Journal of Communicable Diseases
Volume 54, Issue 2 - 2022, Pg. No. 83-88
Peer Reviewed & Open Access Journal

Research Article

# The Effect of Giardia lamblia Infection during the Partial Ban Period due to COVID-19 on Micronutrients among Children in Babylon Province, Iraq

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**DOI:** https://doi.org/10.24321/0019.5138.202275

## I N F O

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**How to cite this article:**
Mohammed SJ, Abbood SK, Abies AA. The Effect of Giardia lamblia Infection during the Partial Ban Period due to COVID-19 on Micronutrients among Children in Babylon Province, Iraq. J Commun Dis. 2022;54(2):83-88.

Date of Submission: 2022-03-22
Date of Acceptance: 2022-06-04

## A B S T R A C T

Giardia lamblia is a protozoan parasite and the cause of animportant unresolved health problem in developing countries, as it is related to poor sanitation and management of supplied water, a problem that is exacerbated by the absence of a simple reliable diagnostic test. The present study aimed to determine the impact of Giardialamblia infection on micronutrients in the seraof patients. All stool samples (50) were collected from patients (aged 2 months-14 years) who were suffering from diarrhoea or bloody diarrhoea and had visited hospitals and medical centres in Babylon governorate. The samples were examined by the direct smear method. 14 (28%) faecal samples had G. lamblia and 36 (72%) had some other infection. On the basis of microscopic examination, the result of the current study showed that the highest prevalence (52.6%) of the infection was among patients < 1 year of age and the age group of 1-5 years showed a prevalence of 16.5% based on microscope examination. Blood samples were collected from the patientand control groups to estimate zinc and copper levelsby using the spectrophotometer technique. The results revealed that the levels of zinc in serum were significantly decreased (p = 0.000) and those of copper were significantly increased in patients infected with G. lamblia as compared with the control group.

**Keywords:** Giardia Lamblia, Children, Zinc, Copper

## Introduction

The intestinal parasite is one of the most wide spread pathogens in the world, it infects around 3.5 billion persons a year, and the majority of them are children. It is mainly represented by protozoa and helminths, and they are transmitted by water and contaminated food.[1] Giardia lamblia is a protozoan parasite causing an important unresolved health problem in developing countries, as it is associated with poor sanitation and management of supplied water, a problem that is exacerbated by the absence of a simple reliable diagnostic test.[2] The prevalence of G. lamblia ranges from 2%-7% in industrialised countries and 20%-60% in developing countries.[3] The majority of infections are probably asymptomatic but some are associated with subacute or chronic diarrhoea and intestinal irritation,[4] which contribute to malabsorption and nutritional deficiency, especially in children.[5,6] G. lamblia is more common in children but it affects all age groups in epidemic areas.[7] Infants under 1 year of ageare less likely to be infected than older children.[8] The life cycle consists of cysts that are resistant forms and are responsible for the transmission of giardiasis and trophozoites. Both cysts and trophozoites can be found in the faeces (diagnostic stage). The cysts are hardy and can survive several months in cold water.[9] The diagnosis of giardias is has been classically based on the detection of cysts or trophozoites in stool and duodenal aspirates specimens by direct microscopic examination and duodenal endoscopic biopsy specimen by histology. It has been recently proventhatthe ELISA method for detection of Giardia-specific antigen in faecal specimens has 97.5% sensitivity and 99.5% specificity.[10,11] The link betweenpoor nutrition and intestinal helminths infection has been well-recognised by many researchers who settled several conclusions regarding age groups at greatest risk and the effect of such infections on growth parameters, especially weight.[12] The intensity and type of parasitic infection contribute to its effect on nutrition.[13] Intestinal parasitic infections may cause malnutrition as a result of impaired nutrient absorption and reduction of appetite.[14] Zinc is the second most abundant transition metal in the body after iron and is an essential trace element that plays a crucial role in cell metabolism, growth, tissue repair, production of neurotransmitters, and antioxidant defences.[15] Copper, an essential trace element, is present in the human body in an approximate amount of 100 mg. The normal Cu intake by an adult is about 1-4 mg/day, with vegetables being one important source.[20] It is involved in a plethora of biological processes, from immune and neural functions to bone and blood health, or antioxidant defence. Most of these roles are performed by metalloproteinase and enzymes, to which copper is mostly bound, acting both structurally and as a cofactor for catalytic activity.[16] For adequate development and growth, children need vital micronutrients, the most common of which are copper and zinc. They are needed for maintaining a healthy child with intact immunity as they participate in a lot of enzymatic and biological processes.[17] The link between poor nutrition and intestinal helminths infection has been well-recognised by many researchers who settled several conclusions regarding age groups at greatest risk and the effect of such infections on growth parameters, especiallyweight and height.[18,19]

