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The use of Enzyme Linked Immunosorbent Assay (ELISA) and Direct Fluorescent Antibody (DFA) Methods for Diagnosis of *Giardia intestinalis*

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SUMMARY: The aim of this study was to evaluate the value of the direct fluorescent antibody (DFA) techniques reported to have high sensitivity and specificity and the enzyme linked immunosorbent assay (ELISA) test used to determine antigens in stool samples in the routine diagnosis of *Giardia intestinalis*. When 44 stool samples in which G. intestinalis cysts and/or trophozoites had been seen during native Lugol examination were investigated, positivity detected with the trichrome staining method, monoclonal ELISA method and monoclonal DFA method was found to be 37 (84.0%), 39 (88.6%) and 35 (79.5%) respectively. DFA detected *Crytosporidium parvum* cysts in addition to *G. intestinalis* in one sample. Twenty-seven (61.4%) of the samples were positive with all three methods. When compared with the DFA method, the ELISA method had a sensitivity of 88.6%, a specificity of 88.8%, a positive predictive value of 79.5% and a negative predictive value of 20.0% while the trichrome staining method had a sensitivity of 85.7%, a specificity of 77.8%, a positive predictive value of 81.1% and a negative predictive value of 22.2%. There was no statistically significant difference between the DFA and ELISA tests and between the DFA test and the trichrome staining method for diagnosing *G. intestinalis* ($p>0.05$).

Key Words: *Giardia intestinalis*, ELISA, Direct Fluorescent Antibody Method

Giardia intestinalis Tanısında Enzym Linked Immunosorbent Assay (ELISA) ve Direkt Fluoresan Antikor (DFA) Yöntemlerinin Kullanılması

ÖZET: Çalışmamızın amacı, *Giardia intestinalis* (*G.intestinalis*)'in dışkı örneklerindeki rutin tanısında antijenlerinin, duyarlılığı ve özgüllüğü yüksek olarak bildirilen DFA (Direct Fluorescent Antibody) ile ELISA yöntemleri kullanılarak değerlendirilmesidir. Nativ-lugol incelemede şüpheli *G.intestinalis* kist ve/veya trofozoitlerinin görüldüğü 44 dışkı örneğinin 37'si (%84) Trikrom boyama, 39'u (%88,6) monoklonal ELISA ve 35'i (%79,5) monoklonal DFA yöntemleri ile pozitif olarak değerlendirilmiştir. DFA yöntemiyle bir dışkı örneğinde *G.intestinalis* ve *Cryptosporidim parvum* birlikte tespit edilmiştir. Örneklerin 27'sinde (%61,4) her üç metodla da pozitiflik saptanmıştır. DFA yöntemiyle karşılaştırıldığında ELISA yönteminin duyarlılığı %88,6, özgüllüğü %88,8, pozitif prediktif değeri %79,5, negatif prediktif değeri %20 olarak tespit edilirken Trikrom boyama yönteminin duyarlılığı %85,7, özgüllüğü %77,8, pozitif prediktif değeri %81,1, negatif prediktif değeri %22,2 olarak saptanmıştır. G.intestinalis'in tanısında DFA ile ELISA ve DFA ile Trikrom boyama yöntemleri arasında istatistiksel olarak fark saptanmamıştır($p>0.05$).

Anahtar Sözcükler: *Giardia intestinalis*, ELISA, Direkt Floresan Antikor Yöntemi

INTRODUCTION

The diagnosis of *Giardia intestinalis (G.intestinalis)* in infected persons is made by observing the cysts or trophozoites microscopically in the stool or duodenal fluid or by the examination of small intestinal samples and biopsies. Such traditional methods require a lot of effort and experienced staff. These methods also lead to false results in 10-50% of the cases when a single stool sample is examined as the cyst may be excreted intermittently in stools and the number of cysts in the stool is low. When infection is known to be present, it may not be possible to detect the parasite in 20-50% of the cases even when concentration methods are used in addition to routine stool examination methods. This

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has led to the development of rapid and reliable methods for the diagnosis of giardiasis. The ELISA method where purified monoclonal antibodies are used to detect *G. intestinalis* cyst antigens in the stool (sensitivity 88-99%, specificity 100%) and the direct fluorescent antibody (DFA) where fluorescent monoclonal antibodies binding specifically to *G. intestinalis* cysts are used (sensitivity 100%, specificity 100%) are tests that have been employed recently for the diagnosis (6, 8, 13).

Our aim in this study was to evaluate the place of the monoclonal ELISA test which is based on searching antigens in the stool and the monoclonal DFA technique which has high sensitivity and specificity for routine *G. intestinalis* diagnosis.

