Received: 18 Apr 2024 | **Accepted:** 05 Aug 2024 **Genel Tıp Derg. Volume** 34/**Issue** 4 (August), 542-546
DOI: 10.54005/geneltip.1470640

ORIGINAL ARTICLE

Evaluation of Adhesin Antigen Test Results in Samples Sent with Suspicion of Amebiasis

Amibiyazis Şüphesiyle Gönderilen Örneklerde Adezin Antijen Test Sonuçlarının Değerlendirilmesi

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How to cite ?

Beder D, Esenkaya Taşbent F. Evaluation of Adhesin Antigen Test Results in Samples Sent with Suspicion of Amebiasis. Genel Tıp Derg. 2024;34(4):542-6.

ABSTRACT

Objective: In this study; it is aimed to evaluate E. histolytica-specific ELISA adhesin antigen test results in stool samples sent to the medical microbiology laboratory with suspicion of amebiasis.
Material and Methods: ELISA (Cellabs, Entamoeba Celisa Path, Brookvale, NSW Australia) adhesin antigen test results, examined on stool samples sent to the medical microbiology laboratory with suspicion of amebiasis in a two-year period between January 2022 and December 2023 were evaluated retrospectively. The data including the test results, gender, age, date and clinical information of the cases of the examined sample were obtained from our hospital's laboratory data software system. Fisher's exact chi-square test was used in statistical analyses.
Results: Of the 1120 samples in the study, 578 (51.6%) belong to male patients and 542 (48.4%) belong to female patients. The ages of the patients ranged from 0 to 94 years, and the average age was 33.92 (standard deviation: ±24.33). There were 335 (30%) samples from pediatric patients, and the mean age was determined as 4.48 ±4.78. One hundred and sixty-one (14.3%) samples were found positive with the ELISA adhesin antigen test specific to E. histolytica. Of patients with positive test results, 77 (47.8%) were male and 84 (52.2%) were female. Among the positive samples, there were 55 (34.1%) samples from pediatric patients. While there was no statistically significant difference between the positivity status and age and gender of the patients (p>0.05), the seasonal difference was considered significant (p<0.05).
Conclusion: In our study, E. histolytica specific ELISA adhesin antigen test positivity was determined as 14.3%, and this rate was found close to the country's literature data. It has been determined that amoebiasis is more common in tropical and subtropical climates. With the use of ELISA adhesin antigen test specific to E. histolytica, which is cheap, fast and does not require experienced personnel, the rate of misdiagnosis can be reduced and unnecessary treatment applications can be reduced.

Keywords: Amebiasis, Entamoeba histolytica, ELISA adhesin antigen test

ÖZ

Amaç: Bu çalışmada amibiyazis şüphesiyle tıbbi mikrobiyoloji laboratuvarına gönderilen dışkı örneklerinde E. histolytica'ya spesifik ELISA adezin antijen test sonuçlarının değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntem: Ocak 2022 ile Aralık 2023 tarihleri arasındaki iki yıllık dönemde amibiyazis şüphesiyle tıbbi mikrobiyoloji laboratuvarına gönderilen dışkı örneklerinde incelenen ELISA (Cellabs, Entamoeba Celisa Path, Brookvale, NSW Avustralya) adezin antijen testi sonuçları retrospektif olarak değerlendirilmiştir. Olgulara ait test sonuçları, cinsiyet, yaş, tarih ve örneğin geldiği klinik bilgileri hastanemiz laboratuvar bilgi sisteminden elde edilmiştir. İstatistiksel analizlerde Fisher kesin ki-kare testi kullanılmıştır.
Bulgular: Çalışmadaki 1120 örneğin 578 (%51.6)'i erkek, 542 (%48.4)'si kadın hastalara aittir. Hastaların yaşları 0 - 94 yaş aralığında olup, yaş ortalaması 33.92 (standart sapma: ±24.33) olarak bulunmuştur. Çalışmada çocuk hastalara ait 335 (%30) örnek bulunmaktadır ve bu hastaların yaş ortalamaları 4.48 (standart sapma: ±4.78) olarak tespit edilmiştir. E. histolytica' ya spesifik ELISA adezin antijen testi ile 161 (%14.3) örnek pozitif bulunmuştur. Pozitif örneklerin 77 (% 47.8)'si erkek, 84 (%52.2)'ü kadın hastalara aittir. Pozitif örnekler içinde çocuk hastalara ait 55 (%34.1) örnek bulunmaktadır. Hastaların pozitiflik durumu ile yaşı arasında ve pozitiflik durumu ile cinsiyeti arasında istatistiksel olarak anlamlı bir farklılık görülmemişken (p>0.05), mevsimler arasındaki farklılık anlamlı kabul edilmiştir (p<0.05).
Sonuç: Çalışmamızda E. histolytica' ya spesifik ELISA adezin antijen testi pozitifliği %14.3 oranında belirlenmiş ve bu oran ülke literatür verilerine yakın bulunmuştur. Amibiyazisin tropikal ve subtropikal iklim özelliklerinde daha sık görüldüğü belirlenmiştir. Ucuz, hızlı ve deneyimli personel gerektirmeyen E. histolytica'ya spesifik ELISA adezin antijen testi kullanımı ile yanlış tanı oranı düşürülerek gereksiz tedavi uygulamaları azaltılabilmektedir.

