Study The Prevalence of *Giardia duodenalis* and Detection IgM, IgG , IgA in Some Patients with Giardiasis

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Abstract

This study was aimed to determine the prevalence of *Giardia duodenalis* from human stool samples during a period started from September 2012 to May 2013 in Al-Karkh region/Baghdad by using direct microscopic examination and to measure the concentration of immunoglobulin IgM, IgG, and IgA in serum of **50** infected patients by Enzyme Linked Immunosorbent Assay (ELISA). **Results** revealed, from **1194** human stool samples *Giardia duodenalis* was detected in **256** samples and the infection rate was **21.44%**.Sex of the patients had significantly influence on the total infectivity rate, in males, females were **20.58%** , **22.93%** respectively. A significant effect of age on incidence of infection was noticed, the higher rate was recorded in children aged **≤ 10** years old **27.18%** in comparison with **14.54%** of patients aged **> 10** years old. The mean concentration antibodies specific for *Giardia duodenalis* in serum samples showed Significant differences (**p< 0.05**) between infected and non- infected humans and also among age groups of infected patients. The higher concentrations of **IgM, IgG,** and **IgA** were obtained in patients aged **2-12** years old in comparison with other age category .The **conclusion** of this study demonstrated significant differences in *Giardia*-antibodies levels between infected and non-infected individuals and among age groups. ELISA can be used for detection and differentiation between acute and chronic infection .

Key word: *Giardia duodenalis,* ELISA, Antibodies detection

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دراسة انتشار جياردیا الاثنا عشرية وتشخيص IgM,IgG,IgA في بعض مرضى الجيارديات

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الخلاصه

أجريت هذه الدراسة للتحري عن انتشار الطفيلي *Giardia duodenalis* في عينات من براز الانسان للفترة من أيلول 2012 الى مايس 2013 في منطقه الكرخ / بغداد بأستعمال الفحص المجهري المباشر و حددت تراكيز الاجسام المضاده IgM, IgG و IgA في مصل 50 مصاب باستخدام تقنيه الاليزا (مقايسة الممتز المناعي المرتبط بالأنزيم). أظهرت النتائج: تشخيص طفيلي *Giardia duodenalis* في 256 عينة براز للانسان من مجموع 1194 عينة وأن معدل نسبة الاصابة بلغت 21.44% كما لوحظ تأثير معنوي للجنس على نسبة الاصابة بالطفيلي حيث بلغت في الذكور والاناث 22.93%, % 20.58 على التوالي .في حين التاثير المعنوي للعمر على حدوث الاصابة أوضح بان نسبة الاصابة بالاطفال بعمر 10 سنة أو أقل 27.18% مقارنة مع 14.54%بعمر أكثر من 10 سنوات. لوحظ وجود فرق معنوي ($p<0.05$) في معدل تركيز الاجسام المضاده IgM, IgG, IgA والخاصة بطفيلي *Giardia duodenalis* في عينات المصل للاشخاص المصابين مقارنة مع الغير مصابين ،سجلت اعلى التراكيز للاجسام المضاده في المصابين بعمر 12-2 سنة مقارنة مع الفئات العمرية الاخرى.الاستنتاج لهذه الدراسة تمثل بوجود فروق معنوية بمستوى الاجسام المضاده للجياردیا بين المصابين وغير المصابين وضمن مجاميع العمر .يمكن استخدام الاليزا في التشخيص و التفريق بين حالات الاصابة الحاده والمزمنة.

الكلمات المفتاحيه:جياردیا الاثنا عشرية،اليزا،تشخيص الاجسام المضاده

Introduction

Giardia duodenalis is a flagellated unicellular microorganism that have the ability to infect different species including human [1]. *Giardia duodenalis* (synonymous with *Giardia lamblia Giardia intestinalis*) is the causative agent of gastrointestinal infections. In human, the disease may occurs in three types mild , severe as well as chronic, while in domestic animals its show clinical importance and economic significant losses [2,3] .

