Diagnosis of *Giardia Lamblia* Infections by Detection of Specific Antigen

Giardia Lamblia Enfeksiyonlarına Özgül Antijen Saptanmasıyla Tanı Konulması

Hatice Akay[1], Semra Kuştimur[1], Ufuk Beyazova[2]

[1]*Gazi University, Faculty of Medicine, Department of Microbiology, Ankara, Turkey*
[2]*Gazi University, Faculty of Medicine, Department of Pediatrics, Ankara, Turkey*

SUMMARY

In our study, the results of a commercial (EIA)test for the diagnosis of giardiasis was compared with the standard microscopic examination. 280 patients who were admitted to Gazi University Hospital Microbiology Laboratory with diarrhea and 60 controls were included in the study. Stool specimens were collected from 340 children with gastrointestinal symptoms who were5 to 11 years old. All stool specimens were examined with microscopy and EIA. In the 78 of 280 samples *Giardia Lamblia* was identified by microscopy, whereas only 74 of 280 samples gave positive reaction by the EIA assay. EIA sensitivity exceeded 92% and its specificity was 99%, by both colourimetry and direct visual interpretation. EIA can be useful in epidemiological examination and may also have a role in confirming clinical diagnosis of giardiasis.

KEY WORDS: *Giardiasis, Enzyme Immuno-assay (EIA), Direct microscopy*

ÖZET

Çalışmamızda, Giardiazisin tanısı için EIA ile rutin mikroskopik yöntem karşılaştırılmıştır. Çalışmaya, Gazi Üniversitesi mikrobiyoloji laboratuarına ishal şikayeti ile başvuran 280 hasta ve 60 kontrol grubu alındı. Yaşları 5-11 arasında değişen toplam 340 çocuktan dışkı numuneleri toplandı. Tüm dışkı numuneleri mikroskopik ve EIA yöntemleriyle incelendi. EIA ile 280 örneğin 74'ü pozitif reaksiyon verirken, mikroskobik yöntemle 280 örneğin 78'sinde pozitiflik saptanmıştır. EIA yönteminin duyarlılığı %92, özgüllüğü %99 olarak belirlenmiştir. EIA epidemiyolojik çalışmalarda yararlı olabileceği gibi giardiazisin klinik olarak tanısını doğrulamada da önemli bir role sahiptir.

ANAHTAR KELİMELER: *Giardia lamblia, EIA, Direkt mikroskopi*

INTRODUCTION

Giardia lamblia is a common human intestinal parasite of worldwide distribution found in both developed and devoloping countries. In endemic areas, high prevalance rates for *Giardia lamblia* infection have been reported in children, mainly among nursery and primary school children.[1]

Giardia lamblia is a flagellated enteric protozoan parasite. There are two forms in the parasite life cycle. An infections cyst form that is the parasitic stage that is passed into the environment through defecation and a trophozoite stage which is found within the infected organism.[2,3] In Turkey, presently giardiasis is the most commonly observed parasitic infection among children. About 10 to 25%of Turkish children are infected. Infection is transmitted directly from person by fecal-oral contamination of cysts or indirectly by water and food.[3,4,5]

Although new approaches to the diagnosis of giardiasis are being sought,[4,5,6,7] most laboratories still rely on microscopical demonstration of *Giardia lamblia* in faeces, intestinal secretions or small intestinal biopsies.[8,9] In general, the examination of giardia is performed in a single stool sample. However, as this parasite presents a variable pattern of excretion, misdiagnoses have been common and actual prevelance may be underestimated.[1] Furthermore microscopical examination of multiple stool samples requires manpower well-trained in identification of Giardia. Small intestine biopsy or duodenal aspiration are invasive and impractical, especially for children.

Corresponding Author:
Hatice AKAY, MD
Cevizlidere mah 16.sok 5/22 06520 Balgat/Ankara
Phone: (0312) 473 32 96
Fax: (0312) 220 10 22
e-mail: haticeakay@mynet.com

Recently, it has been reported that isolation of a Giardia-specific stool antigen (GSA-65) is useful in the diagnosis of giardiasis.[10,11,12] GSA-65 possesses the qualities of a diagnostically ideal stool antigen because it is specific to *Giardia lamblia*. It is stable in the host gastrointestinal tract as a specific antigen which is present in immunologically detectable quantities.[13] Moreover, GSA-65 may be the most abundant immunologically detectable Giardia antigen in the stool of a giardiasis patient.[10] The sensitivity of EIA method, compared to microscopic examination of the faeces in patiens showing signs and symptoms of the infection has been investigated in this study.

Patients And Methods

The 340 children who were included in the study were between 5-11 years. 280 stool specimen from children with gastrointestinal symptoms were obtained at the pediatric outpatient Clinic of the the Gazi University Hospital. 60 control stool specimens were obtained from healthy children who had no recent history suggesting gastrointestinal disease.

