Prevalence of *Giardia lamblia* in Asymptomatic Patients by Direct Examination and ELISA Methods

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Summary

Stool samples from 182 asymptomatic patients were collected from two hospitals in Baghdad (Al-Yarmok and Central Child) during the period from the beginning of March to the end of June/2014 and subjected to microscopic and ELISA diagnostic techniques for *Giardia lamblia* detection**;** the sensitivity and specificity for ELISA were calculated. Cysts of *G. lamblia* were observed in 37 and 49 stool samples of asymptomatic patients from a total 182 examined samples with prevalence recorded 20.32, 26.92% by direct examination and ELISA respectively. Of 145 samples showed negative result in microscope examination, 12 samples were positive in ELISA, sensitivity and specificity of ELISA were 75.51 and 100%. Higher prevalence rates were observed in asymptomatic children aged 2-8 years old 27.84% (22+ve/79) in contrast with 26.21% prevalence rate in asymptomatic adults aged 21-57 years old (27+ve /103). According to gender, males showed higher prevalence 28.94% (33+ve/114) while females showed lower prevalence rate 23.52% (16+ve/68). No differences in the level of infection in both age and gender were recorded. Conclusion: The superior sensitivity of the rapid enzyme assay in detecting asymptomatic cases of giardiasis when a single stool specimen is analyzed, the availability of an immunodiagnostic assay which can detect small amounts of antigens in the feces which have a potential tools to improve the diagnosis.

Keywords: *Giardia lamblia*, Asymptomatic giardiasis, Prevalence, Diagnostic methods, ELISA.

Introduction

Giardia lamblia is an intestinal protozoan parasite caused by giardiasis in human and animals; it is considered a neglected disease in both developed and developing countries (1). It is well documented that in developing countries, infections are associated with poor sanitary conditions, poor water quality and overcrowding (2), whereas in developed countries cases are usually associated with international travel and immigration (3). Giardiasis is transmitted via the fecal-oral route with the ingestion of the infective stage (cyst); personal contact and contaminated water and food are considerd to be the primary routes for transmission. The incubation period is usually 3-25 days but may be longer, with a median of 7-10 days (4). Infections with this parasite leads to malabsorption and diarrhea in adults and children and a widely prevalent in children aged less than 5 years (5), but, most often it occurs asymptomatic, infections in children have been shown to have a negative impact on growth and development. Clinical manifestation ranges from asymptomatic carriage to acute and chronic gastrointestinal infections (6).

Techniques for *Giardia* diagnosis based on microscopic examination are usually applied in laboratory routine; the method is time and labour intensive and depends on the skill of an experienced microscopist (7). In addition, it displays low sensitivity because difficulties are encountered in the detection which due to intermittent excretion of the parasite so that several consecutive samples may be needed from each patient for diagnosis and confirmation of giardiasis (8 and 9) compared to immunological and molecular methods (10 and 11). The main objectives of this study was to determine the prevalence of asymptomatic giardiasis in patients and to determine the sensitivity and specificity of a commercial ELISA kit for detection of *G. lamblia* antigen in asymptomatic stool samples.

Materials and Methods

Area of the study: Two hospitals in Baghdad were selected for this study (Central Child and Al-Yarmok) during the period from

the beginning of March to the end of July/ 2014. A hundred and eighty two (182) stool samples were collected randomly in sterile plastic cups from patients attend hospital laboratories for general stool examination, age, sex, and medical history were obtained. These samples included 103 from patients aged 21-57 years old (68 males and 35 females), and 79 from children aged from 2-8 years old (46 males and 33 females).

Direct examination (wet-mount method): The microscopic examination (40 X power lens) for each sample was done immediately (without preservation) using wet-mount method to determine the presence of cysts of *G. lamblia* (12). For confirmation microscopic examination twice times was done on each sample and samples were stored at -20 until use. This work was done at the laboratories of the hospitals.

