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GIARDIA LAMBLIA INFECTION: REVIEW OF CURRENT DIAGNOSTIC STRATEGIES FOR CHILDREN

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Abstract
Giardiasis has a global distribution and it is a common cause of diarrhea in both children and adults and is transmitted via the fecal-oral route through direct or indirect ingestion of cysts. The laboratory diagnosis of Giardia spp. is mainly based on demonstration of microscopic cyst or trophozoite in stool samples but several immunological-based assays and molecular methods are also available for giardiasis diagnosis. The aim of this study was to conduct a review of the applied methods in medical laboratory and to highlight pitfalls and challenges of them for diagnosis of giardiasis. In this article we have evaluated the Giardia diagnostic methods with a broad review of literature, electronic databases and books. The search has covered the articles and some textbooks that have published up to 2018.
It has been concluded that traditional microscopy combination with stool concentration method should still be held in the routine medical laboratory due to economical and high sensitivity and immunological-based assay and molecular methods which are recommended to use as a complementary test to the traditional technique.

Keywords: Giardia, Diagnosis, Methods, Test

Introduction

The etiological agent of Giardiasis, *Giardia duodenalis* (syn. *G. intestinalis, G. lamblia*) is one of the most prevalent intestinal protozoan flagellate of the human. The life cycle of *Giardia* species is simple and it is included of two active trophozoite and cystic forms.
This parasite transmits via fecal-oral route through direct or indirect ingestion of infectious cysts. The incubation period varies from 9 to 15 days after ingestion of cysts. Symptoms of infection are varied from the absence of symptoms to acute watery diarrhea, nausea, epigastric pain and weight loss (1,2).
Giardiasis has a global distribution and it is common in both children and adults. The prevalence of *Giardia* infection is higher in developing countries. More than 200 million cases of giardiasis are annually diagnosed worldwide. Since 2004, *Giardia* has been included in the "neglected diseases initiative" by World Health Organization (3). The infection rate in asymptomatic children has been reported from 8% to 30% in developing countries and 1-8% in industrialized regions (4). The occurrence of giardiasis is probably higher in individuals with diarrhea.

The prevalence of human giardiasis in different regions of Iran has been reported from 1.2% to 38% (5). In immunocompromised patients, *Giardia* is not considered as an opportunistic pathogen causing prolonged symptoms and enteritis. In HIV-infected individuals, symptoms of giardiasis are similar to HIV-negative individuals and its prevalence has been reported between 1.5% and 17.7% (6). The prevalence of giardiasis was reported 3.1% in HIV/AIDS patients in Iran (7).

Correct diagnosis of giardiasis is important for treatment and prevention of diseases. The laboratory diagnosis of *Giardia* spp. is mainly based on finding and demonstration of microscopic cyst in stool samples, but immunological-based assay and molecular methods also are available and are used for diagnostic or research proposes in developed countries. All diagnostic methods provide different sensitivity and specificity. This condition depends on some factors such as the method of test, the skill of operations and the stage that the test has been performed (8). Since it is important for treatment and control of giardiasis which diagnosis method has been employed? Some methods that are accurate, cheap and relatively easy are required to routine laboratory diagnosis and for large-scale population screening. There are various studies that were carried out to introduce the suitable diagnostic method of giardiasis (8-10). The aim of this study was to conduct a review of the main methods which used in clinical and research laboratory for diagnosis of human giardiasis.

Methods

These articles had used at least one method such as stool examination, immunodiagnostic methods, Plymerase chain reaction (PCR) and culture for diagnosis of Giardiasis.

Results

Diagnostic Methods

Fecal microscopy examination

The microscopic identification of *Giardia* spp. in fecal samples is considered as the gold standard method for the diagnosis of giardiasis. This method is performed to detecting cysts and trophozoites. The sensitivity of microscopy techniques depends on using direct or concentration methods, the number of examined fecal samples and employment of professionally trained persons (11,12).

