

The diagnostic value of Western blot method in patients with cystic echinococcosis

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SUMMARY

Cystic echinococcosis (CE) is the larval cystic stage (called echinococcal cysts) of a small taeniid-type tapeworm (*Echinococcus granulosus*). Carnivores such as dogs are usually definitive hosts. Intermediate hosts are typically herbivores such as sheep and cattle. CE can be detected using various imaging techniques such as ultrasonography or radiology. Moreover the primary diagnosis has to be confirmed by serological tests since the clinical signs of the disease are non-specific. This study examined the antigenic band patterns useful for serologic diagnosis of hydatidosis. We also report on the post-operative evolution of patients treated for this disease and also determined the diagnostic performance of Western blot IgG kit. Twenty-five (16 females and 9 males) non-operated patients with hydatid cysts (NOP) and 33 (21 females and 12 males) operated patients with hydatid cysts (OP) were included as study group and 22 healthy individuals (14 females and 8 males) with no known chronic diseases were included as a control group. The ages of the patients and control group individuals were between 16-83 years. Patient and control groups were matched for age and sex. Cyst hydatid IgG antibodies were detected in the sera from all patient groups but no antibodies were found in the sera from the control group using ELISA IgG method. Twenty-three (92%) non-operated patients and 18 (54.5%) operated patients exhibited positive results when Western blot IgG kit was used. The P7 band pattern was detected in the sera from all operated and non-operated patients. Twenty-seven of these positive cases had p7 and (p7+p16/18), (p7+p24/26) or (p7+p16/18+p24/26). No antibodies against p7, p16/18 ve p24/26 band patterns were seen in sera from the control group. A statistically significant difference was detected between operated and non-operated patients for Western blot positivity. ($p < 0.01$). $p: 0.018$ - $X^2=5,604$ - $OR: 0.176$ - $95\% CI: 0.037- 0.841$.

The sensitivity, specificity, positive prediction and negative prediction values of *Echinococcus granulosus* Western blot kit for 25 cases with CE and 22 healthy controls were calculated as 92%, 100%, 100% and 91.7%, respectively.

In conclusion, we suggest that monitoring p7 in all non-operated patients may be useful to determine the efficacy of medical treatment and that monitoring p7 antibodies using serological and Western blot methods in operated patients may be useful for the screening of post-operative evolution in patients with hydatid cyst.

KEY WORDS: Cystic echinococcosis, Western blot

Received April 07, 2010

Accepted November 11, 2010

INTRODUCTION

Cystic echinococcosis (CE) is the larval cystic stage (called echinococcal cysts) of a small taeniid-

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type tapeworm (*Echinococcus granulosus*). Carnivores such as dogs are usually definitive hosts. Intermediate hosts are typically herbivores such as sheep and cattle. Humans function as accidental hosts because they are usually a 'dead end' for the parasitic infection cycle. CE particularly infects the liver but other organs such as the spleen, brain, heart and kidneys can be also targets. There are many species in *Echinococcus* genus and *E. granulosus* and *E. multilocularis* are

the most prevalent species as the causative agents of CE and alveolar echinococcosis, respectively (Eckert *et al.*, 2000; Sadjjadi, 2006; Dalimi *et al.*, 2002). CE is transmitted through the gastrointestinal or respiratory tract and placental transmission of *E. granulosus* eggs (Zhang *et al.*, 2003; Siracusano *et al.*, 2008).

