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Changing Trends in the Diagnosis of Parasitic Diseases – An Indian Scenario*

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Abstract

Parasitic diseases are major problem worldwide. Until recently, the parasitic methods of the diagnosis which depended on the detection and identification of various morphological forms of the parasites in different body fluids including stool of the infected host was considered as a key and indispensable method in the diagnosis. Of late, these methods have been relegated to the background with the advent of non-microscopic methods. There have been also rapid strides in the developments in immunodiagnostics with the use of enzyme immunoassays, radioimmunoassays, chemiluminescence and nephelometry. The use of stool, urine, tear, saliva etc as alternate clinical specimen instead of blood in the diagnosis of parasitic infections is another important development in the diagnosis of amoebiasis, cystic echinococcosis and neurocysticercosis. Further the advent of molecular methods of diagnosis have revolutionized this field with PCR in certain cases like that of amoebiasis changing the philosophy of its diagnosis by showing that most infections are caused by non pathogenic species. The rate at which these new tests have been emerging suggests that the next few years would see even more research and development in this field.

Keywords: *Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii,* amoebiasis, neurocysticercosis, cystic echinococcosis, serodiagnosis, LPCB, malaria

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Introduction

The load of parasitic diseases globally has been greatly increasing in the recent times. The number of humans affected by these diseases ranges into millions and the morbidity due to these diseases may vary from relatively innocuous to fatal. One of the misconceptions about parasitic diseases had been that they are prevalent only in the tropical areas but now it has been shown that they affect populations in temperate regions as well. As a result of the HIV/AIDS epidemic many hitherto unknown and unimportant parasitic diseases have come under close scrutiny as opportunistic pathogens. An early diagnosis and treatment of the condition significantly has the potential to reduce mortality and morbidity due to these diseases to a great extent. In keeping with the rapid strides and development taking place in all the frontiers of infectious diseases, there have also been rapid and fast changes in the study of parasitic infections, especially in the field of laboratory diagnosis of these conditions (1).

Laboratory diagnosis of parasitic infections

The clinical diagnosis of parasitic infections is hampered by the low prevalence, late development and lack of specificity of major clinical signs. Therefore, laboratory methods play an important role in diagnosis of parasitic infections.

One of the exciting modes of diagnosis to have come up in the recent past has been the imaging procedures. X-rays have been used for the diagnosis of hydatid cysts where these show up as round lesions with irregular margins. Calcified cysts are much easier to make out. The ultrasound has also been used with great effect in the diagnosis of hydatid disease as well as filariasis. The 'water lily' sign depicting cysts with floating membrane is pathognomonic of hydatid disease. The method is also helpful in the medical follow up of patients treated with chemotherapy, successfully treated cysts becoming hyperechogenic (2). The advent of advanced imaging techniques like CT and MRI aided in the diagnosis of some of the parasitic diseases. These techniques were more accurate than either X rays or ultrasound. In fact, the diagnosis of the widely prevalent disease of neurocysticercosis is based mostly on CT scan. But these imaging methods have limitations. Their results are still inconclusive. For example, it continues to be a problem to distinguish other lesions such as tuberculoma of the brain

from that of the NCC. Further, these facilities are expensive and are restricted to tertiary health care hospitals, corporate hospitals, etc.

These and other such drawbacks have seen a renewed interest in the development of laboratory based methods which are cheaper, easier to execute and have easy applicability in the field. The rapid development of new methods and techniques in the area of molecular biology has gained new insights into the genetic and structural features of a considerable number of human pathogens. Understanding of characteristics of any parasitic infection and the use of appropriate diagnostic procedures accompanied by a complete understanding of the limitations of each procedure has become very important.

The field of diagnosis of parasitic infections has thus been continuously evolving over the years in our country. There was already a rich body of research and discoveries by independence. Prominent amongst the scientists who contributed to the field of parasitology in the pre-independence era was Sir Ronald Ross to whom the credit of discovering that malaria was spread by mosquitoes of *Anopheles* sp. goes. He was awarded the Nobel Prize in Medicine in 1902 for his remarkable work in the field of malaria. The diagnostic modalities available in the pre independence era were the examination of stool, blood, etc by microscopy and a few non specific tests like the Antimony test of Chopra. Even these modalities have evolved over time in the post independence era.

