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PROBLEM IN AMOEBIASIS DIAGNOSIS IN CLINICAL SETTING: A REVIEW FROM CONVENTIONAL MICROSCOPY TO ADVANCE MOLECULAR BASED DIAGNOSIS

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Abstract
Amebiasis is an infection of the large intestine, and sometimes the liver and other organs, caused by a single-celled parasite (protozoa) Entamoeba histolytica, or amoeba. Amoebiasis, an intestinal protozoan disease caused by the bacterium Entamoeba histolytica, is a public health problem. Amoebiasis tends to occur in areas where sanitation is inadequate. About 50 million people around the world are infected with amebiasis each year, and it causes approximately 100,000 deaths. Therefore, laboratory detection of pathogenic E. histolytica bacteria and distinguishing them from non-pathogenic bacteria plays a crucial role in treating patients. The most sensitive methods for laboratory diagnosis of intestinal amebiasis include a variety of ELISA tests. To diagnose amebiasis, your doctor will collect stool samples for analysis. The best way is to test the stool for a protein released by the amoeba (antigen tests), or to use the polymerase chain reaction (PCR) technique to detect the genetic material of the amoeba in the stool. This technique produces copies of Many of the amoeba's genetic material, thus making the amoeba easier to detect. Antigen tests or polymerase chain reaction (PCR) are more useful than microscopic examination of stool samples, which are often inconclusive. Laboratory diagnosis of extraintestinal amebiasis is challenged by the lack of definitive detection of current infections and commercially available point-of-care tests. For both types of amebiasis, there is still a need for sensitive, highly specific, rapid and cost-effective tests for use in developing countries where the disease is common. In recent years, new molecules of diagnostic value have been discovered and new tests have been developed. Advances in “omics” technologies allow the discovery of new biomarkers that may help differentiate between different stages of infection.

Introduction

Amoebiasis is a parasitic disease caused by Amoeba or Entamoeba histolytica, a human-specific protozoan and a colon-selective parasite. In 1903, Chaudin explained the epidemiology and circumstances of this disease. In 1968, tissue transmission of amebiasis was also observed [1]. The disease has been described as an endemic disease and not an epidemic disease, meaning that its appearance remains linked to health, hygiene and climatic conditions, as it is transmitted directly through contaminated hands, food and drinks. Amoebiasis is still among the most widespread infectious diseases in the world, as this disease affects between 40 and 50 million people annually [2], 3-9% of whom are exposed to complications and the transmission of amebiasis through tissues (liver, lung, and brain), which kills approximately 100,000 cases per year. The spread of this disease is increasing in less developed regions. In Mexico, for example, there are 1 million cases of circulating amoeba infection [3,4].

There are many cases of patients with liver abscesses of the amoebic, pyogenic, and fungal types, in addition to the difficulty of clinical diagnosis of these types [5,6], as well as the importance of accurate differential diagnosis for each of them, as treatment is possible and follow-up varies depending on the type [7,8], in addition to our societies' ignorance of health methods. cultural and disease prevention. Amoebiasis, as well as negligence and excessive negligence by the medical and laboratory community in the correct and proper diagnosis of this disease in general and histological amebiasis in particular [9], all of this prompted us to conduct such research.

Diagnosis Laboratory

The diagnosis of the E. histolytica parasite is based on microscopic examination of the initial form of the parasite, especially in the case of bloody diarrhea, within half an hour to an hour of the samples arriving at the laboratory. The presence of erythrophagocyte trophozoites in stool samples indicates an infection with amoeba histolytica [10], and microscopic examination was considered by researchers to be 100% specific for diagnosing amoeba disease Fotedar Despite this, cases of bloody diarrhea are rather few, and in the absence of active phases that devour red blood cells [11], the sensitivity of the microscope is limited; This is due to the inability to distinguish between samples containing amoeba histolytica and those containing amoeba variants, which are more than ten times more prevalent. Also, the occurrence of suspicious cases between amoeba histolytica and other non-pathogenic amoeba or white blood cells in the samples often results. This leads to a misdiagnosis of amoeba disease. In recent years, molecular biological techniques have developed to be the basis for diagnosis and investigation of histolytic amoeba [12]. However, the high cost and time consumption that characterize these techniques, in addition to the lack of experience, make microscopic examination the only test used in Poor countries, while other modern tests are limited to developed countries and research laboratories only [11,13]. Laboratory diagnosis is carried out in several ways:

- ***Direct Examination***

This method includes observing the stool visually and identifying its phenotypic nature that may suggest infection with amoeba histolytica [14], which in the case of acute infection with E. histolytica is bloody and mucus-like, as active phases are seen in such samples. Only, where swabs are made from this stool from different areas of the sample and examined under the minimum power (10X) and then the high power (40X) after staining it with iodine dye or without staining it [15]. As for hard stool It contains only the cystic stages, which are characterized (only mature ones) by containing four nuclei with the presence of rod-shaped chromatid bodies with rounded ends. Thus, it can be distinguished from the cystic phase of non-pathogenic E. coli, where the chromatin bodies have blunt ends [13]. There are several dyes that can be used in this method to help diagnose the cystic and active phases of the amoebic dysentery parasite. For example, Lugol's Iodine dye, which dyes the nucleus dark brown and the cytoplasm yellow or light brown. Buffered blue wet mount dye is used to dye the active phase and the cystic phase. This is because the Iodin dye kills the active phase, and other dyes are used to stain the sample, such as Iodin tricome dye and Iron haematoxylin dye [12,15].

