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Diagnostic Trends of Human Toxocariasis

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Abstract

Several methods for diagnosing human toxocariasis have been reported in the literature. Definitive diagnosis is by histopathological examination and morphological and morphometric identification of L2 in tissue samples. However, it is often not practical to obtain biopsy material to examine for larvae. The most effective laboratory diagnostic approach for toxocariasis in a patient suspected of being infected is serology combined with other diagnostic studies. The parasite and its components may be detected by molecular techniques, medical imaging techniques and hematological and biochemicalstudies.

Keywords: diagnosis, human toxocariasis, serology

Introduction

Human toxocariasis is a worldwide zoonotic parasitic disease caused by the larvae of *Toxocara* species, include *T. canis, T. cati* and possibly *T. vitulorum*. This disease is one of the most common zoonotic helminthiases in temperate countries [1] and occurs periodically in urban and rural areas as a result of accidental infection. It normally presents as either visceral or ocular disease although some infected patients are asymptomatic. The disease is largely caused by a dog parasite (*T. canis*) and to a lesser extent a cat parasite (*T. cati*).

Toxocaral larva migrans is recognized as a cause of potentially serious human infection. Although the majority of clinical manifestations vary according to the affected organ, the most common characteristic found is persistent eosinophilia. Other manifestations include hepatomegaly, fever, pneumonitis, visual field defects and neurological disturbances. The severity of symptoms depends on the burden and location of the larvae, including the duration of infection. Cases of visceral larva migrans (VLM) are mostly asymptomatic or mild without obvious abnormalities. Ocular larva migrans (OLM) can result in chronic inflammation and permanent damage to the eye; it may cause posterior uveitis, vitreous body infiltrates, epiretinal membranes and subretinal granulomas. The most frequent clinical findings in children, called covert toxocariasis, are fever, anorexia, headache, abdominal pain, nausea, vomiting, lethargy, sleep and behavior disorders, pharyngitis, pneumonia, coughing, wheezing, limb pains, cervical lymphadenitis and hepatomegaly. Severe pneumonia, cardiac disease or neurological disease are rarely found with toxocariasis, but can lead to death.

A definitive diagnosis of human toxocariasis is often a challenge for the clinician since the clinical signs and symptoms of the disease are non-specific. Therefore, patient history regarding occupational and household chemical exposures,

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drug exposures, asthma, travel to tropical areas, contact with domestic animals, particularly puppies, consumption of raw vegetables or undercooked meats should be obtained. Laboratory investigations useful for the diagnosis of human toxocariasis normally rely on a combination of various methods.

Parasite and its component detection

The gold standard for diagnosing infections caused by protozoa or helminths is microscopic or macroscopic observation of the parasite. Parasitological diagnosis of human toxocariasis can be confirmed by pathological examination, including morphological and morphometric identification of L2 in the affected tissue [2,3]. In OLM, one or more mobile larvae may be directly found under the retina using ophthalmoscopy [4]. Molecular techniques, such as DNA hybridization, polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) or sequencing of *Toxocara* nuclear ribosomal DNA (rDNA), mitochondrial DNA (mtDNA) or repetitive DNA extraction from tissue, have been used to diagnose infections and to analyze genetic variation among nematodes and to evaluate the population genetics of *Toxocara* [5]. PCR has been reported to detect the DNA of *T. canis* in liver tissue of experimentally infected mice [5,6] and provide specific identification, diagnose infections and analyze genetic variation in this nematodes [7]. Tissue biopsy is not usually recommended and larvae are not simply found migrating in biopsy material [8].

Medical imaging techniques

Contemporary imaging modalities, including ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI), can be used to support a tentative diagnosis of toxocariasis, especially in patients exhibiting VLM whose clinical signs include granulomatous lesions and focal inflammatory changes due to migrating *Toxocara* larvae in the liver [9] and nervous system with resultant eosinophilia in the CSF [9-13]. Abdominal US showed multiple hypoechoic areas in the livers of 14 children who presented with hepatomegaly, eosinophilia and positive *Toxocara* serology [14-16]. Characteristic images on US [17] and CT scan [18] in toxocaral endophthalmitis have also been described.