## Materials and Method

### Stool Samples Collection

The current study was conducted from January to April 2021. The objective of the study was not outbreak investigation. Stool samples were collected from 50 patients aged 2 months-14 years who were suffering from diarrhoea or bloody diarrhoea and hadvisitedhospitals and medical centres in Babylon governorate with individualsas a control. All patients answereda full history questionnaireand provided information like age, address, clinical symptoms, presence of blood in stool, fever, and dryness. Stool samples (the size of approximately 20 gm) were collected and placed in sterilised plastic containers with a tight lid to retain samples' moisture and prevent them from drying. They were then transferred to the advanced parasitology laboratory in the College of Science, University of Babylon. The samples were examined by direct smear method and wet preparation method of normal saline on one side of the distilled slide and a drop of Lugol's iodine on butting of the slide, the by a wood stick. A small amount of stool sample was taken and mixed together with normal saline and Lugol's iodine. It wasthen covered with a cover slide to get a clear vision and was examined under 10x and 40x[16] magnification in hospitals and medical centres' laboratories.

### Blood Sample Collection

From the same patients (50),three ml venous blood samples were drawn usingdisposable syringesof 5ml and were put in a plain tube and centrifuge at 3000 rpm for 5 minutes to separate the serum. They were then placed in sterile tubes and kept in deep freeze at -20˚C until used for theestimation of zinc and copper.

### Assay for Measurement of Levels of Zincand Copper in Sera of Participants in Control and Patient Groups with Amoebiasis and Giardiasis

#### Determination of Serum Zinc by using Zinc Kit

The level of serum zinc was determined according to the Egyptian Company for Biotechnology manual (SAE).

#### Determination of Serum Copper by using Copper Kit

The level of serum copper was determined according to the Egyptian Company for Biotechnology manual (SAE).

## Results and Discussion

### Demographic Data

In the present study, a total of 50 faecal samples of patients suffering from severe diarrhoea were collected from hospitals in Babylon province from January toApril 2021. All patients providedfull history and detailed information. All samples were examined by direct smear method and among them, 14 (28%) faecal samples had G. lamblia and 36(72%) had other infections (Table 1).

Our results agreed with the study of Ma'ala SF[20] in which the infection rate was 13.3% for Babylon province and 15.1% and 16.7% for Karbala and Al-Najaf respectively. According to astudy by Ali JK, out of 492 patients who visited Al-Emam Ali Hospital in Babylon province, 79 (16.05%) were infected

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202275

with G. lamblia and 115 (23.37%) were infected with E. histolytica.[27] Geographical variation of different countries and the endemicity of the parasites in these different areas may have led to these differences.[22] From the results, it can be noted that the low level of infection in the study area can be attributed to the availability of environmental conditions leading to the non-proliferation of these parasites, such as a clean environment and healthy habits for humans in addition to taking into account public health and the lack of pollutants in the environment recently due to COVID-19, as well as the lack of resistance of parasites to chemical disinfectants.[23,28]

**Table 1.Incidence of G. lamblia Infection among Patients**

| Infection Type | Total Number of Samples | No. of Positive Cases | Percentage (%) of Positive Cases |
|---|---|---|---|
| G. lamblia | 50 | 14 | 28 |
| Other infections | 50 | 36 | 72 |

### Microscopic Examination based Percentage Positivity of G. lambliaas per Age Groups

The result of the current study showed that as inferred from the microscopic examination, the highest infection percentage for G. lamblia (52.6%) was seen in the age group of < 1 year and a percentage of 16.5% was seen in the age group of 1-5 years (Table 2).

**Table 2.Infection Percentage G. lamblia according to Age Groups**

| Age (Years) | No. of Samples | No. of Positive Samples | Percentage (%) of Positive Samples |
|---|---|---|---|
| < 1 | 19 | 10 | 52.6 |
| 1-5 | 24 | 4 | 16.5 |
| 6-14 | 7 | 0 | 0 |
| Total | 50 | 14 | 28 |

This result agrees with astudy by Al-Aboody BA et al., where the infection rate was 49% for the age group of less than one year, while it was 4% for the age group over 15 years in Bint Al-Huda and Al-Hussein Teaching Hospital in Nasiriyah City.[29] Dhi Qar governorate also agreedwith the study in Karbala Teaching Hospital for children under 12 years of age with a prevalence rate of5.85%.[26]

In our study, a high infection rate was seen for the age group of < 5 years and the cause could be the nature of this parasite as it affects children and people with low immunity. The practice of artificial feeding can be a source of infection too through unclean feeding bottles along with the tendency of children of this age group to pick up contaminated objects and put unhygienic fingers inside their mouths.

### G. lamblia Infection according to Gender

The present study shows the highest infection percentage for G. lamblia in males (42.8%) while the percentage in females was 9% (Table 3).