Ingestion of contaminated food and water conceder to be the main source of infections, also

person to-person spread was documented [4] . Most infections are asymptomatic while, the rate for symptomatic infection varies from low to high percentage [5,6] . Although *Giardia* is essentially a luminal pathogen in the gut, both local and systemic immune responses are trigger. Antibody specific for *Giardia* in serum give proof for its importance in clearance of the pathogen [7] . The method Enzyme linked immunosorbent assay **(ELISA)** is used for the antibodies IgG, IgM and IgA detection also for the determination of chronic or acute giardiasis [8] . This study was designed to Measure the titer of IgG, IgM and IgA antibodies specific for *Giardia duodenalis* in blood samples of infected patients by using Enzyme Linked Immunosorbent Assay **(ELISA**).

Material and Methods

This study was done in Baghdad AL –Karkh restrict during the period from September 2012 to May 2013.

Sampling

Human stool samples

One thousand and one hundred ninety four stool samples of human were collected in sterile plastic cups approximately **10 g** for each sample, all were examined under microscope by using saline wet-mount method for detection of *G. duodenalis* from both diarrheal and non- diarrheal patients attend hospitals laboratories (**Central child**, **Al-kadhymia for children, Al- yarmouk and Abo-Greab)** including **758** male samples **(63.48%)** and **436** females samples **(36.51%)**, according to age category, **651** samples were less/equal to **10** years old **(54.5%)** and **543** samples were more than **10** years old **(45.4%)**.

Human serum samples

Blood samples were obtained from persons showed Giardiasis (approximately **3ml**) using Gel and Clot Activator Non Blood Collection Tube, serum was separated by centrifuge **3000 rpm** for **5 minutes** and stored in eppendorf tube at **-20C^0** until use. A total of **50** serum samples were collected including **25** samples for each males and females, samples were classified according to age into **3** groups. The first group contains **18** samples (**9 male, 9 female**)

under **2-12** years old, the second group contains **16** samples (**8 male, 8 female**) under **13- 33** years old and the last group contains also **16** samples (**8 male, 8 female**) under **34- 54** years old. For control **18** samples were collected including **3** samples for each males and females under each age group **Enzyme Linked immuonosorbent Assay**

Table(1): Human serum samples associated with age and sex

State	Number/sample	Age/ year	Sex
Infected	9	2- 12	Male
	9		Female
	8	13- 33	Male
	8		Female
	8	34- 54	Male
	8		Female
Non - infected	3	2- 12	Male
	3		Female
	3	13- 33	Male
	3		Female
	3	34- 54	Male
	3		Female

Three kits are used for the qualitative detection of **IgG, IgM** and **IgA** antibodies in serum samples. The dilution (**1:100**) was done for each patient sample with diluents working solution before being measured.

A sufficient number of *Giardia* antigen coated micro well strips should be placed in a frame (available with the kit).

Table(2): Test Configuration of ELISA

Row	Strip 1	Strip 2	Strip 3
a	Stander 1	Stander 5	Sample 2
b	Stander 1	Stander 5	Sample 2
c	Stander 2	Control 1	Sample 3
d	Stander 2	Control 1	Sample 3
e	Stander 3	Control 2	Sample 4
f	Stander 3	Control 2	Sample 5
g	Stander 4	Sample 1	
h	Stander 4	Sample 1	