Hematological and biochemical assessment

A marked peripheral blood eosinophilia induced by T lymphocytes [19] is of particular immunological interest in toxocariasis. Only a few other parasitic diseases provoke such a phenomenon, these include acute schistosomiasis, angiostrongyliasis, liver fluke infections and leaking echinococcal cysts. Although eosinophilia is not specific for *Toxocara* infection, it has been consistently associated with VLM. In contrast, patients with OLM rarely have eosinophilia due to the low larval burden [20]. *Toxocara* larvae are tissue nematodes, blood eosinophil counts imperfectly reflect their presence since they preferentially accumulate in tissue [21]. Some toxocariasis patients may have true disease without eosinophilia. A case was recently reported of active toxocariasis eliciting a chronic irritant cough with a positive specific immunodiagnosis and an elevated titer of eosinophil cationic protein (ECP) [22]. Other laboratory findings include hypergammaglobulinemia and elevated concentrations of total serum IgE [23]. In patients presenting with one or more clinical signs of covert toxocariasis but lack eosinophilia, an increase in the concentration of serum total IgE > 500 IU/ml is further evidence of recent *Toxocara* infection. Magnaval and Glickman [24] reported *Toxocara* infection is usually characterized by marked, chronic eosinophilia (> 2.0 x 109 cells/l or more than 400/mm^3), leukocytosis and hypergammaglobulinemia. These authors recommend patients with negative stool examinations and normal serum IgE concentrations should be examined with specific immunodiagnostic tests for toxocariasis.

Serodiagnosis

At present, serological testing using immunological techniques is recognized as the

most effective approach to the laboratory diagnosis of*Toxocara* infection [23,25]. Serodiagnosis is also useful for epidemiological studies of the prevalence of the disease. Most immunological techniques used have had variable success in detecting anti-*Toxocara* antibodies. Woodruff and Bradstreet [26] first used skin testing to detect *Toxocara* specific IgE antibodies. Extracts of *T. canis* adult worms were tested in 35 patients with a history of asthma, choroidoretinitis or unexplained eosinophilia. Eleven of them were infected with *T. canis* or *T. cati*. Collins and Ivey [27] infected guinea pigs with a small number of *Toxocara* and *Ascaris* larvae and challenged intradermally the guinea pigs with a variety of antigens prepared from *T. canis*. They found the larval antigen induced a more significant and sensitive skin reaction than the adult antigen. Cross-reaction occurred at all infected levels between *Toxocara* and *Ascaris* antigens. de Savigny [28] reported diagnosis of genus specific *Toxocara* infection with a passive haemagglutination test using *Toxocara* excretory-secretion (TES) antigens and no cross-reaction was observed with *Ascaris* serum.

The standard serological test for confirming toxocariasis is an ELISA assay employing TES harvested from *T. canis* larvae [23]. The use of TES antigens from larvae increased the sensitivity and specificity of the ELISA test compared to antigen mixtures from adult worms [29-33] although some cross-reactions with other nematodes occurred [34-36]. Indirect ELISA was used to determine the prevalence of total IgG, IgE and IgM antibodies against TES [25]. An anti-*Toxocara* IgE ELISA test has been recommended as the most useful serodiagnostic test because it will not detect iso-haemagglutinins in the test sera, since these are largely IgG antibodies [37]. However, patients do not always present with elevated total IgE levels, although the presence of specific IgE is likely to be associated with an active infection [38].

