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The efficiency of immunoenzyme assay in the diagnosis of lambliosis

Research Article

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Abstract: The aim of this study is to determine the difference in efficiency of direct immunoenzyme-linked assay (EIA) and conventional microscopy (CM) plus conventional concentration technique (CCT) using comparative analysis in the diagnosis of symptomatic and asymptomatic lambliosis when only one stool sample is to be tested. The study enrolled 577 examinees: 208 patients and 369 asymptomatic examinees. Lambliosis was diagnosed using CM plus CCT (three stool samples) and direct EIA (the first sample). All statistical parameters of the EIA method were 100% in the patients with symptoms of infection. In addition to that, in the group of asymptomatic carriers of *Giardia lamblia* (*G. lamblia*) some very high values of these parameters were recorded too, with sensitivity and negative predictive value being both at 100%. In contrast to the EIA method, CM plus CCT of the first stool sample demonstrated significantly lower sensitivity (66.67%) compared to the reference standard. The study did not demonstrate any statistically significant differences in diagnostic efficiancy between the EIA testing of one stool sample and CM plus CCT (p<0.05). However, the observed difference in diagnostic efficiency between the methods was very close to the cut-off value for statistical significance (p=0.06).

Keywords: Conventional microscopy • Immunoenzyme-linked assay • Lambliosis

1. Introduction

The parasite *Giardia lamblia* (*G. lamblia*) can cause digestive tract infection, actually the infection of duodenal mucosis in humans. The symptoms of lambliosis are very diverse. Most characteristic and frequent are mild to moderate abdominal symptoms, abdominal bloating due to intestinal gasses, pain, belching, and rarely colics [1-4]. Lambliosis should be suspected if the above symptoms last approximately ten days. Diarrhea that lasts less than a week without any therapy is most likely not lambliosis [3].

If chronic lambliosis develops in the affected, the resorption of fat, lactose, proteins and liposoluble vitamins is disturbed, i.e. malabsorption syndrom may develop, especially in children [2,4]. Many of the infected do not have any symptoms and signs of lambliosis, and most of the infected worldwide are in fact asymptomatic carriers [2,5-7]. *G. lamblia* is a cosmopolitan parasite, affecting people of all ages and of different socioeconomic backgrounds [4-8]. High risk groups for infection are: children, personnel in the centers for infant care, persons in closed communities (psychiatric institutions, centers for developmentally compromised children and adults, prisons), then international travellers, promiscuous individuals (especially homosexuals), immunodeficient

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patients and family members of those from the high risk groups [2-9].

The diagnosis of lambliosis, whether symptomatic or not, involves parasite examination in order to detect trophozoites or cysts in the patient material (usually in the stool) [10]. Parasite confirmation is accomplished based on morphologic and morphometric characteristics seen on conventional microscopy (CM) of native or stained specimens prepared from patient material, with or without the use of some of the concentration techniques [10]. Generally, conventional concentration techniques (CCT) are used, in particular the sedimentation method. Diagnostic procedures, when lambliosis is concerned may also involve the methods of cultivation, molecular biology, histological analysis, but they are not usually performed in parasitology laboratories [2,4,9-13]. More recently, in order to diagnose lambliosis, immunodiagnostic tests have been devised to detect antigens of the *G. lamblia* parasite in patient material [14-25]. One of the mentioned tests for the antigens detection of the *G. lamblia* protozoan in the feces is the immunoenzyme assay (EIA).

1.1. Aim of the paper

The aim of this study is to detect possible differences in efficiency of EIA and CM plus CCT in the diagnosis of lambliosis if only one stool sample is to be tested. Apart from this, the aim is also to investigate the sensitivity, specificity and diagnostic efficiency of EIA in the diagnosis of symptomatic or asymptomatic lambliosis.

2. Material and Methods

2.1. Patients

We performed the examination of 208 patients, 110 adults (of the average age of 45±1,5) and 98 pediatric patients (<14 years of age). All our patients had some of the digestive disorders typical of lambliosis (nausea, urge to vomit, abdominal pain, occasional diarrhea).

2.2. Asymtomatic examinees

This group consisted of 369 examinees (222 adults and 147 children) without symptoms of gastrointestinal tract (GIT) infection and with increased risk of infection: adults (122) and children (42) who live in refugee centers, psychiatric patients hospitalized in special psychiatric hospitals (100) and proteges (105) from the institute for care of disadvantaged children.

2.3. Parasitological stool examination

Parasitological examination of fresh stool samples without preservation involved microscopic examination of three successive stool samples in the period of 1-3 days (in a 10 days' period). Stool samples were processed and examined within two hours after sampling.