Procedure assay

One hundred µl of diluted patient serum samples, standards and controls was added into the designated micro well strips, one sealer was used for covering the plate which it was then incubated at room temperature for one hour. The preparation of Anti-Ig G Tracer Antibody working solution was done by **1: 21** fold dilution of the tracer antibody with the Tracer Antibody Diluent for each strip, as **1 ml** of Tracer Antibody diluents was mixed with **50 µl** of Ig Tracer Antibody in a clean test tube. After the sealer was removed, aspiration of the contents of every well was done washing every well for **5** times by adding **350 µl** to **400 µl** of working wash solution into each well and then completely aspirating the contents. One hundred µl of the above diluted tracer antibody working solution was added to each of the well. the plate was covered with sealer and also with aluminum foil to avoid exposure to light and then the plate was incubated at room temperature for 30 minutes. Then sealer removing, aspirating of the contents and washing for 5 times by dispensing 350-400 µl of working wash solution were done . A 100 µl of ELISA HRP Substrate was added into each well. The plate was incubated after covering with sealer and aluminum foil at room temperature for 15 minutes. After removing the sealer 100 µl of Stop Solution was added into each well and mixed gently. The absorbance was read at 450 nm within 10 minutes in a micro plate reader [8] .

Results and discussion

Prevalence rate

A total of **1194** stool samples were examined under microscope by using Saline wet-mount method in laboratories of several hospitals . Cysts of *Giardia duodenalis* were detected in **256** stool samples and the infection rate recorded **21.44%**. (Table 3).

Table(3):Distribution of *G.duodenalis* positive samples in human

Location/hospital	Total cases	No.of positive	Percentage%
Central child	319	78	24.45
Al-kadhymia for children	267	59	22.09
Al- yarmouk	282	40	14.18
Abo-Greab	326	79	24.23
Total	1194	256	21.44

Giardia duodenalis prevalence can be attributed to different environmental conditions, asymptomatic carriers, length of diarrhea, type of epidemiological study and use of diagnosis test of difference sensitivity and specificity, in general the prevalence was strongly associated with a variety of risky factors including host, sociodemograph environmental and zoonotic [(9)] . The higher prevalence recorded in this study which was **21.4%** may be due to high resistance of the cysts (infective stage) to environment factors, the low infected dose (**10cyst**), modes of transmission and poor hygiene [(10)] . This finding is almost similar to the results of a study in Brazil when **366** stool samples were examined and the infection rate recorded **23.8%** [(11)] . Also

Foronda [12] concluded that **34.6%** was the infection rate in Egypt. In Malaysia a total of **321** stool samples were examined and the rate was **23.7%** [6] . The CDC recorded **33%** infection rate of giardiasis in developing countries [13] .

Measurement of antibodies concentration by ELISA

The presence of Immunoglobulin (Ig) in the blood serum and other body fluids consider to be as a part of the immune system's response to antigens presents. Antibodies include several immunoglobulin classes IgM, IgG, IgA, IgD, IgE, the detection of individual class or whole classes can be done by serological testes depending on the test protocol[14]. Each of these immunoglobulin is differs in function and significance : In infections, the antibody IgM, is usually detected first but declined within a few weeks. IgG, often show arises a little later but usually lasts for longer, mainly found in the absence of detectable IgM, and give the evidence for past infection. Recent infection is usually indicates by the presence of IgM, with or without IgG. IgA is present in the serum and it secreted into the mucosal, it is particular association with parasitic and other infections in the gut mucosa [15] .*Giardia duodenalis* recognized as one of the commonly infections of human. Thus, a public health importance was documented [16] . As a result for infection, the antibodies production in serum and mucosal secretion occur [17] . Studies of antibodies are used to help characterize the pathogenesis and pathology, yield epidemiologically information that reflect the prevalence and incidence, in the isolation of organisms and /or clinical diagnosis [18]. Also the difficulty in the antigens identification because of antigenic variation lead to depend on these studies [19] . A total of **50** serum samples of infected human were analyzed for ELISA technique to detect and measure the concentration of antibodies, including **25** samples for each male and female, **18** samples were classified under **2- 12** years old(**9** samples for each male and female) and **16** samples classified under each of **13- 33** and **34- 54** years old(**8** sample for each male and female). **18** samples of non-infected humans were collected including **9** samples for each male and female , **6** samples (**3** male, **3** female) were classified under each stage of age .