Although various imaging techniques such as ultrasonography or radiology easily detect CE in clinical settings, the primary diagnosis needs to be confirmed by serological tests since the clinical signs of the disease are non-specific (Grimm *et al.*, 1998, Doiz *et al.*, 2001). Serological tests such as immunoelectrophoresis, double diffusion in agar, or indirect hemagglutination are being replaced by more sensitive assay methods such as enzyme-linked immunosorbent assay (ELISA), immunoblot (IB), and indirect immunofluorescent antibody test (IFA) (Virginio *et al.*, 2003). The main problems for the serodiagnosis of CEs are often the unsatisfactory performance of the available tests and the difficulties associated with the standardization of antigenic preparations and techniques. To overcome these drawbacks, highly sensitive and specific antigens and antigenic components derived from different developmental stages of *E. granulosus* must be available. The use of an appropriate source of antigenic material is crucial in the serodiagnostic tests (Carmena *et al.*, 2006). The serologic diagnosis of CE is strongly dependent on the antigen used, thus explaining the lack of sensitivity, specificity and concordance among different techniques, standardization of an antigen that enables postoperative follow-up of the disease being necessary (Doiz *et al.*, 2001). B/5-rich fraction or partly purified antigen from hydatid fluid is used almost in all serologic tests (Lightowlers *et al.*, 1995; Siracusano *et al.*, 1991).

Serology has been one of the methods selected for the post-operative control of hydatidosis. However, the long persistence of anti-*E. granulosus* antibodies after recovery hampers the diagnosis of relapse by serology (Todorov & Stojanov, 1979; Zarzosa *et al.*, 1999).

This study examined the pattern of antigenic bands essential for the serologic diagnosis of CE, revealed by immunoblotting analysis. We also report on the post-operative evolution of patients treated for this disease and also determined the diagnostic performance of Western Blot IgG kit.

MATERIALS AND METHODS

This study involved three different groups at Istanbul University, General Surgery and Microbiology Departments of Cerrahpasa Faculty of Medicine and General Surgery Policlinics of Istanbul Education and Research Hospital between January 2008-December 2009 as a cross-sectional and case-control based study.

Twenty-five (16 females and 9 males) non-operated patients with hydatid cysts (NOP), 33 (21 females and 12 males) operated patients with hydatid cysts (OP) and as a control group of 22 healthy individuals (14 females and 8 males) with no known chronic diseases were included in this study. The ages of the patients and control group individuals were between 16-83 years and patient and control groups were matched for age and sex. ELISA cyst hydatid IgG (Euroimmun Labordiagnostica, Germany) and *Echinococcus granulosus* Western Blot IgG (Euroimmun Labordiagnostica, Germany) kits were used for all groups. Cyst hydatid IgG antibodies were detected in the sera from all patients but no antibodies were detected in the sera from the control group individuals. The bands showing the existence of antibodies against *Echinococcus granulosus* antigens were evaluated with *Echinococcus granulosus* Western Blot IgG (Euroimmun Labordiagnostica, Germany) Western blot kits were prepared with antigens reacting against antibodies. These antigens were non specific antigens p39(39kDa) and genus specific but cross-reactive antigens of p24/26(24-26 kDa), p16/18(16-18kDa) with other *Echinococcus* species and also *Echinococcus granulosus* specific p7(7 kDa) antigens. We followed the manufacturer's instructions to evaluate the band patterns. Only p39 band pattern was considered negative and p7 and (p7+p16/18), (p7+p24/26) or (p7+p16/18+p24/26) band patterns were considered positive.

Absence of the p7 band and weak p16/18 and/or p24/26 band patterns were considered negative while follow-up was considered mandatory for patients with strong p16/18 and/or p24/26 band patterns. We used SPSS 11.5 software for statistical calculations and X² method and p<0,05 was considered as the cut-off value for statistical significance. We also calculated the sensitivity, specificity, negative prediction and positive prediction values for *Echinococcus granulosus* western blot kit.

TABLE 1 - The distribution of antibody band patterns in operated and non-operated patients by Western blot method.

Patients	Number of samples	Negative	Positive				Total*	Follow-up
			P1	P2	P3	P4		
Operated follow-up patients	33	8	0	1	6	11	18	7
Non-operated patients	25	1	1	1	5	16	23	1
Total	58	9		2	11	27	41	8

P1: Only 7kDa, P2:(7kDa + 16/18kDa), P3(7kDa + 24/26kDa), P4:(7 kDa + 16/18kDa + 24/26kDa), P5:(24/26kDa). Negative: No bands between 7 kDa and 28 kDa. *X² = Operated X Non operated P<0,01.