Microscopy of stool and other specimens

Intestinal parasitic infections are among the most widespread health problems in the world especially in the developing countries. With the advent of AIDS there is an increase in the incidence of newer parasitic infections caused by coccidian parasites and other hitherto unheard of parasites. The definitive diagnosis of most of the intestinal parasites depends upon the demonstration of the cysts and trophozoites or ova, larvae and rarely adult worms in the stool by stool microscopy in cases of protozoal and helminthic infections respectively (3).

Of late, Ziehl-Neelsen staining is a newer permanent staining method imported from clinical bacteriology to stool microscopy to detect *Cryptosporidium, Isospora, Cyclospora* and other coccidia in stool specimens. These are the opportunistic parasites, which cause infections in the

immunocompetent hosts as well as in the AIDS and other immunocompromized hosts. Lacto-phenol cotton blue (LPCB) is another staining procedure imported from clinical mycology to be used as a temporarily staining agent in wet mount preparation of stool. The LPCB, wet mount of stool offers many advantages over that of saline and iodine wet mounts. In the LPCB wet mount, helminthic ova and protozoal cysts are stained such a deep blue that it is difficult to miss them even during low power microscopic screening which is not the case in saline wet mount preparation where the non bile stained ova are refractile and colourless (4, 5). In addition to this, in the cases of stool collected from the cases of amoebic dysentery, the pus cells present in the stool are lysed in the LPCB wet mount and the intact blue stained cysts of *Entamoeba histolytica/Entamoeba dispar* are easily detected and identified by LPCB stain (6, 7).

Substantial progress has been made in recent years in the development of a variety of non-microscopic methods for diagnosis of intestinal parasitic infections. These include detection of faecal antigen (copro antigen) in stool samples. ELISA has been employed most frequently to detect copro antigens for the diagnosis of intestinal amoebiasis, giardiasis, intestinal taeniasis and a few other intestinal parasitic infections. Many of these tests, unlike stool microscopy are essentially sophisticated, hi-tech and are immensely appealing to the laboratory workers. These tests have the advantage of being highly sensitive and specific, non-dependent on the skill of technicians for the morphological detection and identification of parasites and ideally suited for screening of large volume of specimens without causing any strain to the eye.

Conventionally, diagnosis of malaria is established by demonstration of malaria parasites in peripheral thin and thick blood smears by microscopy. Newer staining methods include acridine orange staining method of blood smears. In this method blood smears are prepared on a slide and are stained with acridine orange. This results in a differential staining of the malarial parasites. Nuclear DNA is stained green and cytoplasmic RNA red and aids in the identification of the malarial parasite .The stained slide is examined with a fluorescence microscope .The method is sensitive and is useful for screening large number of smears without causing much strain to the eye. A modification of this method which has been in use in our

country for some time now is QBC. Quantitative buffy coat (QBC) is a method of microhematocrit centrifugation of the capillary blood which is stained with a fluorescent dye such as acridine orange. Following this the capillary tubes are examined under a fluorescent microscope. QBC is the most sensitive parasitic method of diagnosis in malaria. This is an example of semi automation in parasitic diagnosis, catering to the need of processing and examining large number of blood for malaria. Disadvantage of the method is that it does not allow the identification of the species of *Plasmodium* which is essential for prognosticating as well as making treatment decisions.

I shall be dealing with the various developments in the fields of immunodiagnosis and molecular diagnosis of parasitic diseases by considering specific organisms for the sake of clarity. The conditions being dealt are amoebiasis, cysticercosis and cystic echinococcosis, earlier known as hydatid disease.

Laboratory diagnosis of amoebiasis

As already mentioned, the mainstay of diagnosis in intestinal amoebiasis had been stool examination. But this investigation is not of much use in case of another common condition caused by *E. histolytica* – Amoebic Liver Abscess (ALA). In this and other extra-intestinal forms of amoebiasis serodiagnostic methods have been tried and found to be useful (8). Several novels, efficient and easily implementable tests have been developed and evaluated by the author over the years. These include carbon immunoassay, staphylococci adherence test, coagglutination test, rapid indirect hemagglutination test (IHA) and Protein A IHA (9-13).

