



## Serum Levels of Zinc, Copper, Vitamin B12, Folate and Immunoglobulins in Individuals with Giardiasis

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### Abstract

**Background:** *Giardia lamblia* is one of the most important intestinal parasites. The aim of this study was to measure serum levels of IgA, IgE, zinc, copper, vitamin B<sub>12</sub> and folate in individuals with giardiasis in comparison to normal subjects.

**Methods:** The study was carried out among 49 *Giardia* positive and 39 age and sex matched healthy volunteers. Examination of stool samples was done by direct wet smear and formol-ether concentration method. Serum samples were obtained for further laboratory examination. IgA levels were measured by Single Radial Immune Diffusion (SRID). IgE levels were measured by ELISA kit. Zinc and copper levels were measured by Ziestchem Diagnostics Kit and colorimetric endpoint-method respectively. Vitamin B<sub>12</sub> and folate levels were measured by DRG Diagnostics Kit and Enzyme Immunoassay method respectively. All data were analyzed using SPSS version 17.

**Results:** There was a statistically significant difference in IgA, IgE, copper and zinc levels between positive and negative groups ( $P < 0.05$ ). There was no significant difference between vitamin B<sub>12</sub> and folate levels between the two groups. Mean values of *Giardia* positive and negative groups for IgA were 309.26 and 216.89 mg/dl, IgE 167.34 and 35.49 IU/ml, copper 309.74 and 253.61 µg/dl and zinc 69.41 and 144.75 µg/dl respectively.

**Conclusion:** The results showed levels of IgA may correlate more closely with giardiasis than IgE. Regarding trace elements, giardiasis elevated serum copper levels, while it decreased serum zinc. Finally, there was no significant difference in serum levels of vitamin B<sub>12</sub> and folic acid between the two groups.

**Keywords:** *Giardia*, IgA, IgE, Zinc, Copper, Vitamin B<sub>12</sub>, Folate, Iran

### Introduction

Intestinal parasites are still important a worldwide public health problem. *Giardia lamblia* is one of the important intestinal parasites that cause both acute and chronic diarrheal diseases in human (1). Symptomatic giardiasis is also characterized by epigastric pain, nausea, vomiting and weight loss (2). *Giardia* is a flagellated protozoa, has two forms, cyst and trophozoite. Infection is initiated by ingestion of cysts from contaminated food and water and by person-to-person contact. Trophozoites of *Giardia* colonize the lumen of the

small intestine but do not invade the epithelium or deeper layers of the mucosa (3). Infections with *Giardia* are usually self-limited in immune competent individuals. Mucosal defenses against *Giardia* must act in the small intestine. Secretory antibodies against *Giardia* play a central role in anti-giardial defense and clearance of parasite. Patient deficient for the production of IgA, the major immunoglobulin in mucosal secretions, appear to have slightly increased incidence of *Giardia* infections (3).

Immune responses to *Giardia* occur in the intestinal mucosa and a spectrum of inflammatory mechanisms accompany this infection (2-4). While it is well established that infection by intestinal helminths is frequently associated with immediate hypersensitivity reactions and eosinophilia can cause the polyclonal stimulation of IgE synthesis, the presence of an IgE response in giardiasis is not so clear. A number of reports have described the existence of allergic symptoms in persons with giardiasis. Elevated levels of serum IgE and eosinophilia have also occasionally been described in this infection (5). Despite wide research, the host response to infection remains enigmatic. Therefore, antibody assay is expected to be a useful marker in this field.

Trace elements regulate key metabolic pathways, modulate the immune response, and suppress the incidence of various diseases (6, 7). The most important vital elements in human body are copper, zinc and iron. Zinc is necessary for the immune system functions. Zinc deficiency is associated with decline in lymphocyte, which leads to form acute diarrhea (8, 9). Zinc also helps in production of antibodies and T-cell and other blood cell activity (9, 10). Copper is essential for the production of red blood cells, hemoglobin formation and absorption of iron, and for the activity of various enzymes (8) (11). However, the association between trace elements and giardiasis has rarely been investigated.

Vitamin B<sub>12</sub> and folate deficiency are related to cell proliferation and anemia. Also vitamin B<sub>12</sub> is involved in DNA synthesis (12). *Giardia lamblia*, colonizes in proximal small intestine where these two vitamins are absorbed. Thus, giardiasis may lead to B<sub>12</sub> and folate deficiency (13). The purpose of this study was to investigate serum levels of IgA, IgE, copper, zinc, B<sub>12</sub> and folate in giardiasis in comparison to normal subjects.