RESULTS

Cyst hydatid IgG antibodies were detected positive in the sera from all patient groups but no antibodies were observed in the sera from control group using ELISA IgG method. Twenty-three (92%) of the non-operated patients and 18 (54.5%) of the operated patients were found positive for Western-blot IgG kit. P7 band pattern was detected in the sera from both the operated and the non-operated patients. Twenty-seven of these positive cases had p7 and (p7+p16/18), (p7+p24/26) or (p7+p16/18+p24/26) band patterns. No antibodies against p7, p16/18 and p24/26 band patterns were detected in the sera from the control group. A statistically significant difference was detected between operated and non-operated patients for Western blot posi-

vity.(p<0.01). p: 0.018- X²= 5,604- OR: 0.176- %95 CI: 0.037- 0.841 (Table 1).

The sensitivity, specificity, positive prediction and

TABLE 2 - The diagnostic performance of Western blot kit.

	True Positivity		
	+	-	
<i>E.granulosus</i>	+	23	0
Western Blot IgG	-	2	22
Total		25	22

Sensitivity: 92%. Specificity: 100%. Positive Prediction Value: 100%. Negative Prediction Value: 91,7%.

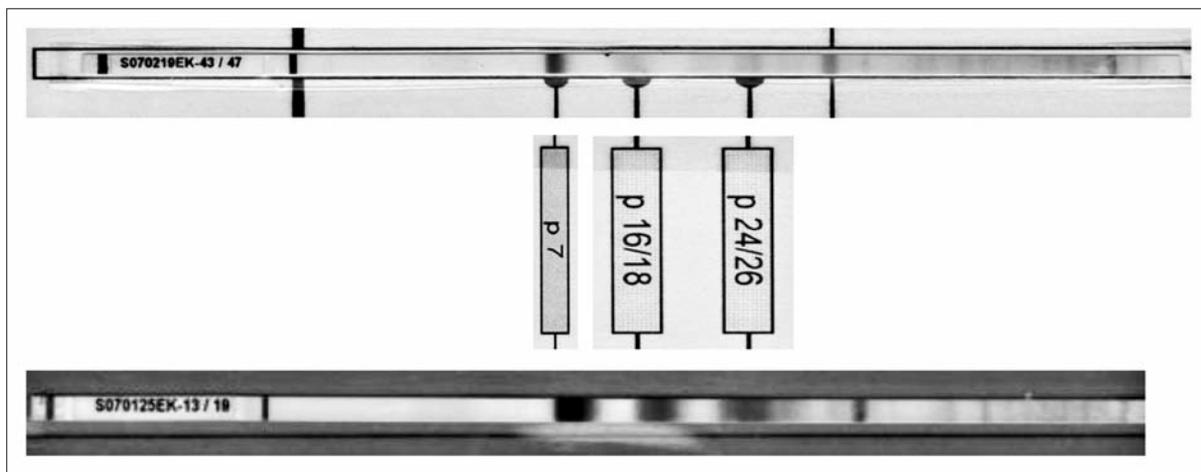


FIGURE 1 - Upper figure shows control strip for western-blot and lower figure shows a positive operated follow-up patient's strip for 7 kDa + 16/18kDa + 24/26kDa.

negative prediction values for *Echinococcus granulosus* Western blot kit for 25 cases with CE and 22 healthy controls were calculated as 92%, 100%, 100% and 91.7%, respectively (Table 2). Upper figure shows control strip for western-blot and lower figure shows an operated follow-up patient's strip positive for 7 kDa + 16/18kDa + 24/26kDa (Figure 1).