Stool specimen examination: The specimens were submitted to the medical microbiology, laboratory of the Gazi University Hospital, Ankara, Turkey. Two stool specimens, one a day were collected from patients and healthy controls and were initially treated with saline and /or iodine solutions before examination.[14] Each specimen was examined by microscopy using low-power (x10) and high-power (x40). Microscopic examination was considered the gold standart. The other unpreserved stool portions were prepared for EIA testing according to each manufacturer's instructions.

Antigen detection in stool samples: Giardia antigen was detected by a commercially available EIA kit (prospect /Giardia,) according to the instruction of the manufacturer. This EIA assay detects a *Giardia lamblia* spesific antigen (GSA-65) associated with the giardia cyst wall. The ProSpecT Giardia microplate assay (Alexon, USA), a monoclonal antibody assay, was performed on each sample (100 μl of rehydrated sample solution, as described above), according to the manufacturer's directions. It detects the GSA 65 *G. lamblia*-specific antigen in stools. The absorbance of each specimen was measured at 492 nm wavelength with a spectrophotometer. The absorbance values were adjusted by subtracting the optical density (OD) of the negative control from the OD of the samples. Specimens which produced an adjusted OD ≥0.241 were considered positive, according to the manufacturers instructions. The sensitivity, specificity, and positive, negative predictive values of the assay were calculated.[15]

Statistical analysis

Values are presented as mean ± SD, with a p value less than 0.05 indicating significance. Differences between the groups were compared using Mann-Whitney test for non-parametric, Student's t-test for parametric variables. All statistical calculations were made using SPSS® for Windows 11.0 (SPSS Inc. Headquarters, Chicago, Ill., USA) software program.

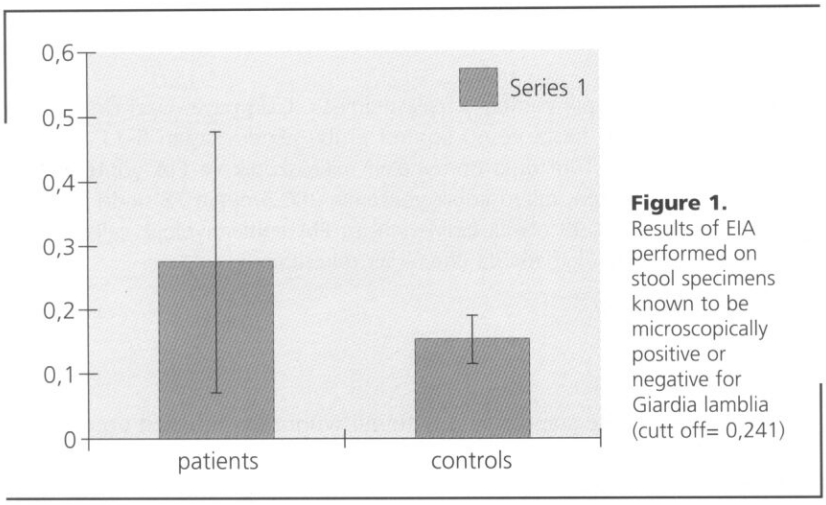

Figure 1. Results of EIA performed on stool specimens known to be microscopically positive or negative for Giardia lamblia (cutt off= 0,241)

Table 1: Clinical Features of 280 Patients with Giardiasis

Symptom	%with symptom
Diarrhea	60.0
Abdominal pain	49.2
Foul stool	49.2
Anorexia	43.8
Nausea	36.9
Distention	3.9
Fatique	29.2
Vomiting	26.1
Headache	24.6
Fever	15.4
Urticaria	15.4
Weight loss	7.7
Mucus in stool	3.1

Results

Symptomatology of giardiasis varies depending on such factors as inoculum size, duration of infection and distinct host and parasite factors. The clinical symptoms observed in our patients are shown in Table 1.

Results of EIA are shown in Figure 1. Mean antigen level of *G. lamblia*

Table 2: Results of ELISA and microscopic examinations for *Giardia lamblia* in stool

	Results of ELISA and Microscopi				Sensitivity[a] %	specifity[b] %	Predictive values	
	Micro (+) ELISA (+)	Micro (+) ELISA (-)	Micro (-) ELISA (+)	Micro (-) ELISA (-)			Positive[c] %	Negative[d] %
Patients(n=280)	72	6	2	200	92	99	98	97
%	25.7	2.1	0.7	71.4				
Controls(n=60)	-	-	-	60				
%	-	-	-	100				

[a] true positives/(true positives+false negatives) *100, [b] true negatives/(true negatives+false positives) *100, [c] true positives/(true positives+false positives) *100, [d] true negatives/(true negatives+false negatives) *100

was 0.275±0.202 in patients and 0.16±0.038 in control ($p<0.01$). 74 of the patients (26 %) displayed higher values than the cut-off point (0.241).

