Methods of the Detection of Parasite Infections in Libya- A Review

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Abstract

Diagnosis of parasitic diseases requires highly sensitive and specific tests. In many cases the identification of parasites concerns their epidemiology and it is important to distinguish between species and subspecies. The current laboratory tests practiced in Libya are based on conventional techniques including serology and microscopy. More rapid and accurate tests are needed to meet the increasing number of patients in Libya. Nowadays, advanced methods are used to improve the diagnosis of parasitic diseases. These include molecular-based techniques such as polymerase chain reaction (PCR). The principle of nucleic acid probes is that a specific sequence of the parasite's DNA is isolated and used in a hybridization assay to identify homologous parasite DNA from infected material. Since DNA normally remains the same during every stage of the parasite's life cycle, this technique has many applications. The effective control and treatment of parasitic diseases requires rapid, reliable and highly sensitive diagnostic test. This review summarizes both the conventional methods and the progress in new approaches in parasite diagnoses, and debates some of the merits and demerits of these tests.

1.Introduction:

Little information is available regarding the diagnosis of intestinal parasites[8-10]. The most common protozoal infections are Blastocystishominis followed by Entamoeba histolytica / Entamoeba dispar or Giardia lamblia and Entamoeba coli among Libyan population. Moreover, Cryptosporidium Spp. infections have been reported in Libya among patients particularly in children with diarrhea [1]. The detection of Cryptosporidium spp is not routinely done in laboratories. For this reason, frequency of cryptosporidiosis and source of infections are not fully known in Libya [1-2]. However, other studies also reported a higher prevalence of giardiasis, as it was 8.7% in out patients in Tripoli, 7.8% in expatriates in Benghazi, 6.24% in children attending hospital in Benghazi 15,5.85% children with gastroenteritis in Benghazi, 7.2% among Libyan patients in Sirte, and 10.29 % in children and neonatus admitted in Ibn-Sina Hospital, Sirte[2].

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In parasitology, usual laboratory diagnosis includes traditional approaches, for instance visual microscopy, used for morphological identification of parasite. However, the occasional difficulty of identifying these parasites structures may decrease the sensitivity of such methods. Currently, because of these difficulties, molecular biology has been employed to detect parasites responsible for parasitic diseases [3]. During the last 10 years, the diagnosis of agents of infectious disease has begun to include the use of nucleic acid-based technologies.

Diagnosis of parasite organisms is an ultimate field of clinical microbiology to integrate these methods, because it's costly to use a new technology add also, the rarity of these parasites in countries where this research is taking a place [4]. The initial tests recently used to diagnose multiple parasitic illnesses have slightly changed since the progress of microscopy by Antonio Van Leeuwenhock. Although, the majority of the recent tests cannot discriminate between past, latent, acute and reactivated inflection and are not beneficial for following response to therapy or for prognosis [5].

Several molecular tests to detect parasites have been developed in the last decade. Their specificity and sensitivity have gradually increased, and parasites that were previously difficult to diagnose using conventional techniques began to be identified by molecular techniques [6]. The objective of the present study was to review both current and new diagnostic techniques for confirmation of parasite infections.

2.Microscopy:

Microscopy has been the only tool available for the detection of parasites through inspection of blood smears, tissue specimens, feces, lymph node aspirates, bone marrow, and even cerebrospinal fluid [7-8]. This lag in diagnostic innovation can be partly explained by the fact that detection and differentiation of cysts, ova and larvae, when performed by well-trained technicians, are simple, fast and inexpensive. In addition, microscopy also

allows for detection of unexpected parasites, which may be particularly appropriate, as well as convenient, when the clinical presentation is atypical or vague. In resource-limited health systems the advantages of microscopy are more obvious. When labor costs are low and investment in new technologies is limited, molecular techniques have little to offer for daily clinical practice. Moreover, basic microscopy procedures are sufficient to detect the most prominent parasite species in regions with a high prevalence and intensity of intestinal parasites. [3-9]. Furthermore, basic microscopy procedures are sufficient to detect the most prominent parasite species in regions with a high prevalence and intensity of intestinal parasites.