MATERIAL AND METHOD

Forty-four stool samples where *G. intestinalis* cysts or trophozoites had been suspected by native lugol examination were kept in SAF (sodium acetate acetic acid formalin) and PVA (polyvinyl alcohol) fixatives at +4°C until the time of study. The samples kept in PVA fixative were examined by the Trichrome staining method. The stool samples kept at -200C were subjected to monoclonal ELISA (*Giardia* CELISA, Cellabs, Australia) and kept in SAF fixative were subjected to DFA (*Giardia/Cryptosporidium* DFA Cellabs, Australia) tests. For comparison with the DFA method (sensitivity 100%, specificity 100%) the ELISA method and the Trichrome staining method the Mc Nemar test was used for statistical analyses.

RESULTS

Of the 44 stool samples suspected to have *G. intestinalis* cysts or trophozoites on native lugol examination, 37 (84%) were positive with the Trichrome staining method, 39 (88.6%) with the monoclonal ELISA method and 35 (79.5%) with the monoclonal DFA test (Figure 1). DFA showed *Crytosporidium parvum* cysts together with *G. intestinalis* in one sample (Figure 2).

Only 27 (61.4%) of the samples were positive on all three methods. When compared with the DFA method where fluorescent monoclonal antibodies binding specifically to *G. intestinalis* cysts are the ELISA method had a sensitivity of 88.6%, a specificity of 88.8%, a positive predictive value of 79.5% and a negative predictive value of 20% while the Trichrome staining method had a sensitivity of 85.7%, a specificity of 77.8%, a positive predictive value of 81.1% and a negative predictive value of 22.2% (Table 1). There was no statistically significant difference between the DFA and ELISA tests and between the DFA test and the Trichrome staining method for diagnosing *G. intestinalis* ($p>0.05$) (Table 2 and 3).

DISCUSSION

Giardiasis is seen worldwide and in all age groups although it is encountered more frequently in children. Several studies from Turkey at different times have reported the incidence of the causative factor as 1.9-37.7% (9, 10). The infection may be asymptomatic or an acute diarrhea of short duration may develop. Most cases complain of oily soft stool, abdominal distention, gas, abdominal cramps and epigastric tenderness. A change in the quality and amount of the mucus in the jejunum has been shown in patients infected with *G. intestinalis*. The concurrent pathological changes caused by the parasite in the intestinal mucosa lead to a clinical picture of malabsorption. Patients in whom the diagnosis has not been made and the proper treatment not provided may suffer from intermittent bouts of diarrhea with periods of normal defecation for months. Cases that spontaneously heal continue to excrete cysts and are a source of infection (3).

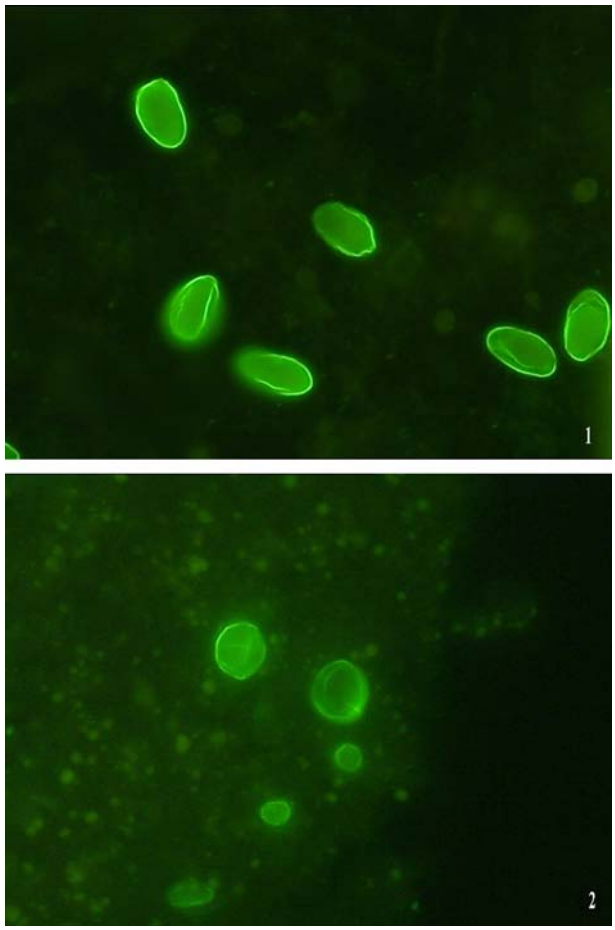

Figure 1. Appearance of *G. intestinalis* cysts with fluorescent microscopy (x40). **2.** Appearance of *G. intestinalis* cysts together with *Cryptosporidium parvum* oocysts on the same preparation with fluorescent microscopy (x40).

