



## Professional article

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## MODERN DIAGNOSTICS OF GIARDIASIS

### SUMMARY

Protozoan *Giardia duodenalis* (GD) is an opportunistic parasite in humans and animals. This protozoan, with the direct life cycle, parasitizes the upper parts of the digestive tract. Because of non-characteristic symptoms of giardiasis, as well as significant prevalence of cyst carriers, it is necessary to do a parasitological examination of feces in order to make the diagnosis of this parasitosis, aiming to detect the causes. The "gold standard" (reference standard) for parasitological diagnosis of GD is a conventional microscopy (CVM) of three or more samples with/without the application of the concentration technique. Lately, the diagnostics of giardiasis has been complemented with up-to-date commercial assays for detection of GD protozoan antigen in feces. These serological tests are characterized by high sensitivity, specificity and diagnostic efficiency, in which case only one sample of material is sufficient for the examination. However, CVM of three and/or more samples of feces remains irreplaceable in the differential diagnosis of the digestive tract parasitic infections.

**Key words:** *Giardia duodenalis*, conventional microscopy, immunodiagnosics

### INTRODUCTION

Protozoan *Giardia duodenalis* (GD) is an opportunistic parasite in humans and animals. This protozoan, with the direct life cycle, parasitizes the upper parts of the digestive tract. After ingestion of infective form of cysts, trophozoites form in the duodenum, adhering to the duodenal mucosa of the host (carrier). Mostly, trophozoites stay in the intestinal lumen, though there have been sporadic cases of GD penetration into the adjacent tissue. The largest number of giardiasis cases is characterized by the infection of the duodenal mucosa. For these reasons, the local mucosa immunity stands for the major defense mechanism of the host during this infection (1).

Human giardiasis can be described as asymptomatic and/or symptomatic infection with the low-degree symptoms and intensity. Even more serious complains have been reported, such as the malabsorption syndrome. The fact that genus *Giardia* has pathologic importance was concluded in the '70-ies of the past century (1). In 1981, the World Health Organization added this genus to the list of pathogenic parasites (2).

Pathologic changes, as well as clinical manifestations of this parasitosis, depend on numerous factors, the most important of which are: the virulence of GD strain, number of inoculated cysts, age of the host and immune reactivity of the host's organism at the moment of infection.

Clinical diagnosis is difficult to make as the symptoms of giardiasis are unrecognizable, non-

characteristic, and with respect to differential diagnosis, it is not easy to set them apart from gastrointestinal complaints of other etiology. Furthermore, the symptoms directly depend on the parasite life cycle, which is the reason of frequent asymptomatic infections (1).

Incubation period lasts 12-19 days and ends with the appearance of cysts in the feces of the host. This period is associated with an acute phase of disease characterized by the appearance of different symptoms of various intensity.

In the case of an immunocompetent, healthy host's organism, the infection subsides spontaneously and the symptoms disappear. Unfortunately, in a certain number of cases, despite normal reactivity of the immune system, the acute phase turns into the chronic one. In such patients, the chronic, recurrent form of giardiasis develops. Besides acute and chronic recurrent form, a large percentage of asymptomatic cyst carriers with the colonization of the duodenal mucosa with the GD parasite has been reported (1).

The findings of one investigation indicate that 60-80% of the children infected with GD have in their surrounding an asymptomatic carrier, either among the family members or the staff of the day care centers. Asymptomatic subjects, the carriers of GD, stand for the main reservoir for the infection spread (3).

Due to non-characteristic symptoms of giardiasis, as well as significant prevalence of cyst carriers, it is necessary to do a parasitological examination of feces in order to make the diagnosis of this parasitosis, aiming to detect the causes.

#### Diagnosics of giardiasis

So far, as the "gold standard" (reference standard) for parasitological diagnosis of GD, the conventional microscopy (CVM) of direct preparations made of the patients' material samples has been recommended. The examination of three stool samples in three consecutive days is considered to enable the detection of causes in a considerable number of cyst carriers. Material for the parasitological examination is most frequently the stool, though this parasite can be detected in the duodenal juice, bile and histopathological finding of the duodenal and gastric mucosa.

