A comparative study of blood cells variations and serum factors between children with *Giardia lamblia* and that of control group

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**ABSTRACT**

*Giardiasis* is an infection caused by flagellated protozoan which is called *Giardia lamblia*. The protozoa is an intestinal parasite and has a global distribution. The prevalence of it varies between 5% -30%. The present study was undertaken to analyze probable correlation between blood cells and immune system status in children aged 2 to 10 years with giardiasis who selected from Iran University of Medical Sciences hospitals. Children with giardiasis underwent precise laboratory analysis with regard to blood cell counts (lymphocytes, monocytes and polymorph nuclear cells) and serum factors (Immunoglobulin and complements components). The examination showed that the blood in affected children with giardiasis has a significant reduction in terms of lymphocytes and monocytes. Whilst otherwise, white cells showed no significant differences. Similarly, serum analysis defined significant reduction in C³ and C⁴ complements components. In contrast, despite observing insignificant differences in amount of IgG, serum level of IgE, IgM and IgA immunoglobulin increased significantly. These differences are possibly related to cellular immune function and complement system.

**Keywords:** *Giardia lamblia*, Blood cells, Serum factors

**INTRODUCTION**

*Giardia*, a flagellated protozoan, inhabits the upper part of the small intestine of its host that has a direct life cycle (1, 2, 12). In humans, the clinical effects of *Giardia* infection range from the asymptomatic carrier state to a severe malabsorption syndrome (3, 4). Giardiasis infection has a worldwide distribution with higher prevalence in children (5, 6). The prevalence varies in terms of socioeconomic and geographical status as well as the state of the host (7, 10, 11). Therefore, it is very essential to be diagnosed and treated in a right way (8, 9, 19). Several studies carried out in order to examine immune responses to the infection that shows variation on type and level of immune responses with regard to the duration of exposure and severity of infection and vast antigenic variety of *Giardia lamblia* (13, 14). Animal models have allowed us to study role of cellular immunity at the mucosal site (15). Due to invasive technique required for harvesting cells at the mucosal site, studies of cell-mediated immunity in human giardiasis have been done with lymphocyte circulating in the blood that have been reported that children born without immunity are significantly more susceptible than immune children to giardiasis (16,17). Cellular immunity response in patients with giardiasis showed a significant difference between CD⁴⁺, CD⁸⁺ lymphocytes in duodenum of symptomatic or asymptomatic giardiasis (18,19). Moreover, significant or non-significant difference in level of secretory IgA and total serum immounoglobuline have been reported in above-mentioned group (20). Some studies shows increase in IgA, IgM and IgG₁ antibody titers in patients with symptomatic giardiasis to that of asymptomatic
patients using Indirect immune Fluorescence Assay (IFA) and Enzyme Linked Immune Absorbent Assay (ELISA) (21,22,23). Meanwhile, antibody IgG anti-\textit{Giardia lamblia} were observed in blood serum of children with giardiasis (24, 25). In present study, alteration of blood cells and serum factors were undertaken in children aged 2-10 years with giardiasis and probable correlations and affectivity of mentioned factors in giardiasis.

**MATERIALS AND METHODS**

A total of 40 children with Giardiasis and 40 children of control group participated in this study; using the method which applies the following formula:

$$n = \left( Z_{1-\alpha/2} + Z_{1-\beta} \right)^2 \times \frac{(S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}$$

A retrospective study was done and data were collected using both observation and questioners. The present study was undertaken in both children aged 2 to 10 years without any history of giardiasis who selected from Iran University of medical sciences hospitals and children with symptomatic giardiasis (diarrhea, vomiting, abdominal pain) who underwent fecal analysis by Direct microscopy and Concentration technique (formalin-ethyl acetate) and subsequently, divided into \textit{Giardia lamblia} positive group (patients with \textit{Giardia} infection) and \textit{Giardia lamblia} negative group (control group). From each of examined children 3$^{rd}$ clotted blood and 3$^{rd}$ anti-coagulant blood had been collected. Total amount of white blood cells (WBC) / mm$^3$ and absolute number of monocytes, neutrophils, lymphocytes and eosinophils were analyzed by anti-coagulant blood samples.

Serum obtained by centrifugation of 3$^{rd}$ clotted blood and stored in the refrigerator at -20 $^\circ$C, in order to analyze IgG, IgM, C3 and C4 serum level using SRID(Single Radial Immune Diffusion) and also to determine IgE serum level using IgE ELISA Kit. Immunoglobulin kits and complements C3 and C4 preparation was done by Bio gene Company and subsequently, the obtained results in both control and patients groups were analyzed Using SPSS.

