ACTA FACULTATIS MEDICAE NAISSENSIS

UDC: 616.993.1:616-078

Scientific Journal of the Faculty of Medicine in Niš 2010;27(1):33-37

Original article ■

Advantages Of Enzyme Immonoassay In Diagnosing Lambliosis Of Population Under Sanitary Supervision

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SUMMARY

Preventive, legally prescribed medical examinations of the population under sanitary supervision, with the aim of diagnosing asymptomatic lambliasis, include the application of conventional microscopic examination (CVM) with concentration technique (CT)of one stool sample.

The aim of the paper was to compare the results obtained by the enzyme imnunoassay (EIA) for detection of *Giardia lamblia (G. lamblia)* antigen in stool, with the results of the investigation of asymptomatic lambliasis by CVM with CT in routine laboratory procedure.

The investigation included 279 healthy subjects under sanitary control. One stool samples of investigated subjects were analyzed by using CVM with CT and by EIA for detection of *Giardia lamblia (G. lamblia)* antigen in stool.

Positive result was found in one sample by CVM with CT (1/279; 0,36%). Using EIA method, two positive subjects with asymptomatic lambliasis were detected (2/279; 0,72%). Having applied EIA, a higher prevalence of this parasitosis within the investigate group was proved.

EIA method for detection of the presence/absence of antigen for *G. lamblia* in stool is a method of choice in the laboratory procedures where one stool sample is used to investigate this parasitosis.

***Key words: Giardia lamblia*, prevalence, enzyme imnunoassay**

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INTRODUCTION

Lambliosis is a disease caused by the presence of *Giardia lamblia* (*G. lamblia*) protozoan in the upper portions of the human digestive tract (DT). Although the first reports on *G. lamblia* protozoan are 325 years old (1), this parasitosis is still diagnosed in many people worldwide as asymptomatic infection of the digestive tract. The fact that asymptomatic lambliosis is more prevalent than symptomatic infection certainly deserves attention (2). In humans, *G. lamblia* most commonly causes diarrhoeal syndrome, associated with reduced resorption of fats, proteins, and liposoluble vitamins. The syndrome of malabsorption, the most serious clinical form of lambliosis, occurs mainly in children (3).

Lambliosis is an infection present worldwide, with identified endemic and hyperendemic regions (4, 5). In some of them, the disease occurs sporadically; however, the sporadic patterns of occurrence can be transformed into epidemics within the environment where large population groups are exposed to the causative agent (travels, migrations, international interpersonal contacts). The existance of a large number of asymptomatic cyst carriers causes the risk of spread of this infection to rise substantially (2).

Some of prevention measures, aiming at the prevention and spread of lambliosis, are law-regulated control health examinations before the inclusion of an individual into a community, and also sanitary surveillance of individuals in close professional contact with foodstuffs. At the end of 1960s, continual surveillance was introduced aiming to establish the incidence of this parasitosis among the people engaged in the production and circulation of foodstuffs in the food industry. Systematically, prevention parasitologic examinations and treatment of the infected have reduced the prevalence of lambliosis in this group of individuals (6).

Conventional microscopy with concentration technique (CVMsTK) of single stool sample is routinely applied to diagnose this parasitic infection of the DT in the form of mandatory health check-ups within the efforts to prevent the spread of lambliosis. In contrast to the above, conventional microscopy (CVM) of three or more stool samples, with or without concentration technique, is still being regarded as a reference method („golden standard“) for parasitologic diagnosis of *G. lamblia* infection (7). It is believed that only the examination of a number of stool samples has an impact on the reduction of rate of false negative results. The introduction of new immunoenzyme (EIA) tests into the diagnosis of lambliosis was the turning point (8-15), since this serologic method can reduce the number of required examinations (of several stool samples) per indivudual, with preserved high sensitivity and specificity, i.e. diagnostic efficacy. Based on these facts, we may assume that this new serologic method can be more efficacious in the detection of *G. lamblia* infection, especially in view of fewer CVMsTK samples and/or of just one stool sample.