Giardiasis can be diagnosed by diagnostic application of parasitological and immunological methods. The methods of molecular biology, like DNA probes and PCR, are available in research centers and are not used in the routine work. DNA probes are usually used to prove the presence of parasites in water samples.

Parasitological diagnostics of the infection/colonization by parasite GD comprises:

- CVM of direct preparations without/with the concentration technique application, preparations stained by the fluorescent dye – acridine orange (AO), permanent preparations stained by Giemsa method
- Cultivation
- Histopathological analysis

**Conventional microscopy of direct preparations** means parasitological examination of fresh, liquid stool samples, and is carried out within 30 minutes from the admission of samples into a parasitological laboratory, while formed samples are processed and microscopically examined within two hours. The examination of duodenal juice sampled during endoscopy requires fast parasitological diagnostics (within 30 min from sampling). Direct native preparation is prepared according to a customary parasitological procedure and is observed under the light microscope as a native undyed/dyed preparation (by iodine. i.e. Lugol's solution) (4).

Aiming to detect the parasitic elements, the methods of concentration (sedimentation and flotation) are used, after which the native microscopic preparations are prepared (4). Nowadays, for parasitological examination the commercial tests providing the application of concentration methods can be used; they are more suitable for laboratory work and provide the survival of parasites in the long run.

For better GD visualization, the fluorescent dye AO is used. The stained GD can be easily differentiated from fungi and bacteria by means of the microscopic fluorescent microscopy. Parasite GD can be also stained by Giemsa method application, which enables the preparation of permanent microscopic preparations.

Cultivation of trophozoites, though being possible, is not performed in the routine work, but only in the research work with the purpose to obtain a large amount of trophozoites and cysts for the further analysis. The methods have not been the standard yet and there are great variations in the results (5,6).

As histopathological investigations require the application of invasive diagnostic procedures, an application of other diagnostic analysis which should precede the biopsy is recommended. However, only the histopathological investigation can resolve the dilemma what is the precise role of GD in the appearance of digestive tract mucosa damages, primarily duodenal mucosa (7-10).

During gastroduodenoscopy, the tissue from the stomach and duodenum can be sampled for histopathological processing. When suspecting the parasite GD, the paraffin sections are stained by

histochemical methods: trichrome staining (Gomori) and Alcian blue, pH=2.5 –Periodic acid Schiff (AB-PAS)(11).

Immunodiagnosics of giardiasis

Conventional microscopy of three stool samples requires the cooperation of patient, clinician and parasitologist. As the procedure lasts for a few days, there has been a need for a new diagnostic procedure that will be faster, include lesser number of stool samples, but with the diagnostic efficacy of the “gold standard”. Immunodiagnosics of giardiasis has arisen a considerable interest in the recent years. The examination of the antigenic structure has contributed to the increase in the number of publications in this field (12, 13).

The results of numerous laboratory studies for detection of GD antibodies in serum have been presented by the application of various serological techniques in confirmed cases of giardiasis (parasitological and/or clinical) (14-17). Serodiagnostic analyses have not showed the difference in response to serum antibodies between symptomatic and asymptomatic patients. As GD trophozoites rarely affect tissues, the systemic immune response has practically never been stimulated, and the research for antibodies in the serum of GD remains an unreliable method. Although there are several commercial assays for determination of anti-Giardia antibodies in infected patients, there are not sufficient data in the literature about their efficiency (13).

On the other hand, proving the presence of antigen in feces by enzyme-linked immunosorbent assay, non-enzyme immune assays, or fluorescein-marked monoclonal antibodies gave the satisfactory

results in the diagnostics of giardiasis (10, 17-19), especially in the assessment of the disease course and screening of GD infection/colonization of the human digestive tract (12). For proving the presence of antigen in feces there are several assays commercially available, which enable determination of small quantity of antigens in feces. By ELISA-GSA 65 technique, the GD-specific antigen (GSA) identified in trophozoites and cysts with molecular weight around 65kDa is determined (20, 21).The assay is commercially available, and its sensitivity and specificity is compared to the microscopic examinations of cysts in the stool (21). Unlike serological analyses for proving the presence of antibodies against GD proteins, ELISA-GSA 65 for proving the presence of antigens in the stool has shown an extremely high sensitivity and specificity ranging from 98-100% (14). Numerous papers (17-19, 22-25) have shown that by the analysis of one stool sample, i.e. detection of antigens by immunoenzyme assays, excellent results can be obtained. In this way, the procedure is shortened, the patient's comfort is provided (lesser number of hospital visits, delay of invasive diagnostic procedures), faster diagnostics made by doctors, clinicians and parasitologists.

