

Study of *Toxocara* seroprevalence among patients with allergy and healthy individuals in Bulgaria

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SUMMARY

Data in the literature addressing the ability of *Toxocara* infection in humans to induce development of atopic disease are controversial. The aim of our study was to determine the seroprevalence of anti-*Toxocara* antibodies in three groups of people: subjects with allergic symptoms and presence of allergen-specific IgE, subjects with allergic symptoms and absence of allergen-specific IgE, and clinically healthy blood donors. Serum samples from all subjects were tested by ELISA and Western blot for presence of specific antibodies against *Toxocara canis*. The results of our study did not support the link between toxocariasis and allergic manifestations in atopic patients. Among subjects with allergic symptoms and absence of atopy was found seroprevalence of 2.2% in Western blot. Same index in patients with atopy was 0.8%, and in clinically healthy blood donors 4.0%. Our study gives us grounds to consider that it is appropriate persons with allergic reactions, without evidence of atopy to be tested for presence of anti-*Toxocara* antibodies in the course of their diagnostic evaluation. Data from clinically healthy persons suggest that there is a 'hidden' infection among the population, which is not clinically manifested.

Keywords allergy, ELISA, seroprevalence, toxocariasis, Western blot

INTRODUCTION

Human toxocariasis is a parasitic zoonotic disease with prolonged relapsing course and polymorphic clinical mani-

festations. Causative agents are larvae of dogs' and cats' nematodes *Toxocara canis* and *Toxocara cati*. For first time, toxocariasis (*Larva migrans visceralis*) was described by Beaver (1) in 1952 who defined hepatomegaly, pneumonitis and eosinophilia as main clinical signs of the disease. Currently, toxocariasis is one of the most commonly reported zoonotic diseases with worldwide distribution (2). Most often, the diagnosis is conducted with serological tests and determination of specific IgG antibodies. ELISA and Western blot (WB) are used predominantly for routine diagnosis of *Toxocara* infection. The spread of toxocariasis among humans depends on several factors: place of residence (urban or rural), age, socio-economic status and professional exposure (3). Study in Austria recorded seroprevalence rate among farmers of 44%, 27% among veterinarian workers, 25% in employees of slaughterhouses and 17% among hunters (4). In Ireland, seroprevalence rate of up to 16.6% was found among school-age children living in rural areas (5), and in USA, the recorded rate reached 25% among African Americans living in rural areas (3). In regard to the socio-economic status, a study in Spain found seroprevalence rate of 37% among poor children living in the province of Guipuzkoa (6). From the countries with tropical climate, higher seroprevalence rate was found in the Marshall Islands (86.75%) (7).

In Bulgaria, toxocariasis is not notifiable disease, and for this reason, we do not have available data for the exact prevalence of the infection in the country, but from 2004 to 2013 in the Department of Parasitology and Tropical Medicine at the National Centre of Infectious and Parasitic Diseases (NCIPD) were studied 300 serum samples from patients suspected of toxocariasis with symptoms of allergy, collected from different parts of the country. Seroprevalence of 8% was found among them. Study of healthy blood donors ($n = 350$) revealed seroprevalence of 8.6% ($n = 30$) in ELISA and 3.4% ($n = 12$) in WB (8).

The immune response of the host to the migrating *Toxocara* larvae is directed against larval excretory-secretory

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STUDY OF ELISA DIAGNOSTIC FEATURES
FOR DETECTION OF SPECIFIC IgG2 ANTIBODIES
IN PATIENTS WITH TOXOCARIASIS

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(Submitted by Corresponding Member O. Poljakova-Krusteva on May 23, 2012)

Abstract

Toxocariasis is a disease in humans caused by the larvae of dog and cat nematodes – *Toxocara canis* and *Toxocara cati*. The clinical signs of this parasitosis are nonspecific, so the main diagnostic methods are serological for detection of specific anti-toxocara IgG antibodies. In recent years, for improving the diagnosis of toxocariasis, specific subclasses antibodies – IgG1, IgG2, IgG3 and IgG4, whose role in various parasitic diseases is still under investigation were examined. The aim of the study was to investigate the level of specific IgG2 antibodies in patients with toxocariasis in ELISA, follow up their dynamics in the course of infection and determine the potential of the reaction for the diagnosis of the disease.

