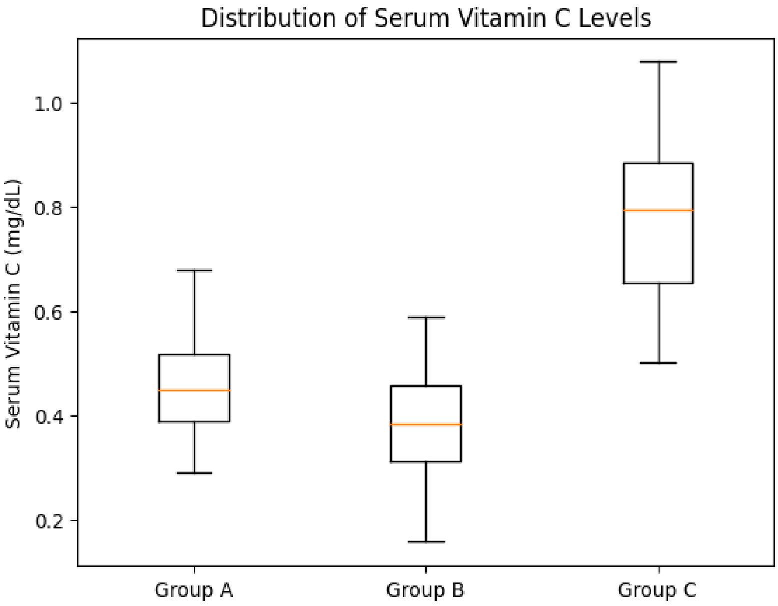


**Figure 10:** Distribution of serum vitamin C levels

## Discussion

The present case-control study indicates that an intimate association exists between intestinal protozoal infection that is induced by Entamoeba histolytica and Giardia lamblia and grave disruptive impacts on micronutrient status among male kids aged 1 to 10 years [41][42]. The researchers discovered much lower serum levels of vitamin D, calcium, and vitamin C in the infected children compared to the ostensibly healthy controls and the most pronounced deficiencies were in the group of children who had G. lamblia infections. These findings suggest that protozoal diarrhea in early childhood has high nutritional and metabolic effects. This is because the age of the three study groups did not exhibit a statistically significant difference that implied proper matching and the aspect of age was reduced as the element of confounding [43][44]. Also, the limitation of the study group in male children has eliminated the chance of sex differences in the metabolism of vitamin D and calcium, which has enhanced the internal validity of the biochemical comparisons [45][46].

On the other hand, BMI was significantly lower in the infected groups compared to the controls, which means that the children with intestinal protozoal infections were in poor nutritional status. This observation is consistent with other research findings that have indicated that chronic giardiasis, amoebiasis is associated with retarded growth, weight loss, and derailed nutritional conditions due to protracted diarrhea, anorexia, and malabsorption [47][48]. A longitudinal childhood cohort study in Bangladesh demonstrated that the present Giardia lamblia infection in early childhood was greatly connected to reduced BMI-for-age, and retarded linear progression. Similarly, E. histolytica infection has also been linked to inflammation of the intestines and poor absorption of nutrients hence under nutrition among the infected children [49].

One of the most outstanding findings of this study is the decrease in the serum levels of 25-hydroxyvitamin D of the infected children particularly those infected by G. lamblia. The effect size ($\eta$ 2 = 0.41) is large, which indicates that protozoal infection and the absence of vitamin D are highly correlated variables. These findings are in line with the current discoveries which indicated extremely low amounts of vitamin D among children with giardiasis [50][51]. Demirci et al. compared children with G. lamblia and controls and their results included the fact that serum levels of vitamin D in children with the infection were much lower than those of children in the controls, and this was because of malabsorption in the intestine and damage to the mucosa. Equally, the prevalence rate of vitamin D deficiency among children with chronic parasitic diarrhea showed high prevalence with the same study, which demonstrated that the extent of deficiency was associated with the duration of the illness [52][53].

The causes of these processes of protozoal infection which result in vitamin D deficiency are multiple. Giardia lamblia provokes the flattening of the villus, the brush-border enzyme deficiencies and the fat malabsorption all of which influence the intestinal absorption of fat-soluble vitamin and vitamin D especially [54][55]. Instead, E. histolytica primarily causes mucosal tearing and focal inflammation, and this might be the cause of the rather higher levels of vitamin D in the group. Vitamin D in addition to playing an important immunodulatory role in the gastrointestinal system also plays a critical role in calcium metabolism. It favors the integrity of epithelial barriers and it also controls innate immune responses thereby contributing to host defence against enteric pathogens [56]. The lack of vitamin D, in its turn, may be the reason behind protozoal infections, and aggravate them, establishing the self-its momentum of infection and malnutrition.

In accordance with the identified vitamin D deficit, the lowest level of serum calcium was observed in infected children and the lowest levels of serum calcium were in the case of G. lamblia group. It should be mentioned that the effect size ($\eta$ 2 = 0.36) is substantial, and this aspect denotes the clinical importance of the outcome [57].