Table (4):The concentration of IgM antibody in serum of infected human with *Giardia duodenalis*(mean ± standard error)

State	Number of samples	Age(year)	Sex	Mean of concentration µ /ml and standard error
Infected	9	2- 12	Male	18.02± 2.46 Aa
	9		Female	16.85± 2.52 Aa
	8	13- 33	Male	4.22± 0.04 Aa
	8		Female	4.42± 1.07 Aa
	8	34- 54	Male	4.23± 0.38 Aa
	8		Female	4.42± 1.07 Aa
Non- Infected	3	2- 12	Male	1.04± 0.76 Bb
	3		Female	1.62± 0.84 Bb
	3	13- 33	Male	2.2± 0.76 Bb
	3		Female	0.94± 0.60 Bb
	3	34- 54	Male	1.90± 0.70 Bb
	3		Female	1.50± 0.53 Bb

Different capital letters denoted significant differences ($p<0.05$) between infected and non- infected individuals

Different small letters denoted significant differences ($p<0.05$) among ages of infected and non- infected individual

The results showed that the mean of IgM concentration in **9** infected male samples aged between **2-12** years old was **18.02 µ /ml** and higher than the mean concentration of **3** non infected samples which revealed **1.04 µ /ml**, for female within the same stage of age the mean concentration was **16.85 u/ml** in comparison with the **3** non infected samples that showed **1.62 µ /ml**.

Under **13-33** years old, **8** of infected male samples were analyzed for measuring the IgM concentration and the mean recorded higher value **4.22 µ /ml** and **4.42 µ /ml** for the **8** female infected samples, in compared with the non- infected samples which recorded **2.2 µ /ml** and **0.94 µ /ml** respectively. Eight of male infected samples classified under **34-54** years old showed **4.23 µ /ml** for IgM mean concentration and for the **8** female infected samples, the mean concentration recorded **4.42 µ /ml** while the non-infected samples recorded lower value **1.90 µ /ml**, **1.50 µ /ml** respectively. The results showed significant($p<0.05$) differences between infected and control individuals and among ages of infected and non- infected individual (Table 4). The concentration of IgA was determined in serum of infected human and the results showed the mean concentration for **9** infected males under **2-12** years old recorded **43.63 µ /ml** and for the **9** infected females was **54.18 µ /ml** in comparison with the lower value of the **3** non infected samples for each male and female which recorded **0.70 µ /ml** , **1.18 µ /ml** respectively. For the **8** samples of infected male under **13-33** years old, the mean concentration revealed higher concentration **39.33 µ /ml** in compared with the 3 control samples **2.08 µ /ml**. Female infected samples recorded **43.69 µ /ml** and the **3** control samples showed **1.93 µ /ml**. Under **34-54** age old stage , **8** samples of infected male and female were analyzed and the mean concentration of IgA was **48.67 µ /ml** and **61.52 µ/ml** respectively. The controls samples within the same age stage recorded **5.27 µ /ml** and **3.53 µ /ml** respectively. The results showed significant ($p<0.05$) differences between infected and non- infected individual and among ages groups of individual (Table 5).

Table(5):The concentration of IgA antibody in serum of infected human with *Giardia duodenalis*(mean ± standard error)

State	Number of samples	Age(year)	Sex	Mean of concentration µ/ ml and standard error
Infected	9	2- 12	Male	43.63± 4.97Aa
	9		Female	54.18± 2.17Aa
	8	13- 33	Male	39.33± 3.92Aa
	8		Female	43.69± 8.76Aa
	8	34- 54	Male	48.67± 8.94Aa
	8		Female	61.52± 9.3Aa
Non-Infected	3	2- 12	Male	0.70± 0.35 Bb
	3		Female	1.18± 0.86 Bb
	3	13- 33	Male	2.08± 0.89 Bb
	3		Female	1.93± 0.80 Bb
	3	34- 54	Male	5.27± 0.80 Bb
	3		Female	3.53± 1.14 Bb

