Detection of *Giardia lamblia* antigen in stool specimens using enzyme-linked immunosorbent assay

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كشف مستضد الجيارديا اللمبلية في عينات البراز باستخدام مقايسة الممتز المناعي المرتبط بالإنظيم (إليزا)

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الخلاصـة: تهدف هذه الدراسة التي أجريت في العراق إلى التعرف على حساسية ونوعية الاختبار المتوافر تجارياً لمقايسة الممتز المناعي المرتبط المرتبط بالإنزيم (إليزا) لكشف مستضد الجيارديا اللمبلية في البراز. شملت الدراسة 84 عينة برازية جمعت من أطفال محافظة دهوك، واتضح أن 42 منها إيجابياً و42 منها سلبياً للجيارديا اللمبلية أو لغيرها من الطفيليات عند استخدام الفحص المجهري المباشر وغير المباشر، وأن حساسية اختبار مقايسة الممتز المناعي المرتبط بالإنزيم لكشف الجيارديا اللمبلية بالمقارنة مع الفحص المجهري 76.4٪، ونوعيته 100٪. وأوصى الباحثون باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم في المسوحات الوبائية في العراق لتأكيد التشخيص لدى المرضى الذين يعانون من أعراض سريرية نموذجية لداء الجيارديات مع سلبية نتائج الفحص المجهري.

ABSTRACT The aim of this study in Iraq was to determine the sensitivity and specificity of a commercial ELISA test for detection of *Giardia lamblia* antigen in stool. Of 84 stool samples from children in Duhok governorate, 42 were positive and 42 negative for *G. lamblia* or other parasites by direct and indirect microscopic examination. The sensitivity of the ELISA test for detection of *G. lamblia* versus microscopy was 76.4% and the specificity was 100%. We recommend using ELISA in epidemiological surveys in Iraq and to confirm the diagnosis in patients with typical clinical symptoms of giardiasis but negative results by direct microscopy.

Détection de l'antigène de *Giardia lamblia* dans des échantillons de selles à l'aide de la méthode immunoenzymatique (ELISA)

RÉSUMÉ L'objectif de cette étude réalisée en Iraq était de définir la sensibilité et la spécificité d'un test ELISA commercial pour la détection de l'antigène de *Giardia lamblia* dans les selles. L'examen microscopique direct et indirect de 84 échantillons de selles d'enfants du gouvernorat de Dohuk a montré que 42 échantillons étaient positifs pour *G. lamblia* ou d'autres parasites, tandis que les 42 autres étaient négatifs. Par rapport à la microscopie, la sensibilité du test ELISA pour la détection de *G. lamblia* était de 76,4 % et sa spécificité était de 100 %. Nous recommandons l'utilisation de la méthode immunoenzymatique dans les études épidémiologiques en Iraq, ainsi que pour confirmer le diagnostic chez les patients présentant des symptômes cliniques caractéristiques de la giardiase mais dont les résultats par microscopie directe sont négatifs.

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Introduction

The protozoan parasite *Giardia lamblia* is recognized as a major cause of diarrhoeal illness in human [*1*]. Difficulties are encountered in the detection of *G. lamblia* in patients' stool because of intermittent excretion of the parasite so that several samples may be needed from each patient for diagnosis and confirmation of giardiasis. [*2*].

G. lamblia is usually diagnosed from stool samples by visualizing the organism, either the trophozoites or the cysts, in unstained wet smears with the aid of a microscope. A number of methods have been investigated for automating the detection of *Giardia* spp., including immunofluorescent assay, enzyme immunoassay, counter-immunoelectrophoresis and radio-immune precipitation assay. An enzyme-linked immunosorbent assay (ELISA) that detects excretory and secretory products of the organism is available now [*3*]. The test can be completed in less than 3 hours and does not depend on complex equipment for interpretation [*4*].

The aim of this study, was to determine the sensitivity and specificity of a commercial ELISA kit for detection of *G. lamblia* antigen in stool samples from children in Duhok, Iraq.

Methods

Sample

This study was conducted from October 2001 to July 2002 on a community sample of children living in Duhok city in the north of Iraq. In a previous study of the frequency of *G. lamblia* in the area stool samples were collected from 1280 children of both sexes, age range 1 to 12 years, who were attending day care centres, kindergartens, primary schools and the local paediatric hospital. A fresh stool sample was collected from each child into a disposable plastic container with the assistance of their parents or supervisors of the centres. Children with negative results had 2 other samples taken at different times over the study period for examination.