Direct examination methods

The diagnosis of giardiasis in most cases is mainly confirmed by stool examination. Fecal suspension in physiological salt solution (0.85 NaCl) or fixation in sodium acetate–acetic acid–formalin (SAF) is used to prepare wet mounts in order to the observation of *Giardia* throphozoite in diarrhea or loose samples. Wet mounts smear can be examined either unstained or iodine stained (2-5% lugol's solution).

Examination of direct wet saline preparation of a fresh stool specimen allows motile trophozoites to be seen, but in stained and SAF preparation smears the trophozoites will be non-motile. If diarrhea stool sample containing trophozoite left too long without fixations or preservatives solution, the organisms tend to degeneration, thus preventing has been recommended for sample transfer and protection of the typical trophozoite morphology. A number of commercial kits with preservative solutions are available or can be made manually. The most commonly used preservation kit contains of 10% buffered formalin, polyvinyl alcohol (PVA), merthiolate-iodine-formalin, and SAF solution (9, 13). Polyvinyl alcohol is suitable for preparation of smear in order to permanent staining.

In the asymptomatic individuals and healthy carrier who do not have diarrhea, the cyst stage is more likely to be seen in a fecal sample examination. Fecal suspension in saline or lugol's solution or in a fixative solution may be used for cyst identification.

Culture methods

Although cultivation of human intestinal protozoa is a useful method for detection and diagnostic purpose, routine culture techniques were not established for *Giardia* spp. in the clinical diagnostic laboratory. Cultivation of *Giardia* spp. is applied in the research laboratory for many types of studies that require a large number of trophozoite.

The *Giardia* spp. is grown in the monoxenic and xenic type of culture system. In monoxenic system, the parasite has been grown in the presence of a single additional flora organism species and in axenic, parasite has been grown in the absence of any other accompanied alive cell (20). Monoxenic cultivation is an introduction to xenic growth; however, *Giardia* spp. can be established directly into axenic media.

The most common and suitable used medium for *Giardia* axenic culture is Diamond's medium "TYI-S-33" which modified by Keister DB (22-23).

Immunodiagnostic tests

A variety of antibody and antigen detection methods have been developed and used for immunodiagnostic of giardiasis during the last three decades. Nevertheless, immunodiagnostic of giardiasis is still has a complementary role for microscopy stool test in the diagnosis of giardiasis. Immunodiagnostic test for *Giardia* spp. diagnostic includes immunoassay techniques such as ELISA for antibody detection and methods dependent on detection of *Giardia intestinalis* antigens in human fecal specimens (13).

Antibody detection

Both cell-mediated and humeral immunorespons stimulated in human giardiasis (24- 25). The presence of IgM, IgG and secretary IgA humeral response to acute giardiasis has been noted previously (24,25-27). In persons with acute giardiasis level of IgM antibody fall to levels of healthy persons between two or three weeks after drug treatment.

This indicates that detection of IgM antibody may be a useful indicator for diagnosis of current infection. IgG antibody response may remain for up to 18 months after infection, so it has been applied in epidemiological studies (4). Smith *et al.,* in 1984 showed specific IgG antibody response to trophozoite is detectable in 81% of infected asymptomatic *Giardia* and only in 12% of healthy control individuals (3).

It is well known that *Giardia* spp. induces a strong production of IgA antibody in human and animal infections. Secretary IgA (sIgA) as the predominant antibody has been detected in duodenal fluid and saliva samples of infected people. The production of secretary IgA has been developed during active giardiasis, so detection and monitoring this antibody may be a useful tool for serodiagnosis (9-10).

A study on *Giardia*-infected children in Egypt has showed that salivary and serum IgA and IgG responses against *G. duodenalis* infection were significantly higher than non-*Giardia* infected children ($p<0.001$) (20).

A variety of assays such as Elisa, IFA, Western blot have been used for the serodiagnosis of giardiasis, but these methods may be problematic as the antibody may be detectable as long times after treatment of acute diseases. Commercially produced kits were not developed for detection of serum antibodies to *Giardia* infection.