AIMS

The aim of this paper was to assess the difference in the prevalence of asymptomatic lambliosis (established by means of immunoenzyme tests) between the individuals on mandatory, law-regulated health check-ups, with regard to the prevalence rates obtained using routine laboratory parasitologic examination.

MATERIAL AND METHODS

Groups of examinees

Our study enrolled healthy individuals (n=279), without symptoms of any DT infection, involved in the system of regular, mandatory health check-ups (so called „sanitary examinations“. The mean age of examinees was 32±14.25 years (range, 16 to 52 years).

Parasitologic analysis

Parasitologic stool examination involved conventional microscopy of non-fixed preparations (fresh stool), with formalin-ether sedimentation concentration (16). The material was processed within 2 hours of receipt. The results were regarded as positive if CVMsTK of the stool sample identified *G. lamblia* cysts.

Serologic analysis

One portion of fresh stool samples was frozen at -20°C immediately after receipt for subsequent testing using the immunoenzyme test (EIA) for *G. lamblia* antigen detection (EIA, Ridascren, Giardia; R-Biopharm, Germany). Our EIA test was performed in accordance with manufacturer's guidelines and by a technician blinded to the results obtained with microscopic stool examination. EIA test results were read on a 450nm microtiter plate reader. Samples were regarded positive in case of absorption 10% higher than „cut-off“ value (cut-off was defined as a mean value of absorption of a negative control plus 0.150). In case that different results were obtained with EIA and CVMsTK, both analyses were repeated.

Statistical analysis

Statistical calculator within the Epi Info program (Ver. 6.04) and SPSS statistical package (Ver. 8.0 Windows) were used to process the collected, systematized, and encoded data. Statistically significant differences were assessed by Fisher exact probability test.

RESULTS

CVMsTK and EIA methods were utilized to examine 279 stool samples from individuals without symptoms of digestive tract infection. Positive *G. lamblia*

finding was obtained in 1 case (1/279; 0.36%) using CVMsTK, confirmed by EIA method. EIA testing identified one more infected examinee (2/279; 0.72%) in this defined group.

The prevalence of *G. lamblia* asymptomatic infection in the examined group obtained by EIA testing was higher by 0.36% compared to the prevalence of this parasitosis established with parasitologic examination. The difference was not statistically significant (Fisher exact probability test, $p<0.05$).

DISCUSSION

Before the introduction of preventive examinations, i.e. mandatory, law-regulated health examinations, lambliosis was detected in a significant number of asymptomatic individuals in our country. Simić et al. established the prevalence of lambliosis in the range from 3.2% to 18.5% in some regions of our country (6).

After these initial studies, in their almost three-decade research, Jevtić et al. observed a drop of prevalence of asymptomatic lambliosis in workers in food industries. During the 1960s, there were 9% identified cyst carriers; after years of instituted prevention measures, the percentage dropped to only 0.8% near the end of 1970s (6). Later on, during the 1980s, regular surveillance of prevalence of this parasitosis revealed that the percentage from the previous decade was preserved - there were 0.8% of cyst carriers out of those involved in the program of health control and surveillance (6).

Based on the data of the Department of Parasitology of the Public Health Institute Niš, it has been established that the prevalence of asymptomatic lambliosis has been reduced in the last five years in individuals within mandatory health surveillance programs (17). After 125.208 analyses in this 5-year period, it has been observed that the prevalence of lambliosis has been continually dropping off in this population group. In 2004, for instance, the prevalence of lambliosis was 0.4% (64/14833), while in 2008 the percentage was 0.16% (53/32814).