A microscopic examination, with detection and identification of *G. lamblia* by direct stool examination and by use of CCT, formalin ethyl acetate sedimentation technique [10], was done. Test results were considered positive if on CM the cysts of *G. lamblia* were found in at least one examination.

2.4. Serologic examination

One part of fresh first stool sample was stored immediately at -20ºC degrees and tested later by immunoenzyme assay (EIA, Redascreen, Giardia; R-Biophfarm, Germany) according to the manufacturer instruction, by one lab technician who was blinded to the microscopy results. EIA results were obtained using a microplate reader with the wavelight setting of 450 nm. The cut-off value in the test was determined by adding 0.15 absorbance units to the measured absorption of the negative control. Samples were considered positive if the absorbance value was higher than 10% above the pre-determined cut-off. If different EIA and microscopy results were obtained, both tests were repeated. EIA results were compared with those obtained by CM. The samples with a positive result in CM were considered true positive.

2.5. Statistical analysis

Data entry and analysis were performed using the Epi Info (Ver.6.04) software and SPSS (Ver. 8.0 for Windows). The performed tests of attributive variables were the χ^2 test and the Fisher's exact test. The agreement of the EIA method and the "golden standard" (CM plus CCT three stool samples) was determined based on the calculated κ (*kappa*) value gradation by Cohen [26]. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), the negative predictive value (NPV), and the diagnostical efficiency (De) of the EIA method and CM plus CCT of the first stool were calculated and compared to the „golden standard".

The relation between sensitivity and specificity of the immunoenzyme method of detection of *G. lamblia* antigen in the stool, as well as the CM plus CCT of the first stool sample, at different cut-off points was represented as a ROC (Receiver Operating Characteristic) curve. The results of p<0.05 were considered statistically significant.

Table 1. Comparison of the CM plus CCT and EIA results.

EIA (N=577 examinees; 577 tests)	CM (N=577 examinees; 1731 tests)		Total
	(+)	(-)	
(+)	36	2	38
(-)	0	539	539
Total	36	541	577

Table 2. Agreement of the EIA method with the reference standard.

GROUPS	*Kappa*	Interpretation
Symptomatic (208 examinees)	1.000	perfect agreement
Asymptomatic (369 examinees)	0.952	almost perfect agreement
Total (577 examinees)	0.971	almost perfect agreement

Table 3. Statistical parameters of diagnostic efficiency of the EIA.

Groups	Se	Sp	PPV	NPV	De
Symptomatic (208 examinees)	100.00	100.00	100.00	100.00	100.00
Asymptomatic (369 examinees)	100.00	99.43	91.30	100.00	99.46
Total (577 examinees)	100.00	96.63	94.74	100.00	99.65

Table 4. Comparison of the results with CM plus CCT (1 sample) and those with "golden standard".

KVM 1 sample; (N=577 examinees; 577 examinations)	Golden standard (N=577 examinees; 1731 examinations)		Total
	(+)	(-)	
(+)	24	0	36
(-)	12	541	541
Total	36	541	577

3. Results

Our study enrolled 577 examinees in total. On CM (3 stool samples), the number of positive examinees was 36; 6.24% (36/577; 95% Confidence interval - CI=4.26-8.21), and using the EIA method the corresponding number was 38; 6.59% (38/577; 95%CI=4.56-8.62). The difference in prevalence of positive findings of 0.35% was not statistically significant (x^2=0.06; p=0.810; p>0.05) (Table 1).

Based on the Cohen gradation, the immunoenzyme test used to detect the presence of *G. lamblia* antigen in the stool demonstrated an extraordinary degree of agreement with the conventional method – microscopic examination of 3 stool samples (κ=0.971). *Kappa* values demonstrated almost perfect agreement of these two methods in the group of asymptomatic carriers of *G. lamblia* (0.952) and perfect agreement (1.000) in the patients (Table 2).

Comparing the results of the applied methods we determined extremely high values of the statistical parameters of diagnostic efficiency of EIA related to the referent standard (Table 3). All statistical parameters (Se, Sp, PPV, NPV, De) of EIA were 100% in the group of patients with the symptoms of lambliosis. Moreover, in the group of asymptomatic *G. lamblia* carriers very high values of these parameters were observed (Sp=99.43%; PPV=91.31; De=99.46%). And Se and NPV were at the value of 100%.