Immunodiagnostic method (ELISA): The antigen detection was done for all stool samples after thawing by Enzyme Linked Immunosorbent Assay (ELISA), using the Cypress Diagnostic kit (HP007) / Belgium and the Asys / Austria device. The work was done at Biotechnology Research Center/ Al-Nahrain University**.**

Preparation of the samples: dilution of a stool sample was made as follows: One ml of parasite sample diluent was added to a properly marked tube, 100 µl of liquid stool was drawn into a disposable pipet and was suspended in the sample stool diluent .If the stool was solid ,an equivalent amount (50-100 mg) was taken with spatula. Homogenize was done by aspiration and ejection with a disposable pipette, after a short time, the clarified supernatant of the stool suspension was carried out in an ELISA machine. If particles found in the supernatant, it should be centrifuged at 5000 rpm for 5 minutes.

First incubation: two drops (100µl) of the positive control, the negative control (sample dilution buffer) and the diluted samples were pipetted into separated wells. Another 2 drops (100µl) of enzyme conjugate were added to each well, mixed gently and incubated at room temperature for 60 minutes.Washing: aspirated was done for all microwells into a waste container with a disinfectant, tapping the inverted plate onto absorbent paper must be done. Then all wells were washed 5 times with 300µl of prepared washing buffer. After the final washing step, residual washing solution was removed by tapping the inverted microwells on absorbent paper. Second incubation: two drops (100µl) of substrate was added into each well, the plate was incubated for 15 minutes at room temperature in the dark. The reaction was stopped by adding one drop (50µl) stop reagent to each well. After carefully mixing, the absorbance was measured 450 nm against an air blank. Quality control: the test was carried out correctly when the positive control showed an absorbance value (Optical density) at 450 nm greater than 0.8 and the negative control showed a value lower than 0.2.

Calculation of results: The threshold (cut-off) was determined by addition of 0.150 absorbance units to the measured absorption of the negative control.

$$\textbf{Cut off} = \textbf{absorbance value of the negative control} + \textbf{0.15}$$

Samples were considered positive if the absorbance value exceeds 10% the calculated threshold and samples with absorbance value 10% below the threshold were considered negative. Sensitivity and specificity: The sensitivity and specificity for ELISA diagnostic method were calculated according to the following formula (11).

$$\textbf{Sensitivity}\ (\%) = \frac{\textbf{No. of positive samples of both microscopic and ELISA X 100}}{\textbf{No. of positive samples of ELISA}}$$

$$\textbf{Specificity}\ (\%) = \frac{\textbf{No. of negative samples of both microscopic and ELISA X 100}}{\textbf{No. of negative samples of ELISA}}$$

Statistic analysis: Data analysis was performed using Statistical Package for Social Science (SPSS) version 16.0. Chi-square test was used to verify the frequencies. P value less than 0.05 ($P<0.05$) as considered as significant.

Results and Discussion

In this study the results revealed that 37 of asymptomatic human stool samples from 182 samples were recorded as positive for *G. lamblia* using direct examination method (20.32%). For ELISA detection method, 49 samples from a total 182 samples showed the

present of *G.lamblia* and a higher prevalence rate was observed 26.92%. There was no significant differences (P<0.05) between the two methods (Table, 1).

Table, 1: Prevalence of *G. lamblia* in asymptomatic patients according to type of detection test.

Direct examination	Total No. of stool examined samples	Positive No.	Percentage %
ELISA	182	37	20.32
		49	26.92
Chi square value: 2.19, P = 0.13, P < 0.05			

Table (2) results revealed that the positive detection for *G. lamblia* in microscopic and ELISA methods were 37and 49 respectively out of 182 total stool samples while, the negative samples recorded 145 and 133 respectively. For ELISA diagnostic method, 12 samples showed positive results in which theses samples were recorded as negative in microscope examination. Thus, the sensitivity for ELISA was 75.51% and the specificity was 100%.

Table, 2: Sensitivity and specificity of ELISA versus direct examination method for *Giardia lamblia*.