Anahtar Kelimeler: amibiyazis, Entamoeba histolytica, ELISA adezin antijen testi

Introduction

Amoebic dysentery (amobiasis) is a parasitic infection caused by Entamoeba histolytica (1). Entamoeba has six known species which are Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba hartmanni, Entamoeba coli and Entamoeba polecki. The only species that causes amebiasis in humans is reported as E. histolytica. Amebiasis is widely detected all over the world and is considered a public health problem (2). Every year, 50 million cases of amebiasis are reported in the world, and approximately 100,000 of these cases result in death (3). The studies in our country have reported that the incidence of infection varied between 0% and 29.5% (1).

Peer-Review: Double anonymized - Two External
Plagiarism Checks: Yes - intihal.net
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Selcuk University Press
Genel Tıp Dergisi | e-ISSN: 2602-3741
https://dergipark.org.tr/tr/pub/geneltip
https://yayinevi.selcuk.edu.tr/

E. histolytica infection was determined endemic in regions where temperate climate prevails, and it is associated with low socioeconomic status and inadequate hygiene conditions in developing countries (4). In developed countries, the cause of this infection is thought as travel to endemic regions (5).

Most of the infected individuals are asymptomatic (3). In 10% of patients, the infection progresses symptomatically and clinical courses such as dysentery, ameboma, acute necrotizing colitis, toxic megacolon, perianal fistula and ulcer and liver abscess may be observed (6,7,8).

In the diagnostic examination of stool samples, differentiating E. histolytica from E. dispar, which is a morphologically identical but genetically different species, is important in terms of planning the correct treatment protocol and preventing transmission. The World Health Organization states that cases with E. dispar should not be treated, and all symptomatic or asymptomatic E. histolytica cases should be treated. (9).

The most commonly used method in the diagnosis of amebiasis is direct microscopy. However, since this method cannot discriminate E. histolytica and non-pathogenic E. dispar, more reliable diagnostic methods are needed (10).

Recently, the use of molecular tests that detect the genetic material of E. histolytica and have higher sensitivity and specificity compared to microscopic examination and culture methods has been increasing. These diagnostic tests work is integrated with automated systems and provides the opportunity to give the results in a short time. Therefore, it gives a great advantage especially in laboratories with high workload. Also, with the use of commercial panels, it has become possible to detect more than one pathogen at the same time. However, its high cost causes limitations in its use (11).

Serological diagnostic techniques for detection of antibodies are especially useful in the diagnosis of extra intestinal amebiasis. However, the fact that the antibody response can be detected for a long time after treatment causes limitations in distinguishing between active and previous infection (12,13).

Antigen-detecting ELISA (Enzyme-Linked Immunosorbent Assay) tests can also differentiate E. histolytica and E. dispar. These tests have advantages such as high specificity and sensitivity, objective and rapid results, no need for experienced personnel, low cost especially compared to the molecular techniques, and the ability to process a large number of samples at the same time (14).

This study aimed to detect the frequency of intestinal amebiasis in stool samples sent with suspicion of amebiasis using ELISA adhesin antigen test specific to Entamoeba histolytica.

Material and Methods

This study was performed with the approval of the Ethics Committee of Necmettin Erbakan University Faculty of Medicine (Date: 01.03.2024 and Decision No: 2024\4832).

ELISA (Cellabs, Entamoeba Celisa Path, Brookvale, NSW Australia) adhesin antigen test results examined on stool samples and sent to the medical microbiology laboratory with suspicion of amebiasis in a two-year period between January 2022 and December 2023 were evaluated retrospectively.