Overall, for diagnostics of symptomatic and asymptomatic giardiasis the immunodiagnostic methods can be used, aiming to detect the GD antigen in one stool sample, or CVM of three and/or more stool samples with/without the concentration technique application. In the case of a positive GD finding the therapy is applied, and in the case of a negative finding, the sequence of diagnostic procedures given in the diagnostics procedure algorithm of clinically suspected cases of giardiasis should be applied (Figure 1) (12).

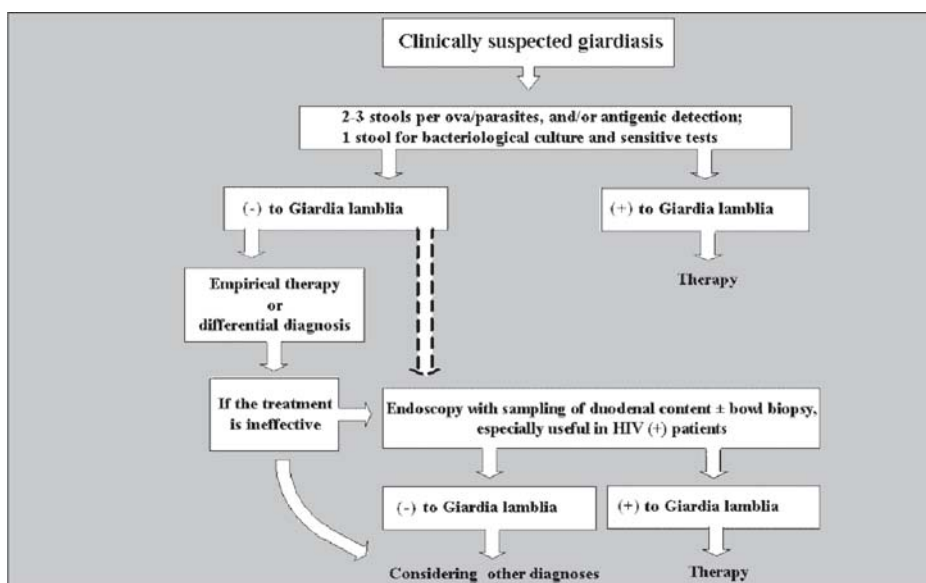


Figure 1. Diagnosis of suspected cases of giardiasis

The novel immune/serological methods for detection of GD antigen in feces are satisfactory alternative to the standard parasitological examinations in the diagnostics of symptomatic and asymptomatic giardiasis. However, in case of the negative GD antigen finding in the stool sample of the patient with the symptoms of infection, only CVM of the

large number of samples can resolve the dilemma whether or not the patient suffers from giardiasis (17). In addition, the advantage of conventional microscopy in the differential diagnosis of other parasitic digestive tract infections should be emphasized (*Figure 1*).