Although the field of diagnostic technology reached advanced level these days, microscopy test of stool specimens maintains essential to the diagnosis of majority of pathogenic intestinal protozoa. Microscopy is, nevertheless, labor-intensive and it takes finesse technologist.

New, highly sensitive diagnostic methods have been developed for protozoa endemic to developed countries, including Giardia lamblia [10]. Giardia intestinal is, Giardia duodenalis and Cryptosporidium spp., using technologies that, if expanded, could effectively complement or even replace microscopic approaches. The incentives for investing in the development and implementation of new routine parasitological tests have therefore been low in these settings. However, in many western countries, conventional microscopy continues to be the first-line diagnostic procedure in most clinical parasitological laboratories [11].

In Libya previously studies included in this review used more than one technique of microscopic examination of stool samples for cysts of E. histolytica, G. lamblia, and Cryptosporidium. There are three species, among others, in the genus Entamoeba that can be found in the human intestine. Moreover, other studies also reported a higher prevalence

of giardiasis, as it was 8.7% in out patients in Tripoli 13, 7.8% in expatriates in Benghazi 14, 6.24% in children attending hospital in Benghazi 15, 5.85% children with gastroenteritis in Benghazi 16, 7.2% among Libyan patients in Sirte 17 and 10.29 % in children and neonatus admitted in Ibn-Sina Hospital, Sirte 18. The microscopic examination of stool specimens remains the backbone of the diagnosis of intestinal protozoa, particularly in developing countries [7-11].

3.Polymerase Chain Reaction (PCR):

In medicine, the species identification of a parasite is important for prevention, diagnosis, and treatment of infectious diseases. PCR-base procedures have changed many aspects of study that's mainly because of the enzymatic amplification of DNA, which can be applied in vitro from little quantities of substance. This is has to do with parasitology, due to it is highly unlikely to get or seclude ample quantity of substance from parasites at their various life-cycle levels for traditional diagnosis. These techniques provide alternative methods for detecting specific pathogens in stool [12]. The PCR makes it possible to perform selective amplification from complex genomes. This technique is based on the process of denaturing a double-stranded genomics DNA template using heat [9]. The PCR technique has high specificity and sensitivity during the differentiation between Leishmaniasis species in comparing with conventional methods. The polymerase chain reaction (PCR), using genomics or kinetoplastid DNA; provides an excellent tool for diagnosis and characterization of Parasite species [13]. The discovery of thermotolerant DNA polymerases and the development of automated PCR processors have facilitated the introduction of PCR into the diagnostic laboratory and have led to an exponential increase in the number of PCR applications [10-14].

Molecular biologic techniques have been applied to the diagnosis of infections with the parasite genus Plasmodium for many years for several reasons. Four species of the parasitic

protozoan Plasmodium infect humans (Plasmodium falciparum, P. vivax, P. malariae, and P. ovale) is responsible for most of the morbidity and mortality. Accurate diagnosis of Plasmodium species is essential for successful treatment [14]. The coccidian parasite Toxoplasma gondii is responsible for widespread asymptomatic infection of the human population worldwide. This infection can cause significant morbidity and mortality in the developing fetus and when reactivated in the immunocompromised individual. Therefore, rapid and specific detection of the parasite within the host is required for accurate diagnosis and treatment, since serologic detection of a host response is inadequate. For these reasons, there has been significant research dedicated to the development of DNA-based diagnosis of T. Gondi in human specimens. Additionally, the people infected with the protozoan parasite, E. histolytica, become asymptomatic carriers, and only 10% develop symptoms of invasive amebiasis. Isolates from asymptomatic carriers are considered to be nonpathogenic, although cysts may be excreted. Diagnosis by microscopy frequently overestimates the prevalence of pathogenic E. histolytic because of the inability to distinguish pathogenic and nonpathogenic strains and the presence of morphologically similar species.