The first diagnostic technique to use is microscopic examination but this method may be inadequate as the excretion of the parasite's cyst form is intermittent. Enterotest, duodenal biopsy and evaluation of brush samples are invasive methods and difficult to use, especially in children. Giardiasis diagnosis needs to be quick and safe (6, 8). ELISA and DFA methods have therefore been used for the diagnosis of giardiasis in various

studies in Turkey. Özekinci et al. (11) have reported 136 (96.4%) of the 141 stool samples they have detected G. intestinalis cysts and/or trophozoites in by direct microscopy as positive with the ELISA method. The ELISA method had a sensitivity of 96.4% and specificity of 80.8% in this study. There was a statistically significant difference between the two methods.

Table 1: ELISA and Trichrom staining sensitivity, specificity, positive predictive value and negative predictive value comparate to DFA

	DFA			
	Sensitivity	**Specificity**	**Positive Predictive Value**	**Negative Predictive Value**
ELISA	88,6	88,9	79,5	20,0
Trichrom	85,7	77,8	81,1	22,2

Table 2. Comparison of DFA results to ELISA results

	DFA				Total	
	Positive		Negative			
ELISA	**n**	**%**	**n**	**%**	**n**	**%**
Positive	31	70,5	8	18,2	39	88,6
Negative	4	9,1	1	2,3	5	11,4
Total	35	79,5	9	20,5	44	100,0

P=0,388

Table 3. Comparison of DFA results to Trichrom staining results

	DFA				Total	
	Positive		Negative			
Trichrom	**n**	**%**	**n**	**%**	**n**	**%**
Positive	30	68,2	7	15,9	37	84,1
Negative	5	11,4	2	4,5	7	15,9
Total	35	79,5	9	20,5	44	100,0

P=0,774

Gödekmerdan et al. (7) have found *G.intestinalis* cysts and/or trophozoites in 20% of the stool samples of 260 patients presenting with various gastrointestinal complaints by direct microscopy. They looked for the *G.intestinalis* antigen with the ELISA method in these samples and found the antigen in 25.4%. The ELISA method had a sensitivity of 100% and a specificity of 93% but there was no statistically significant difference between the two methods. Yılbaz et al. (15) have reported a sensitivity of 92.5% and specificity of 97.7% for the ELISA method while the same rates were 98% and 92% respectively in a study by Değerli and Özçelik (4) . Among foreign studies, Schunk et al. (12) found 22 of 276 stool samples positive with the ELISA method and found parasite cysts/trophozoites in 21 samples with microscopic examination. Taking microscopic examination as the reference method, they reported a sensitivity of 100% and specificity of 99.6% for the ELISA method. Aldeen et al. (1) evaluated nine commercial ELISA kits on 222 stool samples obtained from patients suspected of having giardiasis and reported a sensitivity of 88.6-100% and a specificity of 99.3-100% .

There are only a few Turkish studies on using the DFA method for diagnosing giardiasis. Taylan Özkan et al. (14) have detected *G.intestinalis* cysts and trophozoites in 11% of 272 samples of patients suspected of having *G.intestinalis* by the DFA method and in 9% by the Trichrome staining method. Among foreign studies, Alles et al.(2) have found the parasite cysts/trophozoites in 4.4% of 2696 stool samples by DFA and in 2.9% by routine microscopic examination and have reported the sensitivity of the tests as 99.2% and 66.4% respectively and the specificity as 100% for both tests. They state that there is a statistically significant difference for diagnosis between DFA and microscopic examination. Garcia and Shimizu (5) have used various commercial ELISA and DFA kits and reported the sensitivity of the ELISA method in the diagnosis of giardiasis as 94-100% and the specificity as 100% while both rates were 100% for DFA .

In our study, when 44 stool samples where *Giardia intestinalis* cysts and/or trophozoites had been suspected with native lugol examination were checked, the Trichrome staining method, monoclonal ELISA method and monoclonal DFA (reported to have high sensitivity and specificity method) found 37 (84%), 39 (88.6%) and 35 (79.5%) as positive respectively.

Although direct microscopic examination requires experienced staff, it is more economical and quick and can also detect other parasites. It should therefore be used first. It is thought that the ELISA method of detecting antigens will be beneficial when it is not possible to find the parasite although the patients is symptomatic and especially for monitoring the treatment and for epidemiological studies. The DFA method requires the more costly fluorescent microscope but the high sensitivity and specificity make it ideal for confirming the diagnosis when the infection is suspected clinically but the causative agent cannot be demonstrated.

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