DISCUSSION

The prolonged survival of *Echinococcus granulosus* within the human host indicates that some mechanism is operating to permit parasite evasion of the host immune response. *E. granulosus* larvae survive in spite of cellular and humoral immune responses and cause chronic diseases. Early diagnosis of hydatid cyst results in successful treatment of hydatid cyst patients. The early stages of disease are asymptomatic and serological methods and various imaging techniques such as ultrasonography or radiology help the confirmation of diagnosis (Zhang *et al.*, 2003). The elevated levels of serum antibodies generated during the course of infection can provide a sound basis for the development of sensitive and specific serodiagnostic methods for parasite detection. Nevertheless, serodiagnosis of a number of helminthic infections, such as cystic echinococcosis (CE), remains a challenge. The major problem is that crude or secretory/excretory proteins from the targeted parasite are often cross-reactive with sera from humans and animals infected with other parasites; leading to low specificity in the performance of serodiagnostic assays (Li *et al.*, 2004). *Echinococcus granulosus*-specific antigens must be selected to increase the sensitivity and specificity of detection assays.

gB is a polymeric lipoprotein with a molecular mass of 120 kDa (Oriol and Oriol, 1975). This protein can be measured in patient blood as circulating antigen (Kanwar *et al.* 1994, Liu *et al.*, 1993) and it has been suggested that AgB plays an important role in the biology of the parasite and its relationship with the host (Rigano *et al.*, 2001, Shepherd *et al.*, 2001). AgB is a highly immunogenic molecule (Chordi *et al.*, 1965, Oriol *et al.*, 1971) having value in serodiagnosis. It has three protein subunits with molecular sizes of approximately 8 or 12, 16, and 24 kDa (Leggatt *et al.*,

1992, Oriol *et al.*, 1971). The smallest subunit has proved to be the most useful target in diagnostic studies (Ortono *et al.*, 2000, Rott *et al.*, 2000).

Western blot kits were prepared with antigens reacting against antibodies. These antigens were non specific antigens p39(39kDa) and genus specific but cross-reactive antigens of p24/26(24-26 kDa), p16/18(16-18kDa) with other *Echinococcus* species and also *Echinococcus granulosus* specific p7(7 kDa) antigens. Cyst hydatid IgG antibodies were detected positive in the sera from both patient groups but no antibodies were detected in the sera of the control group by ELISA IgG method. Twenty-three (92%) non-operated patients and 18 (54.5%) operated patients were positive for Western-blot IgG kit. P7 band pattern was detected in sera from all operated and non-operated patients.

No antibodies against p7, p16/18 ve p24/26 band patterns were detected in any sera of the control group A statistically significant difference was detected between operated and non-operated patients for Western blot positivity ($p < 0.01$) $p: 0.018$ - $X^2 = 5,604$ - $OR: 0.176$ - $95\% CI: 0.037$ - 0.841 . We suggest that p7 which was detected in 23 (92%) non-operated patients is an important marker for active disease. The existence of a statistically significant difference between non-operated and operated patients for Western blot positivity show the usefulness of this marker only in half of the post follow-up operated patients (54.4%). This high ratio suggests that this ratio can be depend on the presence of circulating antibodies, reactivation of small cysts and new inoculums during the operation.

Surgery methods are usually used in the treatment of hydatid cyst but nowadays medical treatment is also used with surgery. Medical treatment is designed to make the cysts infertile before surgery. Thus, the risk of infection during surgery is minimized. The screening p7 antibodies serologically with ultrasonography or radiology gains importance for following-up the results of medical treatment.

As a result, we suggest that investigation of p7 in all non-operated patients may be useful to monitor the efficiency of medical treatment and we also suggest that investigation of p7 antibodies with serology and Western blot in operated patients may be useful for the screening of post-operative evolution in patients with hydatid cyst.

ACKNOWLEDGEMENTS

This work was supported by a Research Fund of Istanbul University, project number: 2893. This work was presented in ESCMID Congress, Helsinki, Finland 16-19 May 2009.

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