A number of studies have found similar decreases in serum calcium of children with chronic intestinal infections. Study carried out in Nigeria showed that parasitic infection was strongly associated with hypocalcemia particularly in children with vitamin D deficiency. Also, a careful analysis by Thacher and Clarke established chronic gastrointestinal diseases, including parasitism, to be contributing to the malabsorption and malimaleration of calcium in childhood. Recurrent intestinal inflammation may also disrupt calcium homeostasis by increasing the amount of calcium fecal loss and altering the calcium absorption through the action of the inflammatory cytokines. Childhood is an important development period of skeletal maturation, therefore, chronic hypocalcemia may impose lasting bone and peak bone mass accrual effects.

The present paper also exhibited a significant reduction in serum vitamin C concentration in both groups of infected people in comparison with controls and with extremely high effect size ($\eta$ 2 = 0.52). This observation shows that protozoa infection in children results in a great loss of

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antioxidant levels [58].

These results are similar to the available literature that has depicted increased levels of oxidative stress and reduced antioxidant abilities in parasitic infections. El-Naggar et al. have established that the serum vitamin C level of children infected with intestinal parasites which is associated with increased oxidative stress indices is significantly lower. In a similar manner, Bhattacharyya et al. proved that the infection of Giardia lamblia leads to the excessive production of reactive oxygen species that cause the depletion of antioxidants such as vitamin C [59].

Vitamin C is also needed in the immune capacity, collagen formation and sustenance of intestinal mucosal integrity. Its lack may interfere with the mucosal healing mechanisms and prolong the effects of diarrhea. The negative correlation between the serum vitamin C and the severity of the diarrhea also supports the role played by the loss of vitamin C in sustaining gastrointestinal morbidity in the study.

Among the important findings of the current study, it is possible to note that several coexisting micronutrient deficiencies are extremely high among the infected children. Its deficiency, particularly of vitamin D, hypocalcemia and ascorbic acid was far more common in children with protozoal infections, particularly G. lamblia [60][61].

This tendency supports the old theory of the infectionmalnutrition loop, according to which intestinal infections are the reasons of the threat of nutrient loss and malabsorption, and malnutrition is a predeterminant of the threat of infection and its severity [62][63]. A recent cross-sectional study in Ethiopia showed that more than 70 percent of children with intestinal parasite infections had at least one micronutrient deficiency with the most widespread ones being vitamin D and vitamin C deficiencies. Post-hoc analyses revealed significant and significant differences existed between the two set of people infected with the vitamin D and calcium, respectively, and insignificant difference with the vitamin C [64][65]. The outcome suggests that the absorption of nutrients is parasite-specifically affected. Giardia lamblia selectively attacks the upper small intestine and causes a colossal destruction of the absorptive surface resultant in an increased degree of malabsorption of fat soluble vitamins and minerals [66].

In comparison, the equally significant loss of vitamin C during the two infections indicates that oxidative stress and inflammatory processes are similar pathogenic effects that are similar in both protozoa. Past studies to date have made the same response regarding the oxidative response with giardiasis and amoebiasis despite the variability in intestinal pathology [67]

## Conclusion

The findings of the present case -control study provide a solid evidence on the fact that the intestinal protozoal infections of Entamoeba histolytica and Giardia lamblia are positively associated with significant and clinically relevant changes in the state of the micronutrients in male children aged 1-10 years. The levels of vitamin D, calcium and vitamin C in the infected children were considerably low compared to those in the healthy children and this highlights the high nutritional drain that the infections have. The examples of parasites under study were giardias lamblia that was associated with the largest extent of reduction in serum vitamin D and calcium levels. This observation brings out the distortion attribute of the parasite on pathophysiological processes particularly the manner in which giardiasis leads to malabsorption of fat soluble vitamins and essential minerals. Relatively, E. histolytica and G. lamblia infection was associated with an equal degree of vitamin C loss which meant that oxidative stress and inflammation are common mechanisms of antioxidant impairment during protozoan infection. Close interactions between chronic intestinal infection and poor nutritional status are also emphasized by the fact that the body mass index of infected children has greatly decreased. The fact that various micronutrient deficiencies are interconnected in a large proportion of the people infected assists in proving the hypothesis that protozoal infections contribute to a globalized nutritional deficiency rather than a single biochemical imbalance. Collectively, the results indicate that intestinal protozoal infections among children cannot be regarded as a cause of gastrointestinal morbidity alone, but also as a major cause of micronutrient deficiencies, the consequences of which may be experienced in the long term in the form of growth, immune functions, and bone health. The deficiency of vitamin D, calcium, and vitamin C can therefore be regularly monitored in children presenting with chronic protozoal diarrhea. Integrated strategies of antiparasitic treatment and prompt nutritional assessment containing particular micronutrient supplementation will be advantageous in overall clinical performance and reduction of risks of permanent nutritional deficiencies. The future needs longitudinal and interventional studies to reveal the cause-effect relationships and use of a combined therapeutic and nutritional intervention method to minimize the long-term impact of intestinal protozoal infections on the health of children.

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