## Materials and Methods

The study was carried out at the Health Center Clinics and Children's Hospital of Tehran University of Medical Sciences, during 2009-2010.

Questionnaires were obtained from individuals attended the mentioned centers. Stool samples were collected in sterile clean stool cups from all volunteers. Examination of fecal samples for detection of *Giardia* cyst and/or trophozoite was done by direct wet smear and formol-ether concentration method immediately (8). Among these volunteers a total of 49 positive individuals for *Giardia lamblia* were enrolled as study (positive) group. The control group consisted of 39 age and sex matched healthy volunteers according to the criteria described in the questionnaire. Serum was obtained from both groups and kept in -70 °C freezer for further immunological and biochemical laboratory examination. IgA levels were measured by Single Radial immune diffusion (SRID). IgE levels was measured by IgE ELISA (Enzyme-Linked immunosorbent assay) kit. IgE levels was measured by Elisa reader at 450nm (ELX800 BIOTEK, USA). Zinc and copper levels were measured by Ziestchem Diagnostics Kit (REF 10-517, 5-Br-PAPS, REF 10-546, 3, 5-DiBr-PAESA reagent respectively, and colorimetric endpoint-method. Then were read by spectrophotometer (PD-303 APEL, Japan) at 546 nm and 578nm respectively. Vitamin B<sub>12</sub> and folate levels were measured by DRG Diagnostics Kit REF RIA-1990 (Germany) and Enzyme Immunoassay method. Data were analyzed by SPSS version 17.

## Results

Results of the study are summarized in Table 1, 2 and 3. In Table 1 mean serum levels of immunological and biochemical parameters were compared between *Giardia* positive and *Giardia* negative individuals. There was a significant difference between serum IgA and IgE levels in both groups ( $P=0.001$ ). Furthermore zinc levels in *Giardia* positive is remarkably lower than *Giardia* negative group ( $P=0.001$ ). In addition, there was a significant difference between serum copper levels between both the groups ( $P=0.003$ ). Normal ranges of Immunoglobulins, copper, zinc, vit. B<sub>12</sub> and folate levels and distributions of *Giardia* positive (GP) and *Giardia* negative (GN) was

shown in Table 2. It presents classification of results according to each of the parameters, international normal ranges (8, 14).

Immunoglobulins, copper, zinc, vit. B<sub>12</sub> and folate levels of *Giardia* positive and negative were divided into 3 groups, decreased, normal and increased levels (Table 3). Results for IgA in *Giardia* positive group showed 24.5% of all the cases were in normal range while in 75.5% of the cases IgA levels was increased. In *Giardia* negative group, 68.4% of the cases were in normal range and 31.6% showed increased IgA levels. Results for IgE in *Giardia* positive group showed 70.6% of the cases were in normal range whereas in 29.4% of the cases IgE levels were increased. In *Giardia* negative group, 97.4% of the cases were in normal range and 3.6% showed increased IgE levels. For copper, in *Giardia* positive group, we found 9.1% decreased, 31.8% normal, 59.1% increased, whereas in *Giardia* negative

group, 17.9% of the cases were in normal range and 82.1% showed increased levels.

**Table 1:** Mean serum levels of biochemical & immunological parameters in *Giardia* positive (GP) and *Giardia* negative (GN) groups

Parameters	<i>Giardia</i> Positive (GP)	<i>Giardia</i> Negative (GN)	P values
Serum zinc (µg/dl)	69.41±5.61	144.75±10.71	0.001
Serum cu (µg/dl)	309.74±59.12	253.61±20.77	0.03
Serum IgA (mg/dl)	309.26±17.51	216.89±23.74	0.001
Serum IgE (IU)	167.34±31.99	35.49±6.53	0.001
Serum B <sub>12</sub>	340.28±41.20	341.10±46.85	0.301
Serum folic	7.09±0.28	8.77±1.34	0.150

**Table 2:** Normal ranges of immunoglobulins, copper, zinc, vit. B<sub>12</sub> and folate levels and distributions of *Giardia* positive (GP) and *Giardia* negative (GN)