Immunoenzyme method (EIA), which involves the examination of one stool sample, demonstrated extraordinary values of statistic parameters of diagnostic efficiency in the diagnose of lambliosis. To contrast this finding, using CM plus CCT examination of native preparations of only the first stool samples, we found a smaller number of positive findings (24/577; 4.16%) (Table 4). The difference in the prevalence of lambliosis established by EIA method and CM plus CCT of only the first stool sample was not statistically significant (x^2 = 3.34; p>0.05) for the p<0.05 level, but it was very close to the limit of statistical significance (p=0.06).

In Table 5 we demonstrated the statistical characteristics in the diagnostic efficiency of EIA test and CM plus CCT of the first stool sample related to the "golden standard". While the sensitivity of EIA was 100%, comparing the results with CM plus CCT of the first sample with „golden standard" results, significantly

Table 5. Diagnostic characteristics of EIA and CM plus CCT of the first sample.

	EIA	CM plus CCT
Kappa	0.971	0.789
Se (%)	100.00	66.67
Sp (%)	99.63	100.00
PPV (%)	94.74	100.00
NPV (%)	100.00	97.83
De (%)	99.65	97.92

lower sensitivity values of this method were obtained (66.67%).

Immunoenzyme method was in almost perfect agreement with "golden standard" (*kappa*=0.971), while CM plus CCT of the first stool sample demonstrated significant agreement, where *kappa* was 0.789. The rest of statistical parameters obtained by the comparison of these two methods were relatively high. Both methods demonstrated sufficient diagnostic efficiency (EIA, De=99.65%; CM plus CCT of the first sample, De=97.92%).

In order to evaluate diagnostic characteristics of EIA and CM plus CCT of the first sample, comparing them with the reference standard, ROC analysis was utilized too. The shape of the ROC curve indicated very good diagnostic characteristics of this immunoenzyme method in *G. lamblia* detection in the stool; it was situated almost completely in the upper left corner: AUC was 0.992, while 95% CI=0.739-0.928 (Figure 1).

Characteristics of CM plus CCT of the first stool sample showed relatively low diagnostic significance. The ROC curve was situated at a larger distance from the upper left corner and the surface below the line was smaller (AUC=0.833), 95% CI=0.739-0.928.

Our ROC analysis did not demonstrate any statistically significant difference in diagnostic efficiency of EIA and CM plus CCT of the first stool sample in the diagnosis of lambliosis (p=0.06).

4. Discussion

Conventional microscopy of three stool samples (with or without concentration techniques) is still being recommended as the reference standard ("golden standard") to diagnose infections caused by *G. lamblia* [10]. Near the end of the last century, the introduction of new diagnostic EIA tests to detect *G. lamblia* antigen in the feces was the milestone in the practice of parasitology laboratories, impelling a large group of scientists to assess the sensitivity and specificity of this new method. In the reference literature numerous evaluations could be found that were done after the application of various commercial immunoenzyme tests to diagnose lambliosis [15,16,18-20]. Compared to the "golden standard", the EIA method of *G. lamblia* antigen detection in the feces, has demonstrated high sensitivity (91-100%) and specificity (98-100%) [15,16].

In addition to the comparisons with conventional microscopy of various numbers of samples and commercial tests for material concentration, collateral studies have been done to assess the efficiency of EIA in various patient sub-populations depending on the presence of infection symptoms [21-23].

Figure 1. ROC curve: diagnostic characteristics of EIA[a] method and CM plus CCT of first stool sample related to "golden standard"(N[b]=577).

	EIA	CM+CCT
AUC[c]	0,992	0,833
95% CI[d]	0,978-1,000	0,739-0,928
SE[e]	0,0072	0,0483
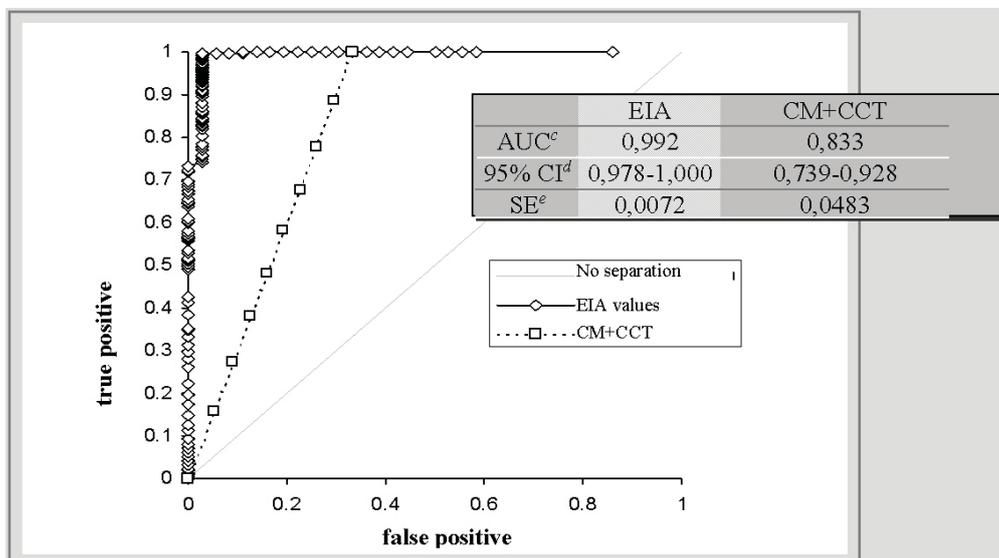
[a]EIA, immunoenzyme method; [b]N, total number of examinees; [c]AUC, surface below the ROC curve/line; [d]95% CI confidence interval; [e]SE, standard error.