In the ELISA method, the stool sample is emulsified with the sample dilution liquid. The diluted stool sample and the conjugate with a monoclonal antibody specific to the parasite antigen are placed in the wells of the microplates. These wells contain polyclonal antibodies that will bind to the E.histolytica antigen. If antigen is present in the stool sample, it combines with the polyclonal antibodies in the microplates and the specific monoclonal antibodies in the conjugate. Non-specific compounds are removed by washing. When the substrate is added, color formation occurs due to the enzyme-antibody-antigen complex.

The data including the test results, gender, age, date and clinical information of the cases of the examined samples were obtained from our hospital's laboratory data software system. The statistical analysis was performed by using SPSS, 22.0 for windows. Descriptive statistics were used to define demographic data, and the outcomes were expressed by number, mean and percentages. Fisher's exact Chi-square test was used in statistical analyses. A value of $p<0.05$ was considered statistically significant.

Results

Of the 1120 stool samples included in the study, 578 (51.6%) were from male patients and 542 (48.4%) from female patients. The ages of the patients ranged from 0 to 94 years, and the mean age was 33.92 years (standard deviation: ±24.33). Of the samples, 335 (30%) of them were obtained from pediatric patients, among them 184 (55%) were boys and 151 (45%) were girls. The mean age of these patients was 4.48 years (standard deviation: ±4.78).

The Entamoeba histolytica ELISA antigen test positivity rate was 14.3% (n=161). Of the 161 patients with a positive antigen test, 77 (47.8%) were male and 84 (52.2%) were female. The mean age of the antigen test positive patients was found as 31.5 years (standard deviation: ±24.48). In the study, 55 (34.1%) samples were positive in pediatric patients, and the mean age of these patients was 4.72 years (standard deviation: ±5.34). Pediatric patients with a positive test result constituted 16.4% of the general pediatric population. Additionally, 22 (40%) of positive pediatric patients were male and 33 (60%) were female. There was no statistically significant difference between the positivity status and age and gender of the patients ($p>0.05$).

The clinical departments where the 161 positive patients included the study were followed are presented in Table 1.

Table 1. Distribution and rates of ELISA antigen test positive patients according to clinical departments

	N	%
Hepatology \Inflammatory bowel diseases	39	24.2
Pediatric emergency	33	20.5
Gastroenterology	26	16.1
Pediatrics	14	8.7
Infectious diseases	11	6.8
Pediatric Hematology-Oncology	8	5
Medical Oncology	7	4.3
Organ and Tissue Transplantation Center	6	3.8
Hematology	5	3.1
Nephrology	5	3.1
Internal Medicine	4	2.5
Other Departments	3	1.9
Total	161	100

When the seasonal distribution of Entamoeba histolytica infection was examined, antigen test positivity was observed at a rate of 9.3% (n=15) in winter, 14.9% (n=24) in spring, 18.6% (n=30) in summer and 57.1% (n=92) in autumn. Additionally, it was determined that this positivity peaked in September with a rate of 33% (n = 54). It was determined that the positivity rate observed in the autumn season was significantly higher than other seasons. (p=0.001)

Discussion

Amebiasis is reported as a cause of serious morbidity and mortality all over the world (15). The prevalence of this infection in the world is reported as 10%, however, this rate increases up to 50%, especially in underdeveloped countries (4,16,8). When the previously performed studies were evaluated, the prevalence of E. histolytica was detected as 4.2% in Bangladesh, 8.4% in Mexico, 15% in Brazil, 20% in Saudi Arabia, 17.1% in Yemen, 25.9% in Tajikistan, and 18% in Tanzania (15,4,16,17). There are many studies in the literature to determine the prevalence of amebiasis in different regions of our country. According to the studies, the prevalence of E. histolytica was 0.5% in Kırıkkale 0.8% in Bursa, 7% in Istanbul, and 7.7% in Mersin (18,19,20,21).

The methods such as direct and trichrome staining techniques and microscopic examination, serological techniques based on antigen or antibody detection, culture and molecular tests are used for the diagnosis of E. histolytica infections (22). The fact that the previous prevalence studies were based on microscopic examination causes a limitation in the reliability of the prevalence of intestinal amebiasis. The low sensitivity of microscopic examination and the inability to differentiate E. histolytica trophozoites and cysts from leukocytes and other Entamoeba species in the feces caused false reporting of the prevalence of the infection (2).

In a study, only 25 (61%) of the 41 stool samples in which E. histolytica positivity was determined by other diagnostic methods could be identified by direct microscopic examination. (14). In a study conducted in Iraq, 47.66% E. histolytica positivity was detected by microscopic diagnostic method (23). In another study, 87 stool samples were suspected for E. histolytica by direct microscopy. E histolytica positivity was reported in 21.7% of these samples by ELISA and in 26.4% by trichrome staining technique (24). In India, 167 stool samples were examined and 9% (n=15) of E. histolytica/E. dispar were detected in microscopic examination, and the rate of E. histolytica was determined as 6% (n=9) by antigen-specific ELISA (25).