Different capital letters denoted significant differences (p<0.05) between infected and non- infected individuals

Different small letters denoted significant differences (p<0.05) among ages of infected and non- infected individual

The mean concentration of IgG antibody in serum of **9** infected male was recorded **31.10 µ /ml** and for the **9** infected female samples was **29.29 µ /ml** which is higher than the **3** non infected samples for each male and female, the mean concentration was **3.84, 3.74 µ /ml**. IgG mean concentration for **8** infected male under **13-33** years old showed **13.94 u/ml** and for **8** infected female **28.04 µ /ml**, this was higher than the mean concentration of the **3** non infected samples which recorded **4.65 µ /ml** and **2.65 µ /ml** respectively. Under **34-54** years old stage **8**

infected male samples were analyzed and the mean concentration showed **21.48 µ /ml** and for female showed **21.47 µ /ml** as compared with the **3** non infected samples that showed **3.52 µ /ml** and **3.69 µ /ml** respectively. The results showed significant($p<0.05$) differences between infected and non- infected individual and among ages groups of individual (Table 6).

Table(6):The concentration of IgG antibody in serum of infected human with *Giardia duodenalis*(mean ± standard error)

State	Sample number	Age (year)	Sex	Mean of Concentration µ / ml and standard error
Infected	9	2- 12	Male	31.10± 3.65 Aa
	9		Female	29.29± 1.62 Aa
	8	13- 33	Male	13.94± 1.99 Aa
	8		Female	28.04± 2.52 Aa
	8	34- 54	Male	21.48± 1.01 Aa
	8		Female	21.47± 3.9 Aa
Non-Infected	3	2- 12	Male	3.84± 0.54 Bb
	3		Female	3.74± 0.62 Bb
	3	13-33	Male	4.65± 1.29 Bb
	3		Female	2.65± 0.78 Bb
	3	34- 54	Male	3.52± 0.30 Bb
	3		Female	3.69± 1.82 Bb

Different capital letters denoted significant differences ($p<0.05$) between infected and non- infected individuals

Different small letters denoted significant differences ($p<0.05$) among ages of infected and non -infected individual

The data indicated that the circulating anti-giardia total IgM, IgG and IgA were significantly higher in infected persons than in non- infected persons. The host defense involves several different immunological and non-immunological mucosal processes [20] . Infection with varying isolates differed in their duration, level of cyst excretion, and ability to induce homologous or heterologous immunity. The development of immune system involves both humeral and cellular mechanisms. Human and animals produce systemic immunoglobulin IgM, IgG, IgA which could prevent or help to control infection by interfering with an important adherence step and preventing adequate colonization by *Giardia* [21] . The higher concentration of antibodies may due to high infection dose , absence of rapid recovery, no treatment and long time interval between infection and blood sampling [18] . The incubation period is followed by the acute phase which is resolves spontaneously in healthy persons showed fully developed in their immune system and the symptoms will disappear but Unfortunately, the acute stage develops into a chronic stage in which, symptoms of the disease will reaper for short and recurrent periods [22]. Kraft in1979 [23] showed that the activation of the classical pathway of complement produced lyses do not play a role in controlling parasite numbers because *Giardia* reside in the lumen of the intestine, while the lyses of parasite by specific antibody play a role in limiting the invasion of tissue. This finding is comforted by Hill [24] who proved that antibodies killed more than **98%** of the parasite. From U.K. and South India a total of **52** patients with Giardiasis were analyzed to detect serum antibodies by using ELISA, the results showed that IgM responses occurred in two patients out of three studied longitudinally, after treatment IgM levels had fallen to normal **2-3** weeks. The specificity and sensitivity of the ELISA were **96%**, the IgG antibody was detected in patients and controls but was unable to distinguish between currency or previous infection[25]. Jokipi [26] also showed that IgG can be detected in **80%** of patients and the titers appear to remain elevated after primary infection for months or years, IgG may be raised in non-infected individuals in endemic areas indicating previous exposure to *Giardia* antigens without production of symptomatic infection. Sullivan[27]) showed that IgM antibody titer raised in **50** serum samples of infected human and had specificity of **93%** as a diagnostic test. In Riyadh, Saudi Arabia a total of **139** positive sera and **97** negative sera were