Parameters	IgA	IgE	Cu	Zn	B <sub>12</sub>	Folate
Normal ranges	≤230 mg / dl	11-188 IU/ml	70-155 µg/dl	63.8-114 µg/dl	160 -970 pg/dl	1.5-17 g/ml
Patient No.	GP GN n=49 n=38	GP GN n=51 n=39	GP GN n=44 n=39	GP GN n=48 n=39	GP GN n=20 n=10	GP GN n=20 n=10

**Table 3:** Distribution of immunoglobulins, copper, zinc, vit. B<sub>12</sub> and folate levels of *Giardia* positive (GP) and *Giardia* negative (GN) groups according to international normal ranges

Parameters	IgA		IgE		Cu		Zn		B <sub>12</sub>		Folate	
	GP	GN	GP	GN	GP	GN	GP	GN	GP	GN	GP	GN
Decreased	-	-	-	-	n=4 9.1%	n=0 0.0%	n=4 50%	n=0 0.0%	n=4 10%	n=0 0.0%	n=4 100%	n=0 0.0%
Normal	n=12 24.5%	n=26 68.4%	n=36 70.6%	n=38 97.4%	n=14 31.8%	n=7 17.9%	n=18 37.59%	n=17 43.6%	n=18 90%	n=10 100%	n=20 100%	n=9 90%
Increased	n=37 75.5%	n=12 31.6%	n=15 29.4%	n=1 3.6%	n=26 59.1%	n=32 82.1%	n=6 12.5%	n=22 56.4%	n=0 0.0%	n=0 0.0%	n=0 0.0%	n=1 10%

Furthermore, in 28.0% of *Giardia* positive group, copper levels were much higher than the normal range (>300µg/dl), on the other hand in *Giardia*

negative group, 15.28% of cases had copper levels much higher than the normal range. For zinc, in *Giardia* positive and negative groups we found

12.5% and 56.4% increased, 37.5% and 43.6% in normal range and 50.0% decreased respectively. In *Giardia* negative group no decrease in zinc levels was observed. Furthermore for Vit B<sub>12</sub> levels, in *Giardia* positive group, we found 10% decreased and 90% in normal range, whereas in *Giardia* negative group, 100% of the cases were in normal range. Results for folate in *Giardia* positive group showed 100% of the cases were in normal range. In *Giardia* negative group, 90% of the cases were in normal range while 10% showed increased folate levels.

## Discussion

Intestinal parasitic infection is, still, an important public health problem, mainly in specific geographical areas and among people with specific socio-economic status (13). Secretory antibodies of the IgA are important candidate for immune defense against *Giardia*, because they are secreted in large quantities into the intestinal lumen and their actions are antigen-specific (2). In a study it was suggested that anti-giardial IgA may exert cytotoxic effects on *G.lambliia* (15). Previous studies has shown an increase in IgA levels while others did not(15-18). However few studies have focused on this matter.

IgE is an antibody which plays an important role in allergy and is especially associated with type 1 hypersensitivity (19). IgE has also been implicated in immune system responses to most parasitic worms (20) and may be important during immune defense against certain protozoan parasites (21). The interaction between IgE-dependent mast cell reaction has been involved primarily to localize eosinophils near the parasite and then enhance their anti-parasitic effects (22, 23). However, increased IgE levels occasionally occur in giardiasis (24, 25). An IgE level is affected by age, sex, race, smoking habits and socio-economic conditions. It may also vary from country to country (22, 26-30). Several studies have been published on serum IgE levels in subjects with helminthic and/or protozoan infections (22, 24, 25, 31, 32). Many investigations showed an increased IgE concentration in patients with

parasitic infections. IgE elevation is higher in worm infection than those of protozoan (2, 22, 24). In another study it was observed significant increase IgE levels in patients with giardiasis which was decreased after treatment (25). Furthermore, serum IgE levels elevation has been reported in helminthic more than protozoan infections (33). However in a previous research there was no increase in IgE levels in intestinal parasitic infection (34).

In this study in *Giardia*-positive group, we found 75.5% increase in IgA level whilst in *Giardia* negative group we found 31.6% increase. This finding shows the importance of IgA level in individuals with proven giardiasis. Moreover, it seems results for IgE levels were more useful tool for determining giardiasis (29.4% increases in *Giardia* positive versus 2.6% increase in *Giardia* negative group).