**RESULTS**

To analyze the results in both case and control groups, T-test and Chi-square test are conducted. The results showed no significant difference between case and control groups in terms of total number of WBC, neutrophil and eosinophil. Besides, there was no significant difference in terms of absolute number of lymphocytes (Chi-square test). However there was a significant of it using $P_v < 0.05$. Therefore, it showed the lower level of absolute number of lymphocytes in cases (patients with \textit{Giardia lamblia}). Total of 73% of cases showed lower level of monocytes which was significant. (T-test, chi-square test $P_v < 0.00$). (Table 1)

There was no significant difference between case and control groups in terms of IgG number (Chi-square & T-test) whereas significant difference were found between two groups in terms of immunoglobulin level and IgA level was higher in cases. Serum IgM level showed no significant difference between two groups (Chi-square ) whereas, significant difference were found using T-test ($P_v < 0.05$) which confirms the higher level of serum IgM in cases. Similarly, higher level of IgE was found in case group than control group. (T-test, Chi-square test) The lower level of C3 was found in case groups rather than control group. (T-test, Chi-square $P_v <0.00$)

A significant difference was found between two case and control groups (T-test $P_v < 0.05$) which confirms the lower level of C4 in case group whereas, no significant difference were found using Chi-square test. (Table 2)
Table 1. Absolute frequency distribution of WBCs and each of their components in case and control groups mm$^3$/blood

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Eosinophil</th>
<th>Monocyte (P&lt;0.000)</th>
<th>Lymphocyte</th>
<th>Neutrophil</th>
<th>WBCs</th>
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<tbody>
<tr>
<td></td>
<td>&lt;450</td>
<td>&gt;450</td>
<td>0-2000</td>
<td>&gt;2000</td>
<td>Total</td>
</tr>
<tr>
<td>Case</td>
<td>32</td>
<td>8</td>
<td>40</td>
<td>30</td>
<td>1500</td>
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<td></td>
<td>10</td>
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<td>800</td>
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<td></td>
<td>38</td>
<td>2</td>
<td>40</td>
<td>4</td>
<td>Total</td>
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<td>Control</td>
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<td>38</td>
<td>2</td>
<td>40</td>
<td>4</td>
<td>Total</td>
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Table 2. Absolute frequency distribution of immunoglobulins, C3 & C4 complement components in both case & control groups mg/dl

<table>
<thead>
<tr>
<th>Frequency</th>
<th>C3</th>
<th>IgG</th>
<th>C4</th>
<th>IgA</th>
<th>IgM</th>
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DISCUSSION

The prevalence of giardiasis varies in terms of geographical and socio-economic states as well as the state of the host (1,2,3). In the present study, no reduction occurred in the number of WBCs of both groups by CBC (complete blood count), serum immunoglobulin level and complement components which showed no reduction in the number of WBCs, (leukopenia). There was an increase in the number of WBCs (leukocytosis) in 4 of control group and one of case group which was not significant, as a result; it confirms some findings on no presence of leukopenia and leukocytosis in patients with intestinal parasites (26).

In the present study, significant reduction was found in terms of monocytes between case and control group. It can be indirectly resulted that immune cell system in patients is most probably more harmed. In addition, some studies considered difference between cellular/humeral immune responses and non-specific immune response in patients (27, 28, 6).

The present findings on giardiasis and serum IgA level also showed an increase in serum IgA level specifically, secretory IgA in intestinal secretion as most of intestinal parasitic infection (29, 30). In present study, serum IgA level was significantly higher in case than control group.

As we know, C3 components are the most important components of immune system and the level of it in serum is more than the other part of the system. Hence, C3 is the factor that activates complement system through two classic and secondary pathways (27, 30).

Although, the present study confirmed C3 deficiency (T-test, Chi-square). It also carries several questions as follows:

- C3 deficiency is the prime factor or it is the consequences of giardiasis?
- C3 deficiency is the malnutrition or it is the consequences of giardiasis?
- Malabsorption of fat-soluble vitamins can play a role on C3 deficiency?
- Secretion of parasite can stop the production of C3?
- Role of C3 against infectious diseases entered systemic blood circulation and visceral factors have been proven but its role is unclear in terms of fighting against intestinal infection. In the other words, it is not yet obvious that how much activity is done by complement components inside the lumen of intestine (26, 29, 31, 32).

T-test showed difference between two groups with regard to C3 components but the evidence is not as strong as of C3. The difference can be due to abnormal level of C3 in two groups. Therefore future study needs to be done on the following hypothesis to find a role of complement system through alternative pathway in fighting against giardiasis.

REFERENCES