- ***Culture methods***

This method has been used to isolate amoeba species for more than eighty years, and the culture medium includes a pure axenic culture and an impure xenic culture, as the E. histolytica parasite can be cultured from fecal samples, rectal biopsy samples, or liver cysts, and laboratory sources indicate that the proportions are Success Cultivation of the E. histolytica parasite ranges between 50-70%. This method is non-routine and less sensitive than the microscopic examination method. It is a difficult and expensive process and requires careful work, in addition to bacteria, fungi, or other protozoa, which are considered a major problem facing the culture. Therefore, this method is not recommended as a diagnostic method. An approved routine for detecting amoeba species in the laboratory [16], while Shnawa et al. (2007) concluded that isoenzyme analysis can distinguish between amoeba species, as it distinguishes between E. histolytica and E. dispar. It was considered the gold standard for diagnosing amoebic infection before the development of modern DNA-based techniques [17].

- ***Serological tests***

Through it, opposites are detected in several ways including: Indirect hemagglutination antibody (IHA) test, Counter immunoelectrophorsis (CIE), Enzyme-linked immunosorbent assay (ELISA), Immuno diffusion (ID) examination, Amoebic gel diffusion test (AGD),Install the Complement Fixation (CF), Indirect immune-fluorescence assay (IFA), and Latex agglutination test (LA) [18].
These methods were used not long ago to distinguish between species of the genus Entamoeba. The ELISA test is one of the most common methods used to determine infection with amebiasis, especially in patients with amoebic liver abscesses. This method has the ability to identify antibodies in patients with a high percentage, where IgG immunoglobulin appears. In the blood one week after infection with colitis and amoebic liver abscess. It is worth noting that some of these tests are specialized in detecting several species belonging to the genus Entamoeba, such *as E. histolytica*, *E. dispar*, and *E. moshkovskii*, while there are other tests. It is specialized in detecting E. histolytica only, and it must be taken into account that these tests may sometimes give false results because the presence of IgG is not considered an indicator in areas where the disease is endemic, but in general, determining infection depends on the presence of IgG or on the presence of IgM, which is formed during the first week of infection, and the main importance of these tests is their lack of reliance on self-diagnosis, as is the case with microscopic examination [9].

Molecular tests

An example of these tests is the Polymerase Chian Reaction test, which has a high ability to diagnose the amoebic dysentery parasite. This is done through DNA extracted from a stool sample. These tests are characterized by high sensitivity, as they can diagnose the presence of the amoebic dysentery parasite even if only one parasite is present in the sample. They also have a high ability to differentiate non-pathogenic species belonging to the genus Entamoeba from *E. histolytica.* One of the obstacles that prevent the widespread use of these tests is the high cost that these tests require, as well as the high level of expertise they require [19]. Methods based on PCR technology have succeeded in detecting the three types of amoeba and have been chosen for clinical studies. And epidemiological studies in developed countries [3], and this technology has developed into a number of methods, the most modern and accurate of which are real-time polymerase chain reaction technology and DNA sequencer technology. The following is a definition of this modern technology:

- ***Real time polymerase chain reaction (RT – PCR)***

It is a modern scientific technique that combines the amplification of a genetic DNA sample with the detection of the quantity and quality of the amplification product in one reaction tube, which protects the samples from contamination as a result of opening and closing the reaction tubes [20]. This technology relies on double dinucleotide reagents, which are a radioactive dye that binds to each reagent that has a sequence complementary to one of the strands resulting from the reaction. A dye

that produces the color fluorophore, which is capable of emitting fluorescence, called the reporter dye, is attached to terminal No. (5) of the detector, and another dye called the absorbent dye is attached to terminal No. (3) of the detector. When the reporter dye is excited by a laser or halogen, this dye absorbs the energy of photons and Then transferred to the absorbent dye via Fluoresce resonance energy transfer (FRET). There are many applications for the use of RT-PCR in medical fields, for example, to diagnose HIV (Human immunodeficiency virus) (Gibellini et al., 2004) and in laboratories to diagnose incurable diseases such as bird and swine influenza. One of the advantages of the real-time polymerase chain reaction test is that it is not affected by amplification. For non-specialists, inflation can be monitored in real time through a screen that has a wide range of motion up to 10^{10} and the rotation is very fast, ranging from half an hour to two hours. In addition, there are no post-test transactions as in PCR [21].