It is possible to reveal the accurate prevalence of E. histolytica infection by identifying other Entamoeba species by using diagnostic techniques with high specificity and sensitivity (26,27). E. histolytica-specific monoclonal ELISA antigen tests are frequently preferred today, especially because they do not show cross-reactivity with other enteric pathogens (28). In the previous studies, it was reported that the results of the ELISA method and molecular techniques used in the detection of E. histolytica were similar. Another study reported that the sensitivity of microscopic examination was 60% and the specificity was 79% while the sensitivity of antigen screening techniques was 80% and the specificity was 99% (3). In the discrimination of E. histolytica/E. dispar sensitivity of ELISA was 95% and the specificity was 93%, when compared to the zymodem technique (29). In our study, the Entamoeba histolytica ELISA antigen test positivity rate was detected as 14.3% (n = 161).

There are studies in the literature reporting that there is no significant relationship between E. histolytica positivity and gender. (30,6). In our study, it was also determined that gender had no effect on the detection of E. histolytica.

In a study in which microscopic examination, culture and isoenzyme analysis diagnostic methods were used to examine the stool samples of children with diarrhea living in the city, E. histolytica infection was detected in 4.2% and E. dispar infection was detected in 6.5%. While in children living in rural areas E. histolytica infection was detected in 1% and E. dispar infection was in 7% (15). In a study conducted in Iran, E. histolytica was reported at a rate of 1% in 10982 children (31). In a study conducted in Ethiopia, E. histolytica positivity was reported at a rate of 13.17% in 501 school children (32). In another study, E.histolytica/E.dispar positivity was established by microscopic examination in 6% of 500 randomly selected cases from the pediatric age group, while it was 3.2% by antigen-detecting ELISA (33). In our study, E. histolytica positivity was determined in 16.4% (n=55) of 335 pediatric patients by ELISA adhesin antigen test.

In a study conducted by using adhesin antigen, regarding the seasons and clinical departments in Kırşehir, positivity was in 4% (n = 6) of the patients, and it was reported that these patients mostly came from the gastroenterology clinic in the autumn season (34). In a study conducted in Sivas, positivity was in 25% (n=65) of the patients with the ELISA adhesin antigen test and it was reported that positivity was highest in 40% (n=22) in winter season. In this study, it was stated

that 28.6% of the patients with a positive antigen test came from the internal medicine clinic, 26.4% from the gastroenterology clinic, 25% from the pediatrics clinic, and 20% from the infectious diseases department (30). In our study, the positivity rate was 14.3% (n=161) and this positivity was the highest in the autumn season at a rate of 57.1% (n=92). In our study, 24.2% of the positive patients came from the hepatology/inflammatory bowel diseases (IBD) department, 20.5% from the pediatric emergency department, 16.1% from the gastroenterology department, 8.7% from the pediatrics department and 6.8% from the infectious diseases department.; We believe that the clinical distribution of E. histolytica positive patients in the studies and the seasonal characteristics when positivity is most common varies based on factors such as the patient's clinical condition, the clinician's differential diagnosis approach, personal hygiene, socioeconomic level and sanitation infrastructure.

Conclusion

In our study, E. histolytica specific ELISA adhesin antigen test positivity was determined as 14.3%, and this rate is close to the country's literature data. It has been determined that amoebiasis is more common in tropical and subtropical climates. The diagnostic algorithm of E. histolytica infections should be based on confirmation of microscopic examination with a different diagnostic technique and discriminating E. histolytica from E. dispar in stool samples. We believe that the use of ELISA adhesin antigen test specific to E. histolytica, which is cheap, fast and does not require experienced personnel, will reduce unnecessary treatment procedures by decreasing the rate of misdiagnosis.

Ethical Approval: This study was performed with the approval of the Ethics Committee of Necmettin Erbakan University Faculty of Medicine (Date: 01.03.2024 and Decision No: 2024\4832).

Authorship Contribution Statement

The authors have contributed to the article as the following:

Spec. Dr. Duygu Beder: Data Collection, Processing, and Reporting, Logical interpretation and presentation of findings, conducting the literature review, and writing the article.

Assoc. Prof. Fatma Esenkaya Taşbent: Generating ideas for the article, supervising, taking responsibility for executing the project, and intellectually examining the study content before submission.

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