Entamoeba histolytica, the causative agent of intestinal amoebiasis and conditions like amoebic liver abscess presents us with a conundrum. Though on light microscopy they cannot be distinguished, based on genetic, biochemical, and immunological studies *E. histolytica* and *E. dispar* are currently recognized as distinct species (14). While *E. histolytica* is the pathogenic species, there has been no evidence that *E. dispar* can be involved in the causation of disease. Similarly another species of amoeba, *E. moshkovskii* has also been documented in the year 1998 in human stool specimens which is also similar in morphology to that of *E. histolytica*. Although the early isolations of this species were from sewage, *E. moshkovskii* can also be found in

environments ranging from clean riverine sediments to brackish coastal pools (15). Since the conventional method of light microscopy doesn't allow differentiating between the pathogenic and non pathogenic species, immunological and molecular techniques have been developed.

Amongst the immunological methods, an ELISA to detect the 170 KDa lectin specific to *E. histolytica* is available commercially. This is a coproantigen test where the ELISA is done on the stool sample of the patient. It is highly specific for the diagnosis of intestinal amoebiasis. PCR based methods for the diagnoses of amoebiasis are gradually becoming popular and more preferred over the conventional methods like microscopy and culture because of their high sensitivity and specificity (16). The PCR based test can detect and differentiate *E. histolytica* / *E. dispar* / *E. moshkovskii* complex by exploiting the differences in their genomes for specific detection and differentiation of the real culprit *E. histolytica* from those of look-alike amoebae (17). A further modification of the method used at the authors centre was to use a nested multiplex PCR targeting the 16S-like rRNA gene for simultaneous detection and differentiation of *E. histolytica, E. moshkovskii* and *E. dispar* directly from stool samples. The species specific product size for *E. histolytica, E. moshkovskii* and *E. dispar* was 439, 553 and 174 bp respectively, which was clearly different for all the three *Entamoeba* species. The multiplex PCR technique was found to be simple, cost effective, sensitive, and enabled the differential detection of *E. histolytica*, *E. dispar* and *E. moshkovskii* in human stool samples (16). Another newer approach to the diagnosis of intestinal as well as extra-intestinal amoebiasis has been the detection of excretory *E. histolytica* DNA in urine. Results of a study conducted at our centre indicate that PCR can also be used to monitor excretion of *E. histolytica* DNA in urine as a prognostic marker during therapy of ALA with specific antiamoebic drugs (18).

Laboratory diagnosis of neurocysticercosis

Neurocysticercosis (NCC) remains a major public health problem in developing and some developed countries. Currently, the best procedures for diagnosing NC are neuroimaging studies supplemented by serological tests. Immunological assays, such as enzyme-linked immunosorbent assay (ELISA), or enzyme-linked immuno-

electrotransfer blot assay (EITB) detect antibodies against *Taenia solium,* or cysticerci (19). The earlier tests like coagglutination test had the advantage of ease of application without the need for automation (20, 21).

The ELISA is a useful method for demonstration of antibodies in both the serum as well as in the CSF but detection of antibodies in CSF provides better reliability. The test has a low sensitivity (75%) and specificity (85%). It shows false positive reactions with sera from other helminthic infections (22, 23). ELISA is useful to diagnose NCC patients with few CNS lesions and relatively mild disease. EITB assay using a purified fraction of glycoprotein (Gp) is highly specific (100%) for detection of antibodies both in the serum and CSF. The presence of 1-7 specific glycoprotein (Gp) bands is considered diagnostic of *T. solium* infection. The test is more than 90% sensitive in NCC patients with more than 2 lesions; sensitivity declines to 50-70% with a single lesion. The assay, therefore, is of limited value in children because most of them have a single lesion. A serum immunoblot assay is more sensitive than the test using CSF. One of the recent approaches evaluated at our centre was the use of EITB to detect antibodies against excretory-secretory (ES) antigens of the cysticerci. The test performed well with a sensitivity and specificity of nearly 95%.

Antibody detection in cysticercosis has 2 important disadvantages: Firstly, it may indicate only exposure to infection. It does not indicate whether the patient is having established and viable recent infection. Secondly, antibody titres continue to persist in the serum even after the parasite has been eliminated through the immune mechanism and/or following the drug therapy.