Direct examination	Total No.	ELISA +ve - ve	Sensitivity %	Specificity %
Positive	37	37 0		
Negative	145	12 133		
Total	182	49 133	75.51	100%

The finding of this study showed the higher prevalence by using ELISA detection method 26.92% in comparison with that recorded in microscope method 20.32%. Giardiasis is usually diagnosed by the direct examination method of stool samples for the identification of cysts and or trophozoites, because cysts are excreted intermittently or, in some cases, released in numbers too small to be detected**,** the use of newer tests, like enzyme immunoassay was needed to have a higher sensitivity for detection (13). Asymptomatic persons can shed the *G.lamblia* cysts in the environment. Thus, the diagnosis of *G. lamblia* infection in asymptomatic with the epidemiological factors contributing to the transmission play an important role in achieving control and transmission (14). Some reports have been documented**,** Rosoff (15) reported that out of the 232 randomly collected specimens, 16 were positive by microscope examination and immunoassay, 6 were negative by microscopic examination but positive by immunoassay. Agreement observed with the results of a study showed that from 54 specimens collected from 30 patients 18 asymptomatically infected with *G. lamblia* and 12 with symptoms were determined to be positive (16). Other result was conducted by (14) in which the prevalence of *G. lamblia* infection among asymptomatic children was 37.93% by microscopy and 76.11% by ELISA.

According to the results, all of 37 stool samples of asymptomatic patients which were positive for *G.lamblia* under the microscope recorded positive by ELISA, while 12 samples were positive by ELISA out of 145 negative samples recorded under direct examination method. The sensitivity is the probability that the assay will be positive when the infection is present. The specificity is the probability that the assay will be negative when the infection is absent thus, the ELISA test versus direct examination method gives sensitivity 75.5% and specificity 100%. The result was comparable with (17) who found that *Giardia* detected by ELISA shows 91% sensitivity and 98% specificity. Researches from United States and Germany concluded that ELISA testing is easier, cheaper and faster compared with other serological tests andis useful for the rapid investigation of a large number of stool specimen's ftflaboratories (18 and 19).

In a study conducted by (17) nine different immunoassay kits for the detection of *Giardia lamblia* were evaluated: the sensitivity ranged from 93% to 100% and the specificity in all kits was above 99%. The authors suggest that the high specificity results are due to the examination of up to seven individual slide preparations on an initially negative microscopic finding. Terri-Lynn (20) concluded that ELISA analysis of stool samples may be further adapted for measuring the intensity of giardiasis infection in humans. Noor (21) showed that out of 1680 stool

samples, 380 specimens (22.6%) were found to be positive for *G. lamblia*. Maximum cases were detected by ELISA test with sensitivity of 100% and specificity of 91.5%. According to age, the present study showed that the prevalence of *G. lamblia* infection among 103 asymptomatic patients aged 21-57 years old was 26.21% as 27 samples were recorded positive by ELISA diagnostic method. The higher prevalence of asymptomatic giardiasis in the children aged 2-8 years old was (27.84%), as detection obtained in 22 stool samples from a total 79 samples. There was no significant difference in the level of infection in both age groups (Table, 3).

Table, 3: Prevalence of *Giardia lamblia* according to age in asymptomatic patients

Age year	Total examined samples	Positive No.	percentage %
21-57	103	27	26.21
2-8	79	22	27.84
Total	182	49	26.92
Chi square value: 0.06, P=0.80, P < 0.05			