**Table 3.Percentage of Infection with G. lamblia according to Gender**

| Gender | No. of Samples | No. of Positive Cases | Percentage (%) of Positive Cases |
|---|---|---|---|
| Male | 28 | 12 | 42.8 |
| Female | 22 | 2 | 9 |
| Total | 50 | 14 | 28 |

Dhanabal J et al. also observed a higher prevalence in males than females but also pointed out that the infections are more likely to be linked to the everyday activities of the individuals rather than gender.[25] In astudy by Al-Ebrahimi HN, it was found that E. histolytica and G.lamblia are more common in males than females and the reason for this is different environmental and occupational exposure.[30] Males are more susceptible than females to infections caused by parasites, fungi, bacteria, and viruses because males generally have a weak erimmune responseas compared to females. These differences are usually attributed to ecological (sociological in humans) and physiological (usually hormonal) factors. Ecological factors include differential exposure to pathogens because of gender-specific behaviour.The other proximate cause is the difference in endocrine-immune interactions.[21]

In another study in Babylon, the infection rate in males was 35%, which was more than that in females (25%). This is because males are more active and have morecontact with external environmental factors. A lack of attention to personal hygiene and infrequenth and washing increases their chances of infection.[23]

Also, sex steroids, specifically and rogens, in males and estrogens in females, modulate several aspects of host immunity. In addition to affecting host immunity, sex steroid hormones also alter genes and behaviours that influence susceptibility and resistance to infection. Thus, males may be more susceptible to infection than females not only because androgens reduce immune competence, but because sex steroid hormones affect disease resistance genes and behaviours that make males more susceptible to infection.[21,24,31]

## Nutritional Factors

### Level of Zinc in Patients'Sera, Other Infection and Control Group

In the present study, every person infected with G.lamblia

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202275

and other infections was found to have significantly lower zincserum levels as compared to controls (Table 4).

**Table 4.Level of Zinc in Study Groups**

| Cases | Mean ± SEM | 95% Confidence Interval | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Control | 78.2500 ± 2.79508 | 71.6407 | 84.8593 | 0.000* |
| G. lamblia | 42.8333 ± 6.73507 | 25.5203 | 60.1464 | |
| Other infection | 29.3810 ± 3.32986 | 22.4350 | 36.3269 | |

*P value > 0.05 was significant

The results of the present study are comparable with those reported by Arbabi M et al.[33] They found that serum levels of trace elements such as magnesium and zinc were reduced with giardiasis and enterobiasis. These findings aresupported by many otherstudies which showedpoor absorption of several micronutrients caused by intestinal parasites.[34] Some studies not only showed that patients with parasitic infections had micronutrient deficiencies, but also their treatment with anti-parasitic medications had improved their serum levels.[35] For example, Olivares JL et al. reported that serum zinclevelshave significantly improved in patients with Enterobius vermicularis and Giardia lamblia infections three months after treatment.[32] Similarly, another study from Mexico reported that the eradication of Giardia lamblia led to a marvellous increase in the mean serum zinc levels in Mexican school children after treatment for six months.[36] The pathophysiology is not clearly understood; however, micronutrient deficiencies may be linked to malabsorption due to mucous affection. Additionally, infection by various parasites affects obviously the level of serum zinc due to its shifting to the liver.[17] Besides, the intestinal parasites use carbohydrates, lipids, minerals, vitamins and other host nutrients in order to gain essential energy fortheir life cycle.[37]

## Level of Copper in Participants' Sera

In the present study, every person infected with G.lambliawas found to have significantly higher copper serum levels as compared to controls and other infections(Table 5).

These results agree with astudy done in Turkey which revealed that children with giardias is had increased serum levels of copper, but lower levels of zinc.[26] Similar findings were reported by other researchers.[32-35] Such controversial results of several studies could be explained by the inability of the body to store zinc causing a decrease in its level to a great extent. Conversely, the storage of copper is mainly in its bound form to ceruloplas min which is considered one of the important acute phase reactants that increases in various infections explaining why serum level of copper increases during such conditions. In the present study, low serum copper could be explained by the low weight and stunted growth of children in addition to inadequate intake of foods with high bio availability of copper such as meat, poultry, and fish. One of the striking observations of the present study is no high difference in serum copper between the control and children with other infections. The explanations for this result could be related to other factors that were not investigated, such as heavy parasitic infections, a small number of study children or some dietetic factors, for example, decreased bio availability of most minerals and increased levels of phytate in the food due to consumption of a diet based mainly on plants.[41]

**Table 5.Level of Copper in Study Groups**

| Cases | Mean ± SEM | 95% Confidence Interval | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Control | 44.2500 ± 4.27096 | 34.1508 | 54.3492 | 0.000* |
| G. lamblia | 80.1667 ± 10.47351 | 53.2436 | 53.2436 | |
| Other infections | 27.0952 ± 5.75942 | 15.0813 | 39.1092 | |

## Conclusion

The study concluded that giardiasis could be a factor along with other hygienic social factors that affects the nutritional status of primary school children in Babylon city.

**Source of Funding:** None

**Conflict of Interest:** None

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ISSN: 0019-5138
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**ISSN: 0019-5138**
**DOI: https://doi.org/10.24321/0019.5138.202275**