One hundred and three sera samples from patients with clinical and serological evidence of toxocariasis, 36 sera from healthy subjects and 23 from patients with other parasitic diseases are included in the study. Positive result in ELISA IgG2 for toxocariasis was found in 44.7% of tested patients sera.

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Sera samples from patients with other parasitoses showed only one positive in ELISA IgG2 and in sera from healthy subjects positive results were not found. The role of specific IgG2 antibodies in toxocariasis is not yet fully understood but studies of other authors indicate that the presence of anti-toxocara IgG2 is specific to this parasitosis.

Key words: toxocariasis, IgG antibodies, subclasses, serological diagnosis

Introduction. Toxocariasis is a parasitic disease caused by the larval stage of dog and cat nematodes – *Toxocara canis* and *Toxocara cati*. People are infected by ingesting embryonated eggs from contaminated sources such as soil, poorly washed vegetables, etc. The main methods of diagnosis are serological – Enzyme-linked immunosorbent assay (ELISA) and Western blot (WB), both of which use excretory-secretory (E/S) antigens derived from *T. canis* larvae maintained in vitro. The tests based on the detection of specific anti-toxocara IgG antibodies often give false positive results because of the cross-reactions with other helminth infections. Therefore, in order to improve the diagnosis of this parasitosis in recent years the possibilities of application of the specific subclasses of IgG antibodies – IgG1, IgG2, IgG3 and IgG4 have been analyzed in diagnosing toxocariasis. Human IgG subclasses have a different serum level and biological function. IgG2 is about 16–48% of the total IgG, with an average serum level of 1.5 to 6.4 g/l [1]. The role of specific IgG2 antibodies in various parasitic diseases is under investigation but according to some authors when they are applied in ELISA for toxocariasis they give the highest sensitivity and can significantly improve the serological diagnosis of this disease [2].

The aim of this study was to elaborate ELISA for detection of specific toxocara IgG2 antibodies, follow up their level in the course of infection and identify the potential of this serological test for diagnostic purposes.

Materials. Human sera:

1. One hundred and three sera samples from patients with clinical evidence of toxocariasis were received at the National Reference Laboratory for Diagnosis of Parasitoses (NCIPD) for the period 2000–2011. All samples were seropositive for total specific toxocara IgG after testing with ELISA and were confirmed in WB.
2. Thirty-six sera samples from healthy individuals were used to determine the threshold (cut off) of the developed ELISA.
3. Twenty-three sera from individuals with other parasitic diseases as follows: echinococcosis – 5, trichinellosis – 5, leishmaniasis – 2, amebiasis – 2, cysticercosis – 1 and toxoplasmosis – 8.

Methods. 1. Commercial ELISA IgG (r-biopharm) – to determine the level of total specific toxocara IgG antibodies in sera samples submitted for clinical indication.

ПРИСЪСТВИЕ И НИВА НА СПЕЦИФИЧНИ IgG И IgA АНТИТЕЛА ПРИ ПАЦИЕНТИ С ТОКСОКАРОЗА И ТЯХНАТА РОЛЯ ЗА УТОЧНЯВАНЕ СТАДИЯ НА ЗАБОЛЯВАНЕТО

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Токсокарозата е широко разпространена паразитна зооноза, причинявана от миграцията на ларви на *Toxocara spp.* в човешкият организъм. Основните методи за диагностика отчитат наличие на специфични IgG антитела, но дългото им задържане налага използването на допълнителни методи, които да подпомогнат определяне активността на заболяването. Проучванията при 130 пациенти с токсокароза показват специфична синтеза на IgG антитела, определени с лабораторен тест ELISA при 95,4% от тях, като са установени различия в нивата спрямо възрастта на пациентите (по-високи нива при децата). IgG антителата се установяват и след една година, с негативиране само при 7 от проследените пациенти (35). Присъствие на антитоксокарни IgA антитела се установи при 26% от изследваните лица. Отсъствието на специфични IgA антитела след 9 месец и най-високите им нива при първичното изследване показва, че определянето им може да се използва като допълнителен маркер, който да подкрепи диагностиката и да позволи определяне стадия на заболяването.