Stool samples were taken immediately to the laboratory of the Department of Microbiology at the College of Medicine, University of Duhok for routine microscopy investigations. For the present study, 82 samples from children with symptoms of giardiasis were randomly selected: 42 samples which were positive for *G. lamblia* and 42 which were negative for *G. lamblia* and any other intestinal parasites by microscopy

Laboratory methods

Microscopy

The samples were processed immediately without preservation. Two types of direct wet film preparation were done for each sample at the same time, 1 slide using normal saline (0.85%) for detecting the actively motile trophozoites and Lugol's iodine (5%) for demonstrating structures. All samples were examined microscopically by the same researchers using 10 × and 40 × power lenses) for the presence of cysts and/or trophozoites of *G. lamblia* and for detection of other parasites. The microscopic examination was done 3 times on each sample for confirmation. The criteria for positive Giardia were active motile flagellated trophozoites and thick hyaline wall of cyst stages.

Enzyme-linked immunosorbent assay

For the ELISA test a 1:10 dilution of fresh or thawed patient stool specimen was prepared. For liquid stool 100 uL of patient specimen was diluted with 1 mL of antigen sample diluent. For solid stools a small portion of patient specimen (volume of a pea) was diluted with 1 mL of antigen sample diluent, mixed well and allowed to stand until heavy particles had precipitated (10 minutes).

The same samples were analysed using a commercial monoclonal microtitre strip ELISA kit for the detection of *G. lamblia* antigen in stool (Novum Diagnostica, Germany), following the manufacturer's instructions. All reagents and controls were supplied by the manufacturer. Diluted patient stool samples, controls and horseradish peroxidase-conjugated monoclonal anti-*G. lamblia* antibodies were pipetted into the microtitre strip well and incubated at room temperature (20–25 °C). After washing to remove unbound material, samples were incubated at 20–25 °C with tetramethyl-benzidine substrate. The absorbance of controls and patient samples was read at 450 nm using an ELISA micro-titre plate reader (Immunoskan-MS, Biological Diagnostic Supplies Limited, UK).

Analysis

The sensitivity and specificity of the ELISA test were calculated as follows: sensitivity = (No. of positive samples by both microscopy and ELISA/No. of positive samples by ELISA) × 100; specificity = (No. of negative samples by both microscopy and ELISA/No. of negative samples by ELISA) × 100.

Results

The comparison between microscopic stool examination (gold standard) and ELISA test for diagnosis of *G. lamblia* is shown in Table 1. The prevalence of infection was 50.0% (42/84) by direct examination and 65.5% by ELISA (55/84).

Of 84 stool samples, 42 were positive and 42 were negative for *Giardia* spp. stages by using microscopic stool examination (Table 1). All the samples that were positive by microscopy were positive by ELISA test and 13 samples that were negative by microscopy were positive by ELISA test. The sensitivity of the ELISA test was therefore 76.4% and the specificity was 100%.

Table 1 Sensitivity and specificity of enzyme-linked immunosorbent assay (ELISA) versus direct microscopy for diagnosis of *Giardia lamblia* in 84 stool samples from children in Duhok

Microscopy	Total	ELISA		Sensitivity	Specificity
		+ve	–ve		
	No.	No.	No.	%	%
+ve	42	42	0	–	–
–ve	42	13	29	–	–
Total	84	55	29	76.4	100.0

Discussion

In our study the ELISA technique was used for the first time to diagnose giardiasis in Iraq. Of a total of 42 stool samples which were positive under the microscope all tested positive by ELISA, while out of 42 stool samples which were negative by microscopy (and checked 3 times), 13 samples were positive by ELISA. This gives a sensitivity of 76.4% and a specificity of 100% for the ELISA test versus microscopic stool examination.

ELISA identified 13 samples as positive that were negative by microscopy. Other researchers found that ELISA was more sensitive and more accurate than microscopic stool examination [5–7]. It is also faster for rapid investigation of a large number of stool samples in laboratories. Similar results have been found in Egypt [8], the United States [9] and Germany [10]. In Turkey, a number of studies have evaluated commercial ELISA kits and other serological tests for detection of *Giardia* antigen in stool. One study found that ELISA had a sensitivity of 88.6% and a specificity of 88.8% for detection of *Giardia* antigen in stool [11], while other studies found a sensitivity and specificity of 96.4% and 80.8% [12] and 98% and 92% respectively [13].

Direct microscopic examination needs experienced staff and is labour intensive. On other hand, the benefit of direct examination is that it also detects other parasites. ELISA testing is easier, cheaper and faster compared with other serological tests and is useful for the rapid investigation of a large number of stool specimens in laboratories. We recommend using the ELISA technique in epidemiological surveys in Iraq and to confirm the diagnosis in patients with typical clinical symptoms of giardiasis but negative results by direct microscopy. Further efforts are needed to prepare an ELISA test from a local strain of *G. lamblia* in order to compare it with the commercially available kits.

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