Results of EIA and microscopic examinations for *G. lamblia* in stool specimens and sensitivitiy and spesificity rates of these tests are presented in Table 2. In 78 of 280 samples the organism was identified by microscopy whereas 74 of 280 samples gave positive reactions by the EIA assay.

44 of samples obtained from patients revealed 5 different types of intestinal parasites other than *G. lamblia* under microscopy. The immunoassay and microscopic examination results are given in Table 3.

All of the 340 cultures set up from the specimens showed normal bacterial flora of the intestine.

Discussion

Giardiasis is seen worldwide and in all age groups but most commonly in children. Because of this, diagnosis of Giardiasis needs quick and safe techiques[3,4] The first diagnostic technique for examination of *Giardia lamblia* is microscopy but this method needs adequate number of the parasite's cyst form in the sample.

In our study, microscopic examination detected *Giardia lamblia* in 78 of 280 specimens. Table 2 shows the results obtained from microscopic examination and EIA. In two cases *Giardia lamblia* was not detected by microscopic examination, but EIA were positive. This is probably due to inadequate number of cyst in feaces.

The results show no cross-reactivity between *Giardia lamblia* and helminths, as can be seen in Table 3.

There are only a few Turkish studies for examination of Giardia lambia using the EIA and most of these are epidemiological studies. Yılbaz et al.[16] have reported the sensitivity as 92 % and the specificity as 97.7% for EIA tests. Gödekmerdan et al.[17] found 66 of 260 stool samples positive with the EIA method and found parasite cysts and trophozoites in 52 samples with native-Lugol preparations. The same study have reported a sensitivity of 100%and specificity of 93 %for EIA method. In our study, taking microscopic examination as the reference method, we found the sensitivity of EIA method 92% and specificity of it 99%. The sensitivity of EIA method, even when multiple stool specimens are examined for ova and parasites, the EIA appeared to be more sensitive and specific for Giardia (Table 2).

The ProspecT Giardia assay provided the best result and had the highest sensitivity (92%). Visual interpretation of the resuls was easiest in the ProspecT assay because the contrast colour density between positive and negative results was the highest in this assay. The assay was easier to perform than microscopic examination, which requires a high level of expertise.

The 92% sensitivity achived by the EIA in the USA was equal in performance to a similar test described previously.[18] However, one site testing in Egypt resulted in poorer sensitivity. The sensivity of the EIA was lower for the specimens tested in Egypt (74%) as compared to the tests done in the USA.

Like others previously,[12,19] we used microscopic examination as the gold standard. On the other hand, some have found that *Giardia lamblia* is not always detected by microscopic examination.[18,20] In our study, EIA positivity in two faecal samples in which microscopy was negative (Table 2) could not be attributable to nonspecific binding. We concluded that this apparent discordance was due to availability of only two stool specimens for each patient or may reflect the variability in the level of excretion of *Giardia lamblia*.

Table 3: List of parasites and result of microscopic examination, EIA for GSA 65 *Giardia lamblia* antigen in children feaces.

No. of specimens with parasite	Intestinal parasite	EIA results (OD)
18	Taenia saginata	(-) neg.≤ 0.241
15	Hymenolepis nana	(-) neg ≤ 0.241
08	Ascaris lumbricoides	(-) neg.≤ 0.241
02	Trichuris trichiura	(-) neg.≤ 0.241
01	Entamoeba histolytica	(-) neg.≤ 0.241

Previous studies evaluated several EIAs for the detection of Giardia antigen in stool.[10,21,22] Our results are in agreement with the findings of Carlson et al., Maraha et al. and Aldeenet et al.[11,12,23] They concluded that the ProspecT assay was one of the best assays evaluated (91% sensitivity, 100% sensitivity and specificity). Mank et al.[24] found that Prospect assay and the Giardia CELISA were more sensitive than microscopic examination when only one stool specimen was examined by microscopy. However, microscopic examination was more sensitive when two samples were examined by microscopy. Our results confirm those of the previous studies and indicate the quality of the ProspecT Giardia EIA.

In conclusion, it would appear that GSA-65 represent an ideal antigen around which to design antigen detection methods useful in of giardiasis. The EIA, in its present form, should be very useful for the rapid investigation of large numbers of samples in clinics and in field conditions where complex equipment may not be available. As the technique is also rapid and can accommodate a large volume of specimens, we suggest that it may be very useful in large-scale epidemiologic studies of *Giardia lamblia* incidence and prevalence.