- ***DNA Sequencer***

It is known as (DNA sequencing), which is the process of determining the exact arrangement of nucleotides within the DNA molecule and includes any method or technique that is used to determine the arrangement of four bases - adenine, guanine, cytosine and thymine in the two strands of DNA. The emergence of rapid DNA sequencing methods has accelerated dramatically. Major in biological and medical research and scientific discoveries [22]. DNA sequencing can be used to determine the sequence of individual genes. The sequence provides the arrangement of individual nucleotides found in DNA or RNA molecules isolated from animals, plants, bacteria, or almost any other source of genetic information. This information is useful for various fields of biology and other sciences, medicine, and forensics. and other fields of study [23]. The method for estimating nitrogenous bases has evolved from oligonucleotide to modern methods such as capillary electrophoresis to estimate nitrogenous bases in DNA.

Conclusion

Laboratory diagnosis of both intestinal and extraintestinal amebiasis has improved with more sensitive and specific tests. For enteric infections, molecular diagnosis is the way forward, with PCR-based assays for well-equipped laboratories and LAMP for low-resource settings. For extraintestinal infections, there is a need to improve serodiagnosis to accurately detect active infection because exclusion of prior infection is challenging for current IgG-based assays. Likewise, the sensitivity of POC rapid diagnostic tests for ALA could also be improved and made commercially available to enable their broader application. Additional areas of research that could be explored include discovering biomarkers that can predict which individuals are susceptible to developing symptoms, distinguishing between different stages of infection and monitoring the success of treatment.

References

1. Castellano E.M. and Marinez P.A. Pathogenesis of intestinal amoebiasis: from Molecules to disease. Clin. Microbiol Rev; 13: 318-330, 2000.
2. Ling Vay e medicine. Com, 2004.
3. 3-Sharma M.P. and Ahuja V. Amebic liver abscess. JIACM; 2: 107-111, 2003.
4. Cabalero J; Viveros M. and Salvatierra B. Seroepidemiology of amebiasis in Mexico. Am. J. Trop. Med. Hyg; 50: 412-419, 1998.
5. Tierney L.M; Mcphee S.J. and Papadolis M.A. Current: Medical diagnosis and treatment. 37th. ed.; 1327-1332, 1998.
6. Haque R; Ali I.K.M. and Petri W.A.Jr. Diagnosis of amebic liver abscess and intestinal infection with the Tech Lab Entamoeba histolytica, antigen detection and antibody tests. J. Clin. Microbiol; 38: 3235-3239, 2000.
7. Angel C; Chand N. and Sankar A. Gastric wall erosion by an amebic liver abscess in a 3 years old girl. Pediatr Surg. Int; 16; 429-430, 2000.

8. Restrepo M.I; Restrepo Z. and Aguirre A. Diagnostic tests for 39 cases of liver Abscess: comparison of ELISA and CIE. Rev. Sac. Bras. Med. Trop; 29: 27-32, 1996.
9. Niscigorska J. and Kaczmarsks A. Amoebic liver abscess Personal Observation. Przegl Epidemiol; 50: 281-286, 1996.
10. Tanyuksel, Mehmet, and William A. Petri Jr. "Laboratory diagnosis of amebiasis." Clinical microbiology reviews 16.4 (2003): 713-729.
11. Fotedar, R., et al. "Laboratory diagnostic techniques for Entamoeba species." Clinical microbiology reviews 20.3 (2007): 511-532.
12. Nath, Joyobrato, Sankar K. Ghosh, and Baby Singha. "Problem in amoebiasis diagnosis in clinical setting: a review from conventional microscopy to advance molecular based diagnosis." J. Acad. Indus. Res 2 (2013): 257-261.
13. Parsons, Linda M., et al. "Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities." Clinical microbiology reviews 24.2 (2011): 314-350.
14. Cook, G. C. "Diagnostic procedures in the investigation of infectious diarrhoea." Baillière's clinical gastroenterology 7.2 (1993): 421-449.
15. Joseph, K. H., V. L. Parmar, and N. B. Manvar. "Diagnosis of Parasitic Infection through Faecal Examination."
16. Pritt, Bobbi S. "Molecular diagnostics in the diagnosis of parasitic infection." Methods in Microbiology. Vol. 42. Academic Press, 2015. 111-160.
17. Shnawa, Bushra Hussain. "Molecular diagnosis of Entamoeba histolytica, Entamoeba dispar, and Entamoeba moshkovskii: An update review." Annual Research & Review in Biology 21.5 (2017): 1-12.
18. MacMillan, A. P. A. A. "Conventional serological tests." Animal brucellosis 206 (1990): 153-197.
19. Ngui, Romano, et al. "Differentiating Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii using nested polymerase chain reaction (PCR) in rural communities in Malaysia." Parasites & vectors 5 (2012): 1-7.
20. Neumaier, Michael, et al. "Fundamentals of quality assessment of molecular amplification methods in clinical diagnostics." Clinical chemistry 44.1 (1998): 12-26.
21. Mallewa, Macpherson. Central Nervous System Infections at Queen Elizabeth Central Hopsital Paediatric Unit, Blantyre, Malawi. The University of Liverpool (United Kingdom), 2009.
22. Metzker, Michael L. "Emerging technologies in DNA sequencing." Genome research 15.12 (2005): 1767-1776.
23. França, Lilian TC, Emanuel Carrilho, and Tarso BL Kist. "A review of DNA sequencing techniques." Quarterly reviews of biophysics 35.2 (2002): 169-200.