## REFERENCES

1. Faubert G. Immune Response to *Giardia duodenalis*. *Clin Microbiol Rev* 2000; 13 (1):35-54.
2. World Health Organization. Prevention and Control of Intestinal Parasitic Infections. WHO Technical Report series 1987; 379:1-47.
3. Pickering LK, Woodward WE, DuPont HL, Sullivan P. Occurrence of *Giardia lamblia* in children in day care centers. *J Pediatr* 1984; 104: 522-526.
4. Kranjcic-Zec I, Mitrovic S, Arsic AV, Djamic A. Medicinska parazitologija i mikologija, prirucnik. Partenon, 1999
5. Ish-Horowicz MS, Korman H, Shapiro M, Har-Even U, Tamir I, Strauss N, Deckelbaum RJ. Asymptomatic Giardiasis in children. *Pediatr Infect Dis J* 1989; 8: 773-779.
6. Feely DE, Erlandsen SL. Effect of cytochalasin-B, low  $Ca^{++}$  concentration, iodoacetic acid, and quinacrine-HCl on the attachment of *Giardia* trophozoites in vitro. *J Parasitol* 1982; 68: 869-873.
7. Belosevic M, Faubert MG, Maclean DJ. The effects of cyclosporin A on the course of infection with *Giardia muris* in mice. *Am J Trop Med Hyg* 1986; 35: 496-500.
8. Brandborg LL, Tankersley BC, Gottlieb S, Barancik M, Sartor EV. Histological demonstration of mucosal invasion by *Giardia lamblia* in man. *Gastroenterology* 1967; 52:143-150.
9. Oberhuber G, Kastner N, Stolte M. Giardiasis: a histologic analysis of 567 cases. *Scand J Infect Dis* 1997; 32: 48-55.
10. Meyer EA, Radulescu S. *Giardia* and Giardiasis. *Adv Parasitol* 1979; 17:1-47.
11. Katic V. Patologija zeluca. Medicinska knjiga Beograd-Zagreb, 1989, 84-128.
12. Gardner TB, Hill DR. Treatment of Giardiasis *Clin Microbiol Rev* 2001; 14 (1):114-128.
13. Faubert GM. The immune response to *Giardia*. *Parasitol Today* 1996; 12:140-145
14. Behr AM, Kokoskin E, Gyorkos WT, Cédilotte L, Faubert MG, MacLean DJ. Laboratory diagnosis for *Giardia lamblia* infection: a comparison of microscopy, coprodiagnosis and serology. *Can J Infect Dis* 1997; 8: 33-38.
15. Miotti PG, Gilman RH, Pickering LK, Ruiz-Palacios G, Park HS, Yolken RH. Prevalence of serum and milk antibodies to *Giardia lamblia* in different populations of lactating women. *J Infect Dis* 1985; 152:1025-1031.
16. Nash TE, Herrington DA, Losonsky GA, and Levine MM. Experimental human infections with *Giardia lamblia*. *J Infect Dis* 1987;156(6):974-984.
17. Aldeen WE, Carroll K, Robison A, Morrison M, Hale D. Comparison of nine commercially available enzyme-linked immunosorbent assays for the detection of *Giardia lamblia* in fecal specimens. *J Clin Microbiol* 1998; 36: 1338-1340.
18. Garcia LS, Shimizu RY. Detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens using the ColorPac combination rapid solid-phase qualitative immunochromatographic assay. *J Clin Microbiol* 2000; 38:1267-1268.
19. Garcia LS, Shimizu RY. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. *J Clin Microbiol* 1997; 35:1526-1529.
20. Rosoff JD, Stibbs HH. Isolation and identification of a *Giardia lamblia*-specific stool antigen (GSA-65) useful in coprodiagnosis of Giardiasis. *J Clin Microbiol* 1986; 23: 905-910.
21. Rosoff JD, Sanders CA, Seema SS, DeLay PR, Hadley WK, Vincenzi FF, Yajko DM, O'Hanley PD. Stool diagnosis of Giardiasis using a commercially available enzyme immunoassay to detect *Giardia*-specific antigen 65 (GSA 65). *J Clin Microbiol* 1989; 27: 1997-2002.
22. Miladinovic Tasic N, Tasic S, Tasic A, Zdravkovic D, Tasic G, Tasic I. Detection of *Giardia lamblia* in stool by enzyme immunoassays. 14th European Congress of Clinical Microbiology and Infectious Diseases, Praha/Czech Republic, 2004; P597.
23. Miladinovic-Tasic N, Kranjcic-Zec I, Tasic S, Tasic A, Zdravkovic D, Tasic G, Tasic I. Osetljivost i drugi parametri dijagnosticke efikasnosti imunoenzimske metode u dijagnostici lamblioze, V Kongres medicinske mikrobiologije, Mikromed 2006; 130.
24. Miladinovic-Tasic N, Tasic S, Kranjcic-Zec I, Tasic A, Tasic I, Radjelovic V, Manojlovic S. Prevalence of lambliosis int examinees without symptoms of infection of the digestive tract, III Macedonian Congress of preventive medicine, 2006, (491), 136.
25. Miladinovic Tasic N. Dijagnosticki i epidemioloski aspekti prisustva protozoe *Giardia lamblia* u digestivnom traktu. Doktorska disertacija. Niš, 2006.