In our study, applying statistical analysis, we found that the immunoenzyme test which was used to detect the presence of *G. lamblia* antigen in the stool showed a remarkable degree of agreement with the traditional conventional method of three stool samples with concentration technique (*kappa*=0.971). Based on the gradation by Cohen, *kappa* values demonstrated almost perfect agreement of the two methods in the group of asymptomatic carriers or perfect agreement in the selected group of patients.

Moreover, it was established that EIA test had the sensitivity of 100% in the diagnosis of symptomatic or asymptomatic lambliosis, where the rest of the parameters of diagnostic efficiency are very high as well. During the study, a positive result was not obtained with conventional microscopy and negative with EIA method, meaning that EIA did not have false negative results in comparisson with CM plus CCT as the "golden standard". In the group of examinees without *G. lamblia* using CM plus CCT, there were two cases in which EIA detected *G. lamblia* antigen in the material. Such a finding could perhaps be explained by the possibility that *G. lamblia* antigen was present in the stool, without finding of preserved parasite on CM [23].

It is well known that as the consequence of intermitent excretion of cysts during the so called "negative phase" of lambliosis and smaller numbers of cysts in the feces, false-negative results of stool examination could be obtained on CM, regardless of whether the material was taken from the individuals with symptomatic lambliosis or asymptomatic individuals with *G. lamblia* colonization of duodenal mucosis [27]. This is the principal reason for the recommendation that *G. lamblia* in the intestines should be identified with CM examination of three stool samples or even more than three, especially in individuals with typical symptoms of lambliosis [15,18,19,23].

In our study, we compared the results of the examination of only the first stool samples with EIA and CM plus CCT . The results obtained with CM plus CCT of the first stool sample, compared to the "golden standard" demonstrated significantly lower sensitivity (66.67%) of the method compared to EIA test results (100%). Using ROC analysis, we confirmed the extraordinary diagnostic potentials of the immunoenzyme method to detect *G. lamblia* antigen in the stool. Based on statistical parameters (the surface below the ROC curve was AUC=0.992), it could be concluded that the infected examinees had EIA test values higher than those without infection in 99.2% of the cases. On the contrary characteristics of CM plus CCT of the first stool sample are of less diagnostic value. ROC curve in this case was at a distance from the upper left corner, the surface below the line was significantly smaller (AUC=0.833), 95% CI=0.739-0.928; CM plus CCT of the first stool sample enabled precise differentiation of infected individuals from those without infection in 83.3% of the cases.

We did not observe a significant statistical difference (at the level of p<0.05) among these methods (EIA vs CM plus CCT) in the detection of *G. lamblia* infection in the first stool sample. The difference in diagnostic efficiency of the methods was very close to the cut-off points for statistical significance (p=0.06), which indicated the importance of this study for routine clinical and laboratory practice. In addition to very high values of statistical parameters of diagnostic efficiency, advantages of EIA are also simple and fast procedure, objectivity of the findings as well as cost-effectiveness. Acording to high sensitivity and specificity, even if only one stool sample is examined, it could well be said that immunoenzyme method should represent the method of choice in the diagnosis of lambliosis if we are unable to repeat the test several times.

Taking into account the fact that prevention measures aiming to detect and suppress the spread of infectious diseases routinely involve the examination of only one stool sample taken from selected individuals. EIA tests could be primarily recommended for routine and/or *ad hoc* epidemiological surveillance, as well as in routine diagnosis in hyperendemic regions. The method could also prove useful in the surveillance of therapy effectivity [21].

5. Conclusion

Our results support the presumption that the EIA method is a satisfactory alternative to conventional microscopy in the diagnosis of lambliosis, but that we should also pay close attention to the fact that CM remains the only method that is able to simultaneously detect the presence of other possible parasites.

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