Elements such as zinc and copper are vital for growth, reproduction and development (35). They are components of some cellular enzyme; in several immune processes they play an important role in the resistance to free radical damage by stabilizing the cellular membrane (6). Low serum levels of copper and zinc could cause impairment in cellular and enzymatic functions (35). Zinc is an element which cannot be stored in the body and therefore it can easily decline in infective diseases (8). Serum zinc levels were lower during protozoan infections. In addition, elevations of serum copper levels are observed in most of the acute and chronic parasitic infections (8). In several studies conducted regarding trace elements in giardiasis has shown a significant decrease in zinc levels while there was an obvious increase in copper levels (36-39). However in few reports there was no significant difference in serum between the two groups (39-41). In this study, we found a decrease in zinc levels in 50% of *Giardia* positive group. In addition, there was a significant increase in copper levels in 59.1% of *Giardia* positive group. Results obtained in our study are compatible with other researchers. As it was mentioned above zinc cannot be stored in the body therefore it could be easily declined in the serum. However, 90% of serum copper is stored

in the bound form to the ceruloplasmin. Elevations of serum copper levels are observed in most of the infections in relation to fluctuations of ceruloplasmin as an acute phase reactant. Vitamin B<sub>12</sub> is involved in the synthesis of important biochemical transmitters in the brain and nervous system. Vitamin B<sub>12</sub> is involved in the synthesis of DNA. It is especially important during growth and cell proliferation (42). Vitamin B<sub>12</sub> is important in transport and storage of folate in the cells (43). Folate is also very important in cell division and DNA synthesis. Folate must be digested by pancreatic juice in duodenum, where *G. lamblia* usually colonizes (44, 45). Damage of intestinal epithelium occurs by adherent trophozoites of *G. lamblia*. It has been proposed as one important mechanism in the pathogenesis of infection (46). Giardiasis can cause vitamin B<sub>12</sub> deficiency,, bowel inflammation and interfere in folate absorption (45,47-49). Few studies demonstrated folate and B<sub>12</sub> deficiency due to giardiasis (49, 50). However some reports found normal absorption of folate during *G. lamblia* infection (50, 51). In this study, no significant difference was observed between the *Giardia* positive and *Giardia* negative groups.

## Conclusion

Our results showed levels of IgA may correlate more closely with giardiasis than IgE. Regarding trace elements, giardiasis elevated serum copper levels, while it decreased serum zinc. Finally, there was no significant difference in serum levels of vitamin B<sub>12</sub> and folic acid between the two groups.

## Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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## References

1. Zarebavani M, Mirhadi F, Rezaeian M (2010). Detection of the “Tim” gene of sheep using “Tim” Gene primers of *Giardia* of with human origin. *Int J Vet Res*, 4 (2): 69-72.
2. Eckmann L (2003). Mucosal defences against *Giardia*. *Parasite Immunol*, 25: 259-70.
3. Langford TD, Housley MP, Boes M, Chen J, Kagnoff MF, Gillin FD, et al. (2002). Central Importance of Immunoglobulin A in host Defense against *Giardia* spp. *Infect Immun*, 70 (1): 11-18.
4. Muller N, Von Allmen N (2005). Recent insights into the mucosal reactions associated with *Giardia lamblia* infections. *Int J Parasitol*, 35 (13): 1339-47.
5. Ertan Kara P, Yereli K, Kasirga E, Kurt O, Sanlidag T, Onag A, et al. (2004). The Examination of Link Between Blood Levels of IgA,IgE,IgG,IgM,CRP and giardiasis in children. *Ege Tip Dergisi*, 43 (2): 91-94.
6. Olivares JL, Fernández R, Fleta J, Rodríguez G, Clavel A (2003). Serum Mineral Levels in Children with Intestinal Parasitic Infection. *Dig Dis*, 21 (3): 258-61.
7. Milner JA (1990). Trace minerals in the nutrition of children. *J Pediatr*, 117 (2): 147-55.
8. Culha G, Sangun MK (2007). Serum levels of zinc,copper,iron,cobalt,magnesium and selenium elements in children diagnosed with *Giardia intestinalis* and *Enterobiosis vermicularis* in Hatay,Turkey. *Biol Trace Elem Res*, 118 (1): 21-26.
9. Kilic E, Yazar S, Saraymen R (2003). Responsiveness of total content changes of magnesium and zinc status in patients infected with *Giardia intestinalis*. *Biol Trace Elem Res*, 96 (1-3): 153-58.