Ключови думи: токсокароза, IgG, IgA антитела, ELISA

PRESENCE AND LEVELS OF SPECIFIC IgG AND IgA ANTIBODIES IN PATIENTS WITH TOXOCAROSIS AND THEIR ROLE IN DETERMINING THE STAGE OF THE DISEASE

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Toxocarosis is a widespread parasitic zoonosis caused by the migration of *Toxocara spp.* larvae in the human body. The main diagnostic methods determine the presence of specific IgG antibodies, but their long persistence requires the use of additional methods assist determining the activity of the disease. Investigation of 130 *Toxocara*-patients showed synthesis of specific IgG antibodies in 95.4% of them, determined by laboratory ELISA test, with differences in age (higher levels in children). IgG antibodies were detected after one year and negative results were observed only in 7 of tested patients (35). The presence of anti-*Toxocara* IgA antibodies was found in 26% of subjects. The absence of specific IgA antibodies after 9 months and their highest levels in the primary study indicates that their determination may be used as an additional methods confirm the diagnosis and allow define the stage of the disease.

Keywords: Toxocarosis, IgG, IgA antibodies, ELISA

Въведение

Токсокарозата е паразитно заболяване, което се причинява от миграцията и присъствието на животински нематодни ларви в човешките тъкани. Основни причинители на заболяването са ларвите на кучешкият паразит *Toxocara canis* и на котешкият – *Toxocara cati*. Клиничната картина на заболяването е описана за първи път от Beaver и съавт. през 1952 година (3), които идентифицират паразитните ларви в чо-

TOXOCARIASIS - WHAT DO WE KNOW? A LITERATURE REVIEW

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ABSTRACT

Toxocariasis is a helminthic zoonosis caused by the presence and migration of animal nematode larvae in human tissue – mostly *Toxocara canis* and *Toxocara cati*. The term visceral larva migrans syndrome was used for the first time in 1952 by Beaver et al. who described the typical clinical presentation. There are difficulties in the diagnosis of toxocariasis because of the variety of symptoms depending on the larva localisation in different tissues and organs. Currently, the most commonly used serological methods are ELISA and Western blot. The disease is characterised by diverse clinical picture and thus toxocariasis is very rarely identified and most patients remain undiagnosed, which requires in-depth study of this widespread but still problematic zoonosis.

KEYWORDS:

Toxocariasis, diagnosis, VLM, OLM

HISTORY OF HUMAN TOXOCARIASIS

The nematode parasites *Toxocara canis* and *Toxocara cati* were described for the first time by Werner in 1782 who initially named the dog parasite *Lumbricus canis*, and Schrank in 1788 naming the cat parasite *Ascaris cati*. In 1947 Perlingiero and György reported the first case of toxocariasis in 2-year-old boy presenting

with typical symptoms – liver involvement, anaemia and fever (1). Two years later, in 1949, Zuelzer and Apt investigated 8 similar cases and described the syndrome observed in young children and characterised by pica, pulmonary involvement with fever, enlarged liver, eosinophilic granuloma, chronic blood eosinophilia, anaemia and hyperglobulinaemia (2). The aetiology of the disease was still unknown until Mercer et al. discovered in 1950 the aetiological agent – ascarid larvae in liver biopsy samples from a child with specific syndrome manifestation (3). Human toxocariasis was first described in 1950 by Wilder C. when he discovered the nematode larvae and their residual hyaline capsules and published a report on ocular granuloma in patients with endophthalmitis (4). The larva was identified later in 1956 by Nichols who performed histological examination of Wilder's samples and determined it as *Toxocara* spp. (5). Two years later, in 1952, Beaver et al. described the clinical manifestation of the disease in children characterised by significant chronic eosinophilia, hepatomegaly, lung infiltrates, fever, cough, hyperglobulinaemia and presence of second-stage larvae of *T. canis* in liver biopsy samples (6). The authors established the term “visceral larva migrans” (VLM) referring to the migration of the larvae through tissues of infected persons and the clinical symptoms caused by their presence in tissues and organs (6). Three decades later Taylor et al. defined the third syndrome of human toxocariasis – covert toxocariasis with non-specific symptoms and signs, associated with increased levels of anti-*Toxocara* antibodies and observed in cases which are not categorised as ocular larva migrans (OLM) or classic VLM syndrome (7).

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AETIOLOGY

The causative agents of toxocariasis are classified in kingdom Animalia, phylum Nematoda, class Secernentea, order Ascaridida, superfamily Ascaridoidea, family Toxocaridae, genus *Toxocara*, species