A major problem with cross-reaction occurs in tropical countries where other soil-transmitted helminths are prevalent, such as *A. lumbricoides*, *Trichuris trichiura*, hookworm and *Strongyloides stercoralis* [32,39-43]. The existence of cross-reactivity among the excretory-secretory antigens of *Fasciola hepatica*, *T. canis* and *A. suum* can occur [44,45]. An indirect antibody competition ELISA employing specific rabbit IgG anti-TES antigen as the competition antibody improves toxocariasis diagnosis with a sensitivity of 60.2% and a specificity of 98% in humans [46,47]. Dot-ELISA gave higher specificity than ELISA in examing the sera of children with suspected toxocariasis. Other methods for developing specific assays are IgM monoclonal antibodies (mAb) which recognize species and genus-specific epitopes [48]. Yokoi *et al* [49] produced a monoclonal antibody to the 120 kDa-TES antigens of *T. canis* larvae which may be useful for determining the parasite burden in early infection and the efficacy of helminthic treatment. No cross-reaction of this mAb with other helminthic infections has been reported. However, a study using a preliminary antigen capture assay employing mAb to TES antigen gave a 25% false positive rate in patients with schistosomiasis and filariasis [40].

Western blot analysis, which is as sensitive as ELISA and relatively specific, should be used as a confirmatory test after screening by ELISA [23]. El-Massry *et al* [50] showed the SDS-PAGE of *T. canis* and *Toxascaris leonina* adult somatic antigens had two similar bands at 90+91.5 kDa and 69.25+70.5 kDa, respectively. Western blotting showed four prominent bands representing immunoreaction between the somatic antigens of *T. canis* adults and serum from rabbits experimentally infected with the corresponding larvae (125.37, 117.73, 90.00 and 69.25 kDa). It appears the bands at 125.37 and 117.73 kDa may used as specific markers for *T. canis* adults. A 55-66 kDa antigen complex has been shown to be responsible for the cross-reactivity between *T. canis* and *A. suum* [46]. Lynch *et al* [32] reported that an 81 kDa antigen was responsible for the strong cross-reactivity between *T. canis* and *Ascaris* extracts. Magnaval *et al* [51] demonstrated four low M_r bands (24, 28 (fused bands), 30 and 35 kDa) and three high M_r bands (132, 147 and 200 kDa) by Western blotting using TES. The lower M_r bands appeared to avoid the problem of cross-reactivity with sera from patients

infected with other helminth diseases [51,52]. Sarimehmetoglu *et al* [53] used TES antigens to diagnose VLM and found protein bands at 24, 28 and 48 kDa in infected mice, and bands at 24, 28, 30, 35, 132, 147 and 200 kDa in humans [54]. A major advantage of using TES-Western blotting is the ability to identify antigens important in serodiagnosis and their discrimination from other cross-reactive antigens in TES. However, Western blotting is more labor-intensive and requires specialized expertise and cross-reactions are still reported.

The presence of cross-reactive antigens in TES limits the use of serodiagnostic methods using TES. The production of large amounts of TES recombinant antigen may provide an alternative method for *Toxocara* serodiagnosis by providing increased sensitivity and specificity compared to native TES. However, recombinant antigens must share important antigenic and immunogenic structures with native TES. Recently, the development of highly specific recombinant antigens from *T. canis* larvae was attempted. Since production of TES is limited to the capacity of parasite culture, a recombinant protein is likely to offer a significant advantage for future diagnosis [8]. Immunological results must be interpreted while taking into consideration the clinical signs of the patient and confirmed by finding the parasite itself or its genome using molecular methods [55]. Recombinant *T. canis* second-stage larva antigens corresponding to the 30 kDa and 120 kDa proteins were developed and tested for their specificity for toxocariasis [8,56-58]. TES-30 is a recombinant protein from *T. canis* second stage larvae corresponding to the 30 kDa antigen from TES. TES-30 appears to be sensitive and specific with no cross-reactions reported with sera from individuals with *Ascaris* and hookworm infections and only minimal cross-reactions with sera from individuals with gnathostomiasis, paragonimiasis and spirometriasis [8,56,57]. The gene encoding TES-120 was cloned into the bacterium *Escherichia coli*, the resultant antigen reacted with all toxocariasis sera tested but did not react with sera from patients with various helminthiasis and protozoan infections [58]. Thus TES-30 and TES-120 recombinant antigens should be evaluated further in order to confirm their potential as diagnostic antigens. The development of IgG-ELISA based on antiserum prepared against the recombinant arginine kinase of *T. canis* has been reported. There were significant differences ($p < 0.01$) in absorbance between infected and control serum samples from infected mice with 100% sensitivity; the authers suggested the recombinant-AK based IgG-ELISA could be applied to the immunodiagnosis of human toxocariasis [59]. However, it is necessary to evaluate this recombinant antigen with heterologous human sera to evaluate the specificity of the test.