REFERENCES

1. Guimaraes S, Sogayar ML. Detection of anti-Giardia lamblia serum antibody among children of day care centers. *Rev Saude Publica* 2002;36: 63-8.
2. Silva AJ, Moser M. An Illustration of the Life Cycle of Giardia Lamblia National Institute of Health: *National Institute of Allergy and Infectious Diseases* 2007; sept.19.
3. John DT, Petri WA. *Medical Parasitology.* 9 th Edition. Saunders Elsevier: St Louis MO, 2006.
4. Wolfe MS. Giardiasis. *Clin Microbiol Rev* 1992; 5: 93-100.
5. Nash TE, Herrington DA, Levine MM. Usefulness of an enzyme-linked Immunosorbent assay for detection of Giardia antigen in feces. *J Clin Microbiol* 1987; 25: 1167-71.
6. Stibbs HH. Monoclonal antibody-based enzyme immunoassay for Giardia lamblia antigen in human stool. *J Clin Microbiol* 1989; 27: 2582-8.
7. Janoff EN, Craft JC, Pickering LK, et al. Diagnosis of Giardia lamblia infections by detection of parasite-specific antigens. *J Clin Microbiol* 1989; 27: 431-5.
8. Knisley CV, Engelkirk P, Pickering LK, et al. Rapid detection of Giardia antigen in stool with the use of enzyme immunoassays. *American Journal of Clinical Pathology* 1989; 91:704-8.
9. Vinayak VK, Dutt P, Puri M. An immunoenzymatic dot-ELISA for the detection of Giardia lamblia antigen in stool eluates of clinical cases of giardiasis. *J Immunol Met* 1991; 137: 245-51.
10. Addis DG, Mathews HM, Stewart JM, et al. Evaluation of a commercially available enzyme-linked immunosorbet assay for Giardia lamblia antigen in stool. *J Clin Microbiol* 1991; 29: 1137-42.
11. Rosoff JD, Stibbs HH. Isolation and identification of a Giardia lamblia specific stool antigen (GSA-65) useful in coprodiagnosis of giardiasis. *J Clin Microbiol* 1986; 23: 905-10.
12. Carlson JR, Sullivan PS, Harry DJ, et al Enzyme immunoassay for the detection of Giardia lamblia. *Eur J Clin Microbiol Infect Dis* 1988; 7: 538-40.
13. Maraha B, Buiting AGM. Evalution of four enzyme immunoassay for the detection of Giardia lamblia antigen in stool specimens. *Eur J Clin Microbiol Infect Dis* 2000; 19: 485-7.
14. Rosoff JD, Stibbs HH. Physical and chemical characterization of a Giardia lamblia-specific antigen useful in the coprodiagnosis of giardiasis. *J Clin Microbiol* 1986; 24: 1079-83.
15. Thornton SA, West AH, DuPont HL, Pickering LK. Comparison of methods for identification of Giardia lamblia. *Am J Clin Pathology* 1983; 80: 858-60.
16. Yılbaz N, Otag F, Kaya Bartunkal S. Giardiyaz tanısında EIA testi ile Giardia lamblia spesifik antijenlerinin gösterilmesi. *T Mikrobiol Cem Derg* 1994; 24: 126-8.
17. Gödekmerdan A, Özkeklikçi A Bulut V, et al. Giardia intestinalis tanısında mikroskopi ve Enzim-Linked Immunosorbent Assay yöntemlerinin karşılaştırılması. *T Parazitol Derg* 1998; 22: 233-8.
18. Giesecke J. *Modern infectious disease epidemiology* 2nd ed. London: Arnold, 1994.
19. Ungar BLP, Yolken RH, Nash TE, Quinn TC. Enzyme-Linked immunosorbent assay for the detection of Giardia lamblia in fecal specimens. *J Infect Dis* 1984; 149: 90-7.
20. Rosoff, JE, Sanders CA, Sonnad SS, et al. Stool diagnosis of giardiasis using a commercially available enzyme immunoassay to detect Giardia-specific antigen 65 (GSA 65). *J Clin Microbiol* 1989; 27: 1997-2002.
21. Nash TE, Herrington DA, Levine MM. Usefulness of an Enzyme-Lınked Immunosorbent assay for detection of Giardia antigen in feces. *J Clin Microbiol* 1987; 25: 1169-71.
22. Vidal MF, Gilman CRH, Ungar BLP, Verastegui MR, Benel AC, Marquis G, Peenny M, Lanata C, Miranda E. Detection of Giardia lamblia antigen in children living in a peruvian periurban shantytown (pueblo Joven). *J Clin Microbiol* 1991; 29: 636-7.
23. Aldeen WE, Carroll K, Robison R, et al. Comparison of nine commercially available enzyme-linked immunosorbent assays for detection of Giardia lamblia in fecal specimens. *J Clin Microbiol* 1998; 36: 1338-40.
24. Mank TG, Zaat JOM, Deelder AM, et al. Sensitivity of microscopy versus enzyme immunoassay in the laboratory diagnosis of giardiasis. *Eup J Clin Microbiol Infect Dis* 1997; 16: 615-9.