Molecular methods

Molecular diagnosis of giardiasis is not used in routine medical laboratories.

PCR-based methods are often restricted to research laboratories and mostly used for sub-typing propose such as determination of assemblages or sub-assemblages of Giardia duodenalis (4, 5). The major target gene sequence which has been used in different molecular studies of Giardia species are genes encoding small subunit (SSU) ribosomal RNA, glutamate dehydrogenase (gdh), triosephosphate isomerase (tpi) and β-giardin genes (a protein in the adhesive disk of *Giardia*). comparison and polymorphisms of glutamate dehydrogenase (gdh), the small-subunit of ribosomal RNA (SSU), and triosephosphate isomerase (tpi) genes, showed that *G. duodenalis* is classified to at least eight distinct genetic groups (A to H) or assemblages (1, 5). All these assemblages are indistinguishable by light microscopy. Two assemblages A and B are mainly isolated from human. Genotyping study of human isolates of *Giardia* in different regions of Uzbekistan and neighboring countries indicated that AII as the most common sub-assemblage is followed by BIII and BIV, respectively (5, 19).

Using multiplex real-time PCR have been described for the simultaneous detection of *Giardia* spp., *Cryptosporidium, Dientamoeba* and *Entamoeba histolytica* with a high sensitivity and specificity (17). In recent years PCR-based methods have been used for detecting *G. intestinalis* and other human parasites in environmental sources such as water, and sewage (18-20).

There is an extensive literature that compares the molecular methods and other diagnostic technique in diagnosing *Giardia* infection (13).

Real-time PCR has been reported to be more sensitive and beneficial than Elisa and faecal microscopy for diagnosing *G. intestinalis* infection (11). Using a real-time PCR-based as routine parasitological examination for the identification of *G. intestinalis* displayed an average 92%sensitivity and 100% specificity (23). Comparison of five diagnostic tests for identification of *Giardia duodenalis* in dog fecal samples has showed that performance of the PCR was poor and the relative sensitivity was 58% and specificity reported 56% (24).
A recent study by Hijjawi *et al.* (2018), the sensitivity and specificity for the Nested-PCR in diagnosis of giardiasis was 89.9% and 82.9% respectively (22).

Conclusion

Giardia spp. is one of the most common waterborne parasites that infected human. Cyst stage of this parasite has been identified in surface waters such as rivers, lacks, and ponds. Contaminated food and water to *Giardia* cysts by the food-handlers can be one of the most important sources of transmission of this parasite to humans (5). The main symptoms of human acute giardiasis are diarrhea, flatulence, epigastric cramps, nausea, vomiting and weight loss (6).
It is well known that no traditional or new methods can detect all cases of *Giardia* infection. Several immunodiagnostic tests of rapid diagnosis of giardiasis have been developed particularly in the last three decades, mainly based on the detection of *Giardia* antigens in faecal specimens. While to the high sensitivity of these methods (Table 1), microscopy stool examination especially using concentration methods, most frequently has performed laboratory procedure worldwide as a good performance diagnostic strategy and should still be held as the golden standard. Non-morphological diagnostic methods particularly immunoassay is recommended to detect coproantigen is recommended as a complementary test to the traditional technique and has been applied in larger laboratories that process a large number of stool samples daily. The stool concentration techniques such formalin-ether method can be used as a routine and economical method in medical diagnostic laboratories in developing countries.

Table 1 Comparison of sensitivity and Specificity of different method in *Giardia* diagnosis

Methods	Sensitivity %	Specificity %	References
Direct stool examination	34.7-55	96-100	8, 16, 68
Stool concentration	65.2-83	85-97	8, 16, 60, 67
Sucrose density gradient	42-94	97-100	8, 20
String test (Entero-Test)	44-73	97-100	9, 15, 32
Antigen detection	44-100	68-100	34, 46, 55-67
Molecular assay	58-92	56-100	51-54

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