Both the early detection of toxocariasis and the evaluation of the public health significance rely greatly on serology [40,60,61]. However, serological evidence of widespread infection does not indicate prevalence of clinical disease [62]. Positive serology is not always evidence of a causative relationship between *Toxocara* infection and the patient's current illness, as residual anti-*Toxocara* antibodies can persist for years [63] and they are not very useful for measuring of success of treatment. The presence of anti-*Toxocara* antibodies alone does not distinguish between current and past infections and such tests should be accompanied by other laboratory tests. such as blood eosinophil count and total serum IgE. Anti-*Toxocara* antibodies detected by ELISA persisted for up to 2.8 years in patients in Switzerland [64], while anti-*Toxocara* antibodies detected by Western blotting can persist for over 5 years.

However, serum anti-*Toxocara* antibodies may not be detected by either ELISA [65] or Western blotting [66] in some cases of parasite-identified toxocariasis. In some OLM patients, antibodies cannot be detected in the serum and the number of eosinophils may be normal or mildly elevated even in patients with severe ocular manifestations due to a physiological barrier between blood and ocular fluids [20,67,68]. The anti-*Toxocara* titers in vitreous and aqueous humor fluid are usually elevated and found to be higher than those in serum obtained from patients with ocular

toxocariasis [69]. The sensitivity of ELISA for diagnosis of OLM is lower than for VLM which is probably related to the low larval burden or to the long period between onset of infection and serodiagnostic testing, which can be up to 2 years for OLM but usually less than 6 months for VLM [70]. Criteria for the diagnosis of OLM is usually based on the the presence of ocular lesions, such as retinal or peripheral granulomas, endopthalmitis, or eosinophilia of the vitreous humor, and an elevated antibody titer to *T. canis* larva antigen [71]. Anti-*Toxocara* antibody titers in VLM cases are consistently higher than those in OLM but lower than those with concurrent VLM and OLM. The detection of circulating *Toxocara* antigen has been reported [40,72] and remains an option for serodiagnosis; however, the specificity of antibody capture and the requirement to detect uncomplexed circulating *Toxocara* antigen limit its application.

Until now, no standardized tests have been conducted among patients infected with *Toxocara* larvae in Thailand. There is a clear need to develop definitive diagnostic tests for toxocariasis infection when other tissue nematodes are endemic. An attempt has been made to evaluate various subclass antibodies by indirect ELISA. ELISA has been widely used in several parasitic infections with satisfactory results. However, there are no published studies of subclass antibodies accompanied by TES antigens using ELISA.The recognition of IgG2 antibodies to the epitopes of TES antigens gave a high total sensitivity (98%) (16/16 of patients from Scotland and 33/34 of patients from Thailand) [73]. The IgG3 subclass also gave a high sensitivity (78%). IgG2 and IgG3 responses appear to be directed primarily against carbohydrate or polysaccharide antigens which comprise most of the components of TES antigens [74].

Conclusion

This study reviewed*Toxocara* species, a ubiquitous parasite responsible for some of the most challenging clinical diseases. Traditional parasitological diagnostic approaches can have significant limitations in regard to the specific identification of larvae. We reviewed the various approaches to the diagnosis of human toxocariasis which can be used to differentiate it from other parasites, which can give precise information about and important implications for investigating their life cycle, epidemiology and population biology. At present, the accepted serodiagnostic tests are the IgG-TES ELISA and Western blotting. However, further research is needed to develop or improve assays that can determine the course of the disease and the efficacy of treatment. Recombinant antigens may offer further solutions for *Toxocara* serodiagnosis by providing increased sensitivity and specificity of native TES.

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