examined, the results revealed that IgM was detected in **97.5%** of positive samples and the sensitivity of the test was **84%** and the specificity was **97%** [28] . In another study, in Denver, the results showed that sera levels of IgM, IgG, IgA increased in infected persons in comparison with the control samples and suggest that levels of IgM may reflect exposure to the parasite early in life, levels of IgA may reflect recurrent exposure to *Giardia lamblia* [29] . Immune defense against *Giardia* include the production of IgA ,which are secreted in large amounts into the lumen of the intestine and their actions are antigen-specific , indirect evidence suggests a role of **IgA** in control of experimental infection [30,31]. Clinical studies have provided an increased incidence of infection in patient with **IgA** deficiency , they have low but detectable levels of serum antibodies IgA. Therefore, most of these patients can still produce low levels of **IgA**, which may be sufficient for controlling *Giardia* infection. The results are similar to a study in India which concludes that about one third of patients have detectable **IgA** mainly those with active infection. However, raised titers were not found in local control subject suggesting that the presence of **IgA** is an indication of current infection [32]. Also Nash [21] showed there was an important intestinal IgA response to acute infection. In 2004, Orquideal [33] found that the level of **IgA** was significantly higher in **66** infected children from **95** examined samples of the children. In Turkey a total of **31**samples were analyzed, results showed that **IgA** levels were increased in **12** cases, IgG were under normal range in **8** cases and IgM levels were increased in **10** cases of patients [34] . Zarebarani [35] showed that the levels of **IgA** recorded significant difference between positive and negative groups ($p<0.05$). According to ages, data obtained in this study provide significant ($p<0.05$) differences between control and infected individual and indicate that exposure to the parasite occurs at an early age. Examination of the three classes of the Ig showed a slowly increase in the mean titers of *Giardia*-specific antibody within age, the IgM showed the largest increase which suggest that exposure to *Giardia* was more prevalent at early age old **2-12** years, the failure to demonstrate any long-term association between elevated antibodies titers and age (growth) makes it un-likely that this infection could account for a significant part of the progressive growth observed in these patients given the high potential for infection or re-infection, the pathogenic nature of the parasite and the

relatively immature immune system of this age. The prevalence of Ig in serum was used for diagnosing infection with *Giardia* . Goka [36] reported that a correlation was detected between specific **IgM** titers and the presence of giardiasis. In developing regions, there is increased prevalence in the young as compared with adults as shown by Miotti [37] . According to 2003-2005 data from the CDC, the greater number of reported cases occurred among children aged **1-4** and **5-9** years and adults aged **35-44** years. [38] . In Turkey , a total of **31** children aged **2-15** years-old with **50** age-matched healthy children were examined and the results showed that IgA level was found to be within normal range in patients, IgG levels were found under normal range and the IgM levels were found higher than normal range ($p<0.05$) [34]. Another study showed that giardiasis affects people of all ages, infection is rare during the first **6** months of life in breastfed infants, but young children have an increased susceptibility to infection. Age-specific prevalence of giardiasis continues to rise through childhood and begins to decline only in adolescence [39] . In a study the results showed that for **34** children under **10** years old with giardiasis, levels of **IgM**, **IgG** and **IgA** were assessed [40] . Roberto[41] showed that from **3461** serum samples tested only **1914(55.3%)** were positive for antibodies against *Giardia* and the seropositivity was age-specific which increased in adolescents **10-19** years of age and in adults more than **40** years of age.

Conclusion

significant differences in *Giardia*-antibodies were recorded between infected and non-infected individuals and among age groups. ELISA can be used for detection , differentiation between acute and chronic infection and for epidemiological studies.

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