10. Jendrezko A, Sodowska H (1993). Zinc deficiency in children infected with *Giardia lamblia*. *Wiad Lek*, 46 (1-2): 32-35.
11. Celiksoz A, Acigoz M, Degerli S, Alim A, Aygan C (2005). Egg positive rate of *Enterobius vermicularis* and *Taenia* spp. By cellophane tape method in primary school children in Sivas, Turkey. *The Korean J Parasitol*, 43 (2): 61-64.
12. Oh R, Brown DL (2003). Vitamin B<sub>12</sub> deficiency. *Am Fam Physician*, 67 (5): 979-86.
13. Olivers JL, Fernandez R, FletaJ, Ruiz MY, Clavel A (2002). Vitamin B<sub>12</sub> and folic acid in children with intestinal parasitic infection. *J Am Coll Nutr*, 21 (2): 109-13.
14. Burtis CA, Ashwood RA, Brunis E (2008). *Tietz fundamentals of clinical chemistry*. 6<sup>th</sup> ed. Saunders, Elsevier, pp.: 840-49.
15. Stager S, Gottstein B, Sager H, Jungi TW, Muller N (1998). Influence of antibodies in mother's milk on antigenic variation of *Giardia lamblia* in the murine mother-offspring model of infection. *Infect Immun*, 66 (4): 1287-92.
16. Lai Ping So A, Mayer L (1997). Gastro intestinal manifestations of primary immunodeficiency disorders. *Semin Gastrointest Dis*, 8 (1): 22-32.
17. Strober W, Sneller MC (1991). IgA deficiency. *Ann Allergy*, 66: 363-75.
18. Soliman MM, Taghi-kilani R, Abo-Shady AF, et al. (1998). Comparison of serum antibody responses to *Giardia lamblia* of symptomatic and asymptomatic patients. *Am J Trop Med Hyg*, 58 (2): 232-39.
19. Gould H, Sutton BJ, Beavil AJ, Beavia RL, McCloskey N, Coker HA, et al. (2003). The biology of IgE and the basis of allergic disease. *Annu Rev Immunol*, 21: 579-628.
20. Erb KJ (2007). Helminthes, allergic disorders and IgE-mediated immune responses: where do we stand?. *Eur J Immunol*, 37 (5): 1170-73.
21. Duarte J, Deshpande P, Guiyedi V, Mécheri S, Fesel C, Cazenave P, et al. (2007). Total and functional parasite specific IgE responses in *Plasmodium falciparum*-infected patients exhibiting different clinical status. *Malar J*, 6 (1): 1-13.
22. Hagel I, Lynch NR, Di Prisco MC (1993). Allergic reactivity of children of different socioeconomic levels in tropical populations. *Int Arch Allergy Immunol*, 101 (2): 209-14.
23. Roitt IM, Brostoff J, Male DK (1996). *Medical Immunology*. Gower Medical Publishing, London, pp.: 181-91.
24. Di Prisco MC, Hagel I, Lynch NR (1993). Possible relationship between allergic disease and infection by *Giardia lamblia*. *Ann Allergy*, 70 (3): 210-13.
25. Perez O, Lastre M, Bandera F (1994). Evaluation of the immune response in symptomatic and asymptomatic human giardiasis. *Arch Med Res*, 25 (2): 171-77.
26. White PJ, Potter PC, Malherbe D (1989). A multi-allergen screening test for suspected allergic disease in colored children. *S Afr Med J*, 76 (11): 597-98.
27. Witting HJ, Belloit J, De Fillipi L (1980). Age related serum immunoglobulin E levels in healthy subjects and in patients with allergic diseases. *J Allergy Clin Immunol*, 66 (4): 305-13.
28. Barbee RA, Halonen M, Lebowitz M (1981). Distribution of IgE in a community population sample: correlation with age, sex and allergen skin test reactivity. *J Allergy Clin Immunol*, 68 (2): 106-11.
29. Lynch NR, Lopez RL, Di Prisco MC (1987). Allergic reactivity and socioeconomic level in a tropical environment. *Clin Allergy*, 17 (3): 199-207.
30. Wilhelm D, Klauche M, Görg S (1994). Expression of sCD<sub>23</sub> in atopic and non-atopic blood donors: correlation with age, total serum IgE, and allergic symptoms. *Allergy*, 49 (7): 521-25.
31. Nielsen BW, Lind P, Hansen B (1994). Immun responses to nematode exo antigens: sensitizing antibodies and basophilic histamine release. *Allergy (Denmark)*, 49 (6): 427-35.
32. Buijs J, Egbers MW, LokhorstWH (1995). *Toxocara*-induced eosinophilic inflammation. Airway function and effect of anti-IL-5. *Am J Respir Crit Care Med (United States)*, 151 (3): 873-78.
33. Köse S, Özbel Y, Kokuludag A (1995). Barsak parazitleriile serum IgE seviyeleri ve allerjik