Children with asymptomatic *Giardia* infection serve as unidentified carriers and may be responsible for transmission of the infection ,while the parasite can be spread in different ways, water (Drinking and recreational) is the most common method of transmission (22). In Turkey, the results of a study showed that 94 (5.4%) children were asymptomatic giardiasis from 599 parasitic children (23), while Inabo (24) reported that out of 374 samples examined, 150 (41.45%) were positive for *Giardia lamblia* and the highest prevalence of asymptomatic giardiasis was in the age group 3-5 years (32.9%) while the lowest was in the 0-2 years (11.6%). Another study showed, 124 children with age range 2–12 years which 64 (61.7%) and 79 (65.7%) of children had *Giardia* by direct stool exam and ELISA test, respectively (25). Children are more frequently infected than adults, this indicates that effective immunity towards this parasitic might have acquired, and they are more involved in outdoor activities which might lead to *Giardia* transmission. many factors such as poor health hygiene and toilet training, overcrowding، low socioeconomic status and climatic conditions play a role in the prevalence of giardiasis (26). These differences in the infection rates from our results could be due to many factors mentioned before. Sex distribution of *G. lamblia* infection in asymptomatic patients revealed that males were at a higher risk (28.94%) compared to females (23.52%), but the difference was not significant in both sexes (Table, 4).

Table, 4: Total infection rate of *Giardia lamblia* according to gender in asymptomatic patients

Gender	Number of examined samples	positive No.	Percentage %
Male	114	33	28.94
Female	68	16	23.52
Total	182	49	26.92
Chi square value: 0.13, P=0.71, P < 0.05			

This result probably is due to the higher activity of male and more contact with environment outdoors, compared to females. A previous study had demonstrated that the prevalence of giardiasis was higher in males (50.3%) than females (49.7%) (24). Also (27) reported that males (52.9%) had a higher rate than females (47.1%). Another study showed that the higher infection rate in males was recorded 2.18% while in females was 1.51% (26). The non-significant variation in both sexes is also in agreement with reports of other researchers (28 - 30).

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أنتشار جياردیا اللامبلية في المرضى عديمي الاعراض باستعمال طرق الفحص المباشر ومقايسة الممتز المناعي المرتبط بالانزيم

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الخلاصة

جمعت عينات البراز من 182 شخص بدون اعراض مرضية من اثنين من المستشفيات في بغداد(اليرموك،الطفل المركزي) من شهر آذار – تموز/ 2014 وخضعت لتقنيات التشخيص المجهري المباشر ومقايسة الممتز المناعي المرتبط بالانزيم للكشف عن وجود جياردیا الاثنا عشرية وتم حساب كل من الحساسية والنوعية لمقايسة الممتز المناعي المرتبط بالانزيم. شخص وجود الجياردیا الاثنا عشرية في 37، 49 عينة براز من اجمالي 182 عينة تم فحصها وسجلت معدلات الاصابة 20,32% و 26,92% بتقنية التشخيص المجهري ومقايسة الممتز المناعي المرتبط بالانزيم على التوالي. من 145 عينة أظهرت نتيجة سالبة بتقنية التشخيص المجهري، كانت 12 عينة أيجابية بتقنية مقايسة الممتز المناعي المرتبط بالانزيم وسجلت الحساسية والنوعية فيها 75.5% و100% على التوالي. لوحظت معدلات اصابة أعلى 27,84% (22 /79) في الاطفال الذين تتراوح اعمارهم بين 2-8 سنوات في مقابل 26,21% معدل الاصابة في البالغين الذين تتراوح اعمارهم بين 21-58 سنة (27 / 103). وفقا لنوع الجنس، اظهر الذكور معدل اصابة اعلى 28,94% (33 /114) في حين أظهرت الاناث انخفاض في معدل الاصابة بلغ 23,52% (16 /68). لم يتم تسجيل فروق معنوية في مستويات الاصابة وفقا للعمر والجنس. الاستنتاج: أظهرت النتائج بوضوح الحساسية الفائقة لفحص الممتز المناعي المرتبط بالانزيم في الكشف عن حالات الاصابة بدون وجود اعراض مرضية عند تحليل عينة براز واحدة، كما ان توفر الاختبارات المناعية التي بامكانها الكشف عن الكميات الصغيرة من المستضدات في البراز زاد من القدرة على تحسين التشخيص في نواح كثيرة.

الكلمات المفتاحية: جياردیا اللامبلية، عديمي الاعراض المصابين بالجياردیا، نسبة الانتشار، طرائق التشخيص، ألاليزا.