Nonpathogenic isolates possess different is enzyme patterns and have been shown during the last few years to be genetically distinct from pathogenic strains. Recently, separation of E. histolytic isolates into two species, the pathogenic E. histolytic and the nonpathogenic E. dispar, has been proposed [14]. The reviewed reported suggest that E. histolytic, G. lamblia and Cryptosporidium Spp. may play a minor role in GE among the population in Libya and that infections occur mainly in children 10 years old or younger. In addition, the previously reported high prevalence rates of E. histolytic /dispar reported from Libya could have been due mainly to E. dispar and E. moshkovskii. However, more studies are needed using E. histolytic-specific EIA and/or molecular methods (i.e. PCR) to confirm this observation [11].

4. Serology-Based Assays:

Serology-based diagnosis tools can be divided into two categories: antigen-detection assays and antibody-detection assays. These include the enzyme-linked immunosorbent assay (ELISA), also called enzyme immunoassay (EIA), and all its derived tests such as the Falcon assay screening test ELISA (FAST-ELISA) and the dot-ELISA. Other assays include the hem agglutination (HA) test, indirect or direct immunofluorescent antibody (IFA or DFA) tests, complement fixation (CF) test, and immunoblotting and rapid Diagnostic tests (RDTs) [4-7]. There is a growing willingness of well-equipped laboratories to radically adapt their diagnostic algorithm and introduce high-throughput DNA-detecting assays. The actual process of introducing DNA-detecting assays as first-line routine diagnostic procedures is not only dependent on patient populations and parasite prevalence, but also strongly dependent on sample logistics and the national reimbursement systems [6].

In recent years, several enzyme immunoassays (EIAs) that detect antigens of E. histolytic, G. lamblia, and Cryptosporidium spp. in fresh or frozen stool specimens with 85_100% sensitivity and 93_100% specificity have become commercially available. Only one study in Libya used EIAs to investigate stool samples from diarrheic children in Tripoli [11]. The investigators observed low prevalence rates of E. histolytic [0.8%], G. lamblia [1.3%], and Cryptosporidium spp. [2.1%]. From the previously reported, these three different species of Entamoeba cannot be differentiated by microscope . Furthermore, a study from Saudi Arabia investigated 156 stool samples from diarrheic children for E. histolytic a [5] . The authors concluded that E. histolytic/ dispar in 65% of the samples by microscopy and E. histolytic [2.6%] of the samples by specific EIA [11] . As discussed, immunodiagnostics tests have some serious limitations. Parasitic diseases such as amebiasis, cryptosporidiosis, filariasis, giardiasis, malaria, cysticercosis, schistosomiasis, and African trypanosomiasis do not have commercially or FDA approved antibody detection tests for their diagnosis [2-15].

5. Conclusion:

During the past 10 years, the diagnosis of agents of infectious disease has begun to include the use of nucleic acid-based technologies. Review of the progress towards the development and testing of diagnostic assays that utilize nucleic acids to detect parasites revealed that, although many systems have been developed, few have proceeded towards field trials or large scale clinical evaluation.[16]. Diagnosis of parasitic organisms is the last field of clinical microbiology to incorporate these techniques, due in part to the expense of new technology as well as a scarcity of these parasites in countries where this research is ongoing [4-9].

There is rising require for high-throughput, low-complexity as well as cost effective complements to the labor-intensive microscopy- based approaches and this is in order to protozoan diagnosis [15-16]. These methods are now performed in both diagnostic and research settings. Immunodiagnostic and molecular methods will become increasingly important for the detection and identification of blood parasites. However, light microscopy will still be required for the foreseeable future, and hematologists should maintain their proficiency in the morphological diagnosis of the common blood parasites of humans, especially malaria. In these exceptions, either the parasite level is extremely low, differentiation between morphologically identical organisms is required, or the immune response to the parasite infection is uninformative. PCR-based diagnosis will be too expensive and technically demanding for use in developing countries where the per capita expenditure for total health care is very low. Automation of various steps (sample preparation and processing, amplification, and detection) and the commercial availability of reagents and products would impact on widespread use. However, these techniques will and already have played a role in studies of the epidemiology, taxonomy, and pathogenesis of parasite infection in humans.

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