Detection of antigen in serum or CSF indicates recent or viable infection. ELISA using specific monoclonal antibodies can detect antigen in the serum or CSF. There has been a trend in the recent past to evaluate specimens like urine for the diagnosis of parasitic diseases. This approach is based on the fact that soluble antigens released by the living parasites or after lysis of parasites are found in the blood during active infections. It is probable that the same antigens are released in the urine. This offers many advantages. First and foremost being, it is a non-hazardous specimen. Second, it can be collected easily and frequently collected without causing any inconvenience to the patient and third, it is collected by a non-invasive procedure, which thus avoids

transmission of blood-borne infections (24). A study conducted at the authors centre demonstrated for the first time that cysticercus antigen is excreted in the urine and diagnosis of NCC can be made by detection of the cysticercus antigen in the urine (25).

Laboratory diagnosis of cystic echinococcosis

Cystic echinococcosis (CE) caused by dog tapeworm *Echinococcus granulosus* is one of the few parasitic infections in which serology is used primarily for the diagnosis. The complement fixation test was the first serological test to be used in the diagnosis of cystic echinococcosis (CE). Since then, a rapid progress has been made in the serodiagnosis of CE. A wide number of serological tests have been developed for detection of either antibodies or antigens in the serum (3). Traditional serological tests in the hydatid serology include antibody based tests such as indirect immuno-fluorescence (IIF), immunoelectro-phoresis, radioimmunoassay (RIA), countercurrent immunoelectrophoresis (CIEP), indirect haemagglutination test (IHAT), Protein A-IHA and ELISA (26-29).

Though there are a plethora of antibody based tests available, they suffer from certain disadvantages. Most of them are unable to distinguish between past and acute infections. Further their sensitivity and specificity leave much to be desired. Hence antigen detection tests were developed by many workers around the world. At the author's centre too some simple and rapid antigen detection tests were developed and evaluated. CIEP was found to be moderately sensitive and it could detect circulating hydatid antigen in 55.55% surgically proved and 100% ultrasound proven cases of CE .The test was highly specific. The Coagglutination A (Co-A) test showed a sensitivity of 95% and specificity of 89% in detecting hydatid antigen in the serum (30, 31). A latex agglutination test developed (LAT) showed a sensitivity of 72%, specificity of 98%, positive predictive value of 93% and negative predictive value of 91% (32). Further other specimens than serum such as urine have been evaluated with success. The author's laboratory was the first to demonstrate that hydatid antigen was excreted in urine. Though highly specific, the test at the time of development was not very sensitive (33). A simple and rapid Co-agglutination A test was also developed and evaluated for field use with favourable results (34).

Conclusion

In conclusion, the introduction of LPCB and Ziehl-Neelsen staining methods and non-microscopic alternative methods in stool microscopy, increasingly sensitive and specific high-tech immunodiagnostics for use in large central laboratories for processing specimens in large volume, simple and economical tests for use in poorly equipped laboratory or at field and use of urine, saliva and other body fluids as clinical specimens instead of blood are encouraging trend in the diagnosis of parasitic diseases. The development of molecular diagnostic methods, particularly those utilizing PCR for the detection of parasites will contribute greatly to the identification and control of these pathogens, by increasing speed of diagnosis, specificity and sensitivity, reproducibility and case of interpretation. PCR methods are not without their problems however, and there is a need for laboratory procedures to be refined before PCR-based assays are accepted as the tools of choice for the routine detection of protozoan parasites. These will help not only in the diagnosis of an increasing number of cases but also for large-scale field screening of population in developing countries. Nevertheless with the availability of such a wide variety of tests, choosing a particular test in a laboratory poses a diagnostic challenge for clinicians as well as laboratory workers . The high-tech tests with high sensitivity should not be the only and sole criteria for selection of a test in the diagnosis of parasitic disease .This should also take into consideration the cost of the test, availability of the facilities in terms of equipment, reagents and trained manpower, and the problems peculiar to laboratories in developing country like India. It is believed that all these factors if taken into consideration ,one will definitely make an optimum use of the diagnostics recently evaluated and developed for the diagnosis of parasitic diseases.

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