- deri testleri arasındaki ilkinin celenmesi. *T Parazitol Derg*, 19 (3): 397 - 401.
34. Yenigun A, Suren T, Tanac R (1990). Intestinal parazitozların total serum IgE düzeyine etkii. *Ege Tıp Derg*, 29 (3): 678-80.
  35. Ertan P, Ysereli K, Kurt O, Balcioglu IC, Onağ A (2002). Serological levels of zinc, copper and iron elements among *Giardia lamblia* infected children in Turkey. *Pediatr Int*, 44 (3): 286-88.
  36. Tanyuksel M, Sayal A, Aydın A (1995). Trace element levels in some parasitic disease. *Acta Parasitol Turcica*, 19: 315-21.
  37. Kilic E, Yazar S, Saraymen R (2004). Serum zinc and magnesium levels in patients with blastocystis. *Biol Trace Elem Res*, 98 (1): 21-26.
  38. El Gohari Y, Galal SH, Boulos LM, Moustafa S, Amin SM, et al. (1984). Trace element levels in some parasitic diseases. *J Egypt Soc Parasitol*, 14 (1): 179-87.
  39. Demirci M, Delibas N, Altuntas I, Oktem F, Yonden Z (2003). Serum iron, zinc and copper levels and lipid peroxidation in children with chronic giardiasis. *TJ Health Popul Nutr Mar*, 21 (1): 72-75.
  40. Karakas Z, demirel N, Tarakcioglu M, Mete N (2001). Serum zinc and copper levels in southeastern Turkish children with giardiasis or amebiasis. *Biol Trace Elem Res*, 84 (1-3): 11-18.
  41. Devlin TM (2009). *Text Book of Biochemistry with clinical correlations*. 7<sup>th</sup> ed. New York Willey-Liss, pp.: 723-27.
  42. Cathy Breedon (2009). Guide to nutrition: Vitamin B<sub>12</sub>. *Merit Care Health System*. Available from: [www.google.com](http://www.google.com)
  43. Murray RK, Granner DK, Mayes PA, Rodwell VW (1996). *Harper's Biochemistry*, 24<sup>th</sup> ed. *Appleton & Lange*, UK, pp.: 320-21.
  44. Khademi R, Ghaffarifar F, Dalimi Asl H (2006). In Vitro Effect of Folic Acid and Cobalamin (Vitamin B<sub>12</sub>) on Adhesion and Growth of *Giardia lamblia*. *Iranian J Parasitol*, 1 (1): 47-52.
  45. Sousa MC, Goncalves CA, Bairos VA, Poiars-Da-Silva J (2001). Adherence of *Giardia lamblia* trophozoites to int-407 human intestinal cells. *Clin Diagn Lab Immunol*, 8 (2): 258-65.
  46. Tycker KL, Rich S, Rosenberg I, Jacques P (2000). Plasma vitamin B<sub>12</sub> concentrations relate to intake source in the Framingham offspring study. *Am J Clin Nutr*, 71 (2): 514-22.
  47. Inge PMG, Edson CM, Farthing MJG (1998). Attachment of *G. lamblia* to rat intestinal epithelial cells. *Gut*, 29 (6): 795-801.
  48. Gault MJ, Gillin FD (1987). *Giardia lamblia*: stimulation of growth by human intestinal mucus and epithelial cells in serum free medium. *Exp Parasitol*, 64 (1): 29-37.
  49. Ambrose NS, Hutchinson S, Tejan J (1989). Folate deficiency due to giardiasis. *J R Soc Med*, 82 (1): 48-49.
  50. Askari F, Ghaffarifar F, Dalimi Asl H, Haghi Ashtiani Mt, Delavari M (2007). Study on variation of the sera folic acid, vitamin B<sub>12</sub> and iron level in the 6-12 years old patients infected with *Giardia lamblia* in south Tehran Iran. *J Pediatr*, 17 (2): 149-54.
  51. Hjelt K, Krasilnikoff PA (1990). The impact of gluten on the haematological status, dietary intakes of haematopoietic nutrients and vitamin B<sub>12</sub> and folic acid absorption in children with coeliac disease. *Acta Paediatr Scand*, 79 (10): 911-19.