However, the data concerning the overall prevalence of lambliosis in individuals on sanitary surveillance illustrate the presence of asymptomatic infection with *G. lamblia* parasite established only with CVMsTK of one stool sample. In view of the fact that immunodiagnostic method to detect the antigen of *G. lamblia* parasite almost perfectly matches the reference standard (CVM of three stool samples with or without concentration methods) (6-17), in contrast to CVMsTK of one stool sample, we tried to establish whether the prevalence of asymptomatic lambliosis is higher with this analysis. Utilizing EIA for our study of prevalence of asymptomatic lambliosis in healthy individuals under sanitary surveillance, we established a higher prevalence of this parasitosis.

Based on this higher prevalence (0.72%) with immunodiagnostic methodology, a conclusion may be drawn that the prevalence of asymptomatic lambliosis among healthy individuals under sanitary surveillance is at the levels present in the studies performed during the 1970s and 1980s. Our results reveal the fact that even if modern, immunoenzyme methodology is utilized, which is as an adequate alternative to the „golden standard“ in the diagnosis of lambliosis, the prevalence of healthy cyst carriers remains at a low level, and that prevention measures produced a drop-off of the percentage of healthy cyst carriers in our region.

Certain diagnostic procedures, especially those contained within the prevention programs to detect and check the spread of infectious diseases, involve the examination of only one stool sample of defined subpopulations. These subpopulations include individuals under mandatory, law - regulated sanitary surveillance, children entering closed preschool and school communities, students entering clinical practice in caranteen environment, healthy individuals within the communities with confirmed epidemics of infectious bowel diseases, etc. The program of sanitary surveillance is associated with various problems, such as control post - treatment visits aimed to confirm the eradication of parasites, i.e. the cure of the infection. A control post - treatment visit would involve parasitologic analysis of at least three stool samples in the period of 7 to 10 days. This is, naturaly, time - consuming and costly for the patients.

CONCLUSION

If the problem should be looked into from this point of view too, and if we take into consideration high sensitivity of the EIA method, in our and in other authors' studies (8-15), a conclusion may be drawn that immunoenzyme method to detect the presence or absence of *G. lamblia* antigen in the stool should become a method of choice in the laboratory proceedings involving the examination of one stool sample, then in control check-ups to assess the treatment effectivity, and in all prevention programs aimed to check the epidemics.

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PREDNOSTI IMUNOENZIMSKE METODE U DIJAGNOSTICI LAMBLIOZE KOD LJUDI POD SANITARNIM NADZOROM

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Sažetak

Preventivni, zakonom propisani pregledi ljudi pod sanitarnim nadzorom, sa ciljem dijagnostikovanja asimptomatske lamblioze, podrazumevaju primenu konvencionalne mikroskopije (KVM) sa primenjenom tehnikom koncentracije (TK) jednog uzorka stolice.

Cilj rada bio je ispitati razliku u prevalenciji asimptomatske lamblioze kod ljudi pod sanitarnim nadzorom utvrđenu primenom imunoenzimskog testa (EIA) u odnosu na zastupljenost ove parazitoze dokazanu korišćenjem rutinske analize (KVM sa TK).

Istraživanjem je obuhvaćeno 279 zdravih osoba pod sanitarnim nadzorom. Po jedan uzorak stolice osoba definisane grupe ispitivan je parazitološkom (KVM sa TK) i imunoenzimskom metodom (EIA) za detekciju antigena protozoe *Giardia lamblia* (*G. lamblia*).

Korišćenjem KVM sa TK, utvrđen je samo 1 pozitivan nalaz (1/279; 0,36%), dok je primenom EIA testa zabeležen nalaz asimptomatske lamblioze kod 2 ispitanika (2/279; 0,72%). Uspostavljanjem imunodijagnostike dokazali smo višu prevalenciju asimptomatske lamblioze u izabranoj grupi ljudi koji podležu obaveznom vidu zdravstvenog pregleda.

Direktna imunoenzimska metoda trebalo bi da bude metoda izbora u laboratorijskim procedurama u kojima se za dijagnostiku lamblioze na pregled šalje samo jedan uzorak stolice.

***Ključne reči: Giardia lamblia*, prevalencija, imunoenzimski metod**