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Original Contribution

RESEARCH AND SCREENING OF IMMUNE TESTS IN CHILDREN WITH HYDATIDOSIS

K. Kalinova[1*], K. Georgiev[2], I. Mladenova[2]

[1]Department Pediatric Surgery, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2]Department of Epidemiology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

Echinococcosis is a chronic ongoing helminthosis with multiple organ cysts, affecting humans and animals. PURPOSE: of this study is to develop, standardize and evaluate immunological tests for the confirmatory diagnosis of echinococcosis and to study seropositivity and latent infection by immunoscreen for diagnosis because of the latent course and the late manifestations of clinical symptoms. MATERIALS AND METHODS: In 107 of the surgical children (91.45%), to 2018 years we performed a serological control roll of echinococcus. Any liquid that has been studied for fertility. There are three possible approaches for screening for Echinococcosis in humans: 1) Serological test and confirmation of seropositive imaging methods; 2) Imaging (ultrasound, X-ray) and subsequent immunological examination of patients with cysts; 3) Simultaneous application of imaging and immunological methods Serums from persons examined by immunoscreen. RESULTS: IgG demonstrates the presence of specific antibodies against Echinococcus granulosus in the patient's serum. CONCLUSIONS Mass immuno-screening was performed in conjunction with the medical unit's parasitology.

Key words: echinococcosis, immunological tests, children

INTRODUCTION

Cystic echinococcosis is found in almost every country in the world. She is the leader parasitic zoanthroponosis of great health and social importance in Bulgaria. In our country has seen a dramatic increase in the incidence of 2.3-2.5% ooo in the 1980s to 8.47% ooo in 1996, exceeding baseline values levels of 6.5% Ltd. (1950-1965) before the launch of organized combat measures with parasitosis. In 2008 the primary cases from Bulgaria represented 55.27% (461) and 43.32% (386) respectively of all registered titles in the European Union, which puts us first in terms of echinococcosis (1, 2).

An echinococcal cyst develops which, as it grows, can reach a considerable size. Mostly the liver and lung are affected, but echinococcal cysts can develop in all organs. Immunological methods are used for diagnosis because of the latent course and the late manifestations of clinical symptoms. IgG demonstrates the presence of specific antibodies against Echinococcus granulosus in the patient's serum. Immunological methods are used for diagnosis because of the latent course and the late manifestations of clinical symptoms. Echinococcosis is helminthosis, which occurs as a chronic invasion with multiple organ localization of the cysts and affects humans and animals. We set ourselves the task of implementing and we characterize the diagnostic capabilities of one of the most modern quantitative methods for demonstrating specific IgE - ImmunoCAP and investigating quantitative anti-echinococcal IgE in serum.

Correspondence to: *Krasimira Kalinova MD, PhD, Department of Pediatric surgery, Medical Faculty, Trakia University, Armeiska 18-A-19, 6003 Stara Zagora, Bulgaria, Tel. 359 889424292, E-mail: krasimirakalinova@abv.bg*

PURPOSE

The purpose of this study is to highlight several facts that challenge immune and autoimmune responses to protect E. granulosus from elimination and to minimize severe host pathology.

MATERIALS AND METHODS

In 107 of the surgical children (91.45%), we performed a serological control roll of echinococcus. Any liquid that has been studied for fertility. To evaluate the immunological changes that follow after parole in a child which organization we have used the immunological tests.There are three possible approaches for screening for Echinococcosis in humans : 1. Serological test and confirmation of seropositive imaging methods 2.Imaging (ultrasound, X-ray) and subsequent immunological examination of patients with cysts 3. Simultaneous application of imaging and immunological methods.

In 2016, we developed a research project to 2018 at the Thracian University for the screening of childhood Echinococcosis in families The project showed the importance of good awareness of the population and prevention of groups of people with the same lifestyle with instrumental diagnostics and ultrasound tests.

After sending results, patients with suspected echinococcosis (positive in ELISA and in one or both other tests – ELISA and Western blot, are referred to GPs to clarify the diagnosis. The final confirmation was made after the appointment of imaging diagnostic tests and subsequent treatment.

The diagnosis of CE is mainly based on a combination of serological testing together with imaging approaches. Various serological methods have been developed, mainly based on hydatid cystic fluid, antigen B (AgB), and antigen 5, which are used for the immunodiagnostic of CE, but their results are not satisfactory. Although the use of recombinant or synthetic antigens improves the performance of serological tests, it has not overcome the problem of low sensitivity and cross-reactivity observed in the diagnosis of CE. In addition, the detection of specific antibodies to IgG subclasses has improved the effectiveness of immunodiagnostic tests. This review provides an overview of immunodiagnostic methods, associated antigens, and their characteristics in the diagnosis of CE.

RESULTS

The document highlights the shortcomings and challenges in the serological diagnosis of Echinococcosis. In addition, the limitations of currently available immunodiagnostic tests and the latest developments in the design and application of serological assays for the diagnosis of CE in humans are examined. The detection of specific antibodies to IgG subclasses has improved the efficacy of immunodiagnostic tests. Among the subclasses IgG and IgG2 and IgG4 are considered good markers for diagnosis, and IgG4 as a suitable marker for patient follow-up. The document highlights the shortcomings and challenges in the serological diagnosis of Echinococcosis. In addition, the limitations of currently available immunodiagnostic tests and the latest developments in the design and application of serological assays for the diagnosis of CE in humans are examined. The detection of specific antibodies to IgG subclasses has improved the efficacy of immunodiagnostic tests. Among the subclasses, IgG and IgG2 and IgG4 are considered good markers for diagnosis, and IgG4 as a suitable marker for patient follow-up. This review provides an overview of immunodiagnostic methods, associated antigens, and their characteristics in the diagnosis of CE.

Study of the results of the RPHA at children with echinococcosis - complicated and non-complicated forms.In our clinical study, 97 children (82.91%) responded positively to RPHA. The highest proportion of titre is 1: 1600 in 23 children (19.65%); followed by 1: 400-18 children (15.38%) and 1: 320000 children at 13 children (11.11%). The highest rated title was 1: 204 800 (1.92%) and the lowest was 1: 200 and went Lived at 12 children (10.26%). The ratio of the children to the response of the RPHA with comparatively low titers up to 1: 800 incl. to those with a high titre of 1: 1600 and greater than 1: 1.31.

At the RPHA /in the beginning of investigation/, the results of the results are in the lowest percentage compared to the other reactions, and even with the most complicated forms (at 10 children - 9.4%), finding the best is the best choice Compared with the rest of the methods, the RPHA shows a greater sense of sensitivity in liver hydatidosis - 46 dec. Male (47.91%) versus 43 children (37.39%) by IgG ELISA. In general, in children, we consider the IgG ELISA to be a more sensitive method. These percentages are also confirmed by the congregations of many authors. The results indicate a maximum sensitivity of between 66% and 100%. Positive feedback ELISA children are 95 (82.60%) with a highest titre of 1: 1600 - 29 children (25.22%), followed by 1: 400–23 children (20.00%) and

1: 200– 23 children (20.00%). The highest titer is 1: 1600, and the lowest diagonal titer is 1: 200. Relative between low and high reaction titres (1: 800 and top) is 1: 1.07 **(Tables 1a and 1b).** When analyzing the immunologic point of view of the echinococcosis protection, we found that we had a higher and the diagnostic sensitivity and significance of the RPHA and ELISA, also confirmed by the editorial staff. According to the survey, our higher sense of sensitivity, especially in the case of high titres we found none of our immune systems at the RPHA and ELISA. We believe that the detoxification of the body's immune responses does not exclude the presence of an echinococcal the goat, which even the public does not prove with certainty, which it maintains (2, 3, 4).

Table 1a. *Comparative Diagnostic Sensitivity of RPHA and ELISA for more complicated and complicated forms of hydatidosis in children*

Index	**РПХА**		
	N	%	Ratio
Low titres- up 1:800	42	35,90	1,00
High titres - up 1600	55	47,01	1,31
* Total positive (+)	97	82,91	
Total negative (-)	20	17,09	
Total	117	100,00	

Table1b. ** - p>0,05 (r2 =0,05 до 0,55)*

Index	**ELISA**		
	N	%	Ratio
Low titres - up 1:400	46	39, 32	1,00
High titres - 800 and more	49	41,88	1,07
* Total positive (+)	95	81,20	
Total negative (-)	20	17,09	
Total	115	98,29	

The study of this literature tends to confuse with the fact that in the so-called purulent skin is immunodeficient. agnostics is mail of no value, as reactions are not worth the waste (5, 6). In our study, 60% of cases with superpowered echinococcus were considered to have occurred with it is one of the reactions that other authors have mentioned. Comparative studies on the immune-logical response of RPHA and ELISA in case of adverse or complication hydatid forms.

According to him, in children with ruptured forms, the positive results from the RPHA were 60% versus 50% positive. For hydatidosis in pulmo, the corresponding percentages are 100% for the suppurative forms against 33.33% for the EBS (3: 1). In the negative results, the resulting data had an inverse character (40% at ruptured forms, mean will be 50% at supporative - 1: 1,25). The number of hydatidosis in children with non-complicating forms were 11.54%, 20.00% with pulmo localisation. and 14.20% for rare locations. With ruptured forms, 80% reported positively on the RPHA, as 80% had positive results. of the RPHA in case of non-essential echinoderm(7, 8, 9).

In the case of non-complex forms of rare localization, positive results of RPHA were 85.71 % and 100% for complicated ruptured forms (ratio 1: 1.18). Quite the veracity of the estimate of the differences in the result data for the mesh resolutions. Among the patients with complicated forms in the three localities was proven ($\chi 2 = 5.68$, $p < 0.05$).This tendency is also evident in the study of ELISA in cases of complications and complications. our forms of echinococcosis. Laboratory Western blot meets the reliability criteria confirmatory test - has a very high sensitivity (92.8%) and specificity (94.7%), whose parameters are better than those of ELISA (IgG).

In 115 children (98.29%), we applied a combined immunoassay method. We have positive results in these occasions one of the breakthroughs was positive. Our comparative analysis shows that the higher the percentage of the service the significant.Hydatid probes compared to the pulmo hydatidosis and the relay - respectively 1: 1.02: 2.02 (82, 91%: 82.6%: 63.16%).

DISCUSSION

Population screening is organized for healthy people, including children at risk, who are invited to participate (Holland et al., 2006). Screening is a specific rapid test or examination that is applied to all or part of the population to Screening is used not only for prophylaxis but also as a method for epidemiological study (Paltyshev and Filatov, 1998; Pokrovsky et al., 2012).Compared to serological screening, ultrasound detects a higher frequency of EC and produces higher positive predictive values (Schantz et al., 1997).

The study did not indicate whether these cysts were serologically confirmed or by other diagnostic methods. Serological tests have been improved in recent years, but still have limited value for CT screening. The most commonly used primary screening tests use hydatid antigens and include PLA, RPHA, and ELISA (Craig et al., 1993; Schantz et al., 1995). In some subjects with CE, no antibodies are detected, and the presence of antibodies does not provide information on the location, size, and other parameters of the cyst. Some tests are not highly specific and cross-reactivity with other helminths has been observed (Craig et al., 1993). A practical approach for serological studies of CE is to use a rapid and highly sensitive test, e.g. ELISA, and then confirm with a more specific test (eg immunoblot) (Schantz et al.,1997). The etiology of ultrasound-determined cysts is clarified through immunodiagnostic tests. Bilgin et al. (2009) recommend, as a more effective screening, the combination of ultrasound with ELISA (9). To date, three mass immuno-screening studies have been conducted in Bulgaria for cysts echinococcosis. The first was in (1974–1989) through RSC, RPHA and RLA, which covered 6 343 persons and found seropositivity 6.21% (Boeva-Bangyozova, 1983). The second study was under the National Program for the control of echinococcosis 2004 –2008 by ELISA and immunoblot among 8,506 individuals and revealed a hidden infection rate of 0.68% (Marinova, 2011). Detection of the presence of adult parasites in final hosts is easy. Using standard parasitological methods, the eggs of the tapeworms as well as the members of them, separated by faeces, are detected (10). Methods for the diagnosis of Ex in humans are best developed. It is based mainly on the use of serological tests for detecting antibodies in the body and imaging to locate the location, size and number of echinococcal blisters in the body (11).

According to some authors, there is no correlation between the stages of EC development and the immune response of the macro Organism aereas other (10) have often seen a direct dependency on the immune system. the logical pointers of your life's development of oxygen.

Positive results for suppuration forms according to the authors (12) are due to the reversal of the ecological which fluid through the wall of oxygen in the inflammatory process leading to a higher percentage of positive reactions. The higher the immune-logical reactivity of ruptured forms of echinococcosis, the more complete the antimicrobials are. genital stimulation in children, reported by other authors (13, 14).

In support of the worse, the events of echinococcosis at/1994, in which we have successfully established the SEDC in search. The RPHA is 1: 800, ELISA-1:200, and A.V.M. 4yrs. old, IZ № 806 /1992 with the rupture of the small hydatid cyst of the small liver and the negative our sero-logical reactions: RPHA - (-) open-ended; ELISA- (-) open-label. mmunoCAP for the detection of serum anti-echinococcal IgE has parameters,

confirmatory tests - high specificity (99.09%) and low cross-reactivity (1.72%), which are statistically better than ELISA IgG and superior to Western blot IgG (14.15).

In our opinion, the results of the discovery of the results are a consequence of the severe desulphurisation of the abscesses. It is in oxygen and the loss of its antigenic nature, leading to a negative immune from His speech. In the first case, the inflammatory process is in its initial development and antigenic specificity. The EC has not been disaggregated.

Impress the higher titers ofIgG ELISA in the unsuitable forms: RPHA: NECD – 61.54 %, NEBD – 42.86%; IgG ELISA - 48% for NCD and 65.71% for NCD. Rare titres were predominantly low: RPHA – 50% for in the rare forms of localization and 71.43% for IgG ELISA . The higher share of the low titers in complicated rare hydatid forms is larger than the non-negative ones (RPHA - 100% low titers at complicated rare hydatid forms and IgG ELISA - 100% low titers at complicated forms). Estimate differences in difficulties and Complicated Forms Regulated with You Each titre is reliable, and the percent differences between the three are within the confidence range. and ($\chi 2 = 3.68; 4.81; 5.1; p < 0.05$).

High RPHA titers up to 1: 204 800 have been successfully found at the non-complicated forms, which is not better observed. in the case of more complex forms that confirm the view of the small probability of finding Cleaning them when the EDU is ruptured or sub-purged. We consider that the low titers result from the non-specificity of the immuno-logical test, but may also they will be manifested in both forms of love.

In 59 children (50.43%) we have studied the production of the sepulchral globules of the class There are Ig G, Ig M, Ig A, Ig E preoperatively in the immune-diagnostic diagnostic complex; T and B lymphocytes in 12 of them (10.26%); as well as SIS (circulating immune complexes), anti-nuclear antibody (ANA) and antiphospholipid antibodies in 10 of them (8.55%). Changes in T- and B-lymphocyte levels and ANA in 7 children (5.98%) were not significantly related with the invasion of the invaders and the level of immunoglobulin. The high levels of the C3, alpha1-antitrypsin and SRP protein axis in 50.85% of the studied notes on total lesion processes and have been pointed out to be detached from oxygen something that supports cellular alterations in the liver for years to come They are supported by other authors (2, 3, 5, 6).

According to our study, our Ig G levels increased more frequently (58.24%) than that of Ig M and Ig A (41.18%). The levels of IgE (62.50%) and IgG (62.50%) are higher and higher in many EBDs. ANA (Anti-Nuclear Antibody) titer> 1:40 considered positive and we met in 5 (4.27%) children it is NDD and 2 (1.71%) children with NDD. The higher levels of Ig E correlated with the vitality of the oxygen and we met them more often for more complex forms (nearly 60%). They are also confirmed by other authors' studies (14). Given the high cost of immuno-logical research, we applied them at a limited cost. The number of children and the results are not statistically reliable. They show the important place of the property. EDV bookmarking as a complete method in preflighting ticks (1, 4, 7).

From the analysis done, B cells are normal in more than 75% of the subjects, which indicates that they violate the immunogen levels Bulbuls are for the account of increased cellular activity rather than number . There is a correlation between ours of the eosophilic in the blood of children, the serum lower immunoglobulin (Ig), CIC (circulating immune complexes), alpha and ha ma-in-interferon and HLA class I antigen control in patients with echinoderms, more specifically for EBD, while in solitary or multiple ECDs there are high levels of SIS and low in eosophiliacs You are IgE & IgG. Multiple location logos in the SIS field level abdominals unfortunately, they are high and correlated with the high IgE values. These data show the importance of the place of immunodeficiency in EDV as a supplementary method in pre-operative diagnostics. A.M.Sherbbakov taking attention to the role of Anti-Genetic Housing in the Region Lysing of echinococcal invasion in children (1993). **Table 2** summarizes the results of the application of serological reactions in the study.

Table 2. *Results of serologic tests in recidive hydatidosis*

Localization	Forms	N	%	PIIXA		ELISA	
				Positive	Negative	Positive	Negative
Hepar	Noncomplicate	2	28,57	2		2	1
	Complicate	2	28,57	1	1	1	
Pulmo	Noncomplicate	1	14,29	1		1	
	Complicate	1	14,29	1		1	
Rare localization s	Noncomplicate		0				
	Complicate	1	14,29	1		1	
Общо		7		6	1	6	1

CE- Cystic Echinococcosis , ECD-Hydatidosis of hepar, EBD- Echinococcosis in pulmo,
RLE- Rare localisationEchinococcosis
RPHA-Reaction precipitation and haemaglutinatio
NECD-Non complicated echinococcosis

With the recurrence of the tests, we found that immunologic tests were - RPHA -85, 71% and ELISA –85.71%, which confirms our recurrence of relapse of the hexane embryo in the child's organism. The open-ended immune response does not reliably detect the echinococcal disease for. In the study of one of the children, RPHA and IgG ELISA were negative. If the punctures in the later period after the first 4-7 weeks after the wards may be able to diagnose recurrent echinococcosis.

Bringing us on the basis of the perpetual serologic reactions in children indicate a lack of vitality between the locality or the form and form of development and the resulting immune response. It is not always possible to rely solely on a single reaction, and it needs to be combined, therefore necessary. and complement each other's resolving capabilities. Preoperative diagnosis is solved in a complex with others diagnostic methods. This is cellular activity, but not their number. Other authors confirm this (8, 9).

The levels of IgG immunogenesis increased (10, 11).The increased level of IgA and AFLat (antiphospholipid antibodies) is a marker of the involvement of the vascular endothelium. You are around oxygen. The high IgE level at this threshold is 62.5% and is associated with allergic symptoms both on the body and with the latest T-helper / T-suppressor in favor of the latter (12).

Violations of the cellular immunity of children with skin disease. Absolute lymphocyte counts and T-cell percentages were reduced ($p < 0.001$), supported by other authors (13). More than 54% of children have positive levels of circulating immune complexes (CIC) that correlate directly with the higher IgE levels. The relationship between the immune response and the different localizations in children is disputable.

For the hydatidosis recurrence, the study of RPHA and ELISA are of secondary importance, as in the first case. leading their norms afterwards and then following them upwards their values could be a relapse of the affair. Still in the process of studying the role of the immune-logical study in the community The value of the hydatidosis in children, which is the only point in the overall diagnostic process. Given the high cost of immuno-logical studies, we apply them to a limited number of These and the results do not have statistical credibility.

Large number of primary diagnostic reactions have been developed and implemented in Bulgaria. So far, however, no confirmatory tests have been implemented in Bulgaria. Of these, the most modern and with the highest

diagnostic value is Western blot. World-wide practice uses commonly laboratory-developed variants of Western blot, preferably the antigen prepared from native E.granulosus strains. Recently, attention has also been paid to methods of demonstrating anti-echinococcal IgE, which exhibit high specificity and low cross-reactivity. That's what makes them promising for the development of confirmatory tests. For this reason, we have set ourselves the task of developing, evaluating and implementing a laboratory method for Western blot with an antigen from a local strain of E.granulosus and see what the specific bands are. The literature data on the extremely high specificity and low cross-reactivity of serum anti-echinococcal IgE have led us to evaluate them as a second confirmatory technique. We set ourselves the task of applying and characterizing the diagnostic capabilities of one of the most modern quantitative methods for demonstrating specific IgE - ImmunoCAP and of quantifying anti-echinococcal IgE in serum, which has not been done so far in Bulgaria, and there is little information in the world literature (13).

In addition, we have included a study of serum characteristics in this work-specific IgE in echinococcosis, for which there is scarce data in the literature due to their difficult to prove with routine reactions. Understanding the immune mechanisms of E. granulosus infection in an intermediate human host will provide, we believe, more useful treatment with immunomodulatory molecules and possibly better protection against parasitic infections. Furthermore, the diagnosis of CE has been improved by the introduction of a new molecular tool for identifying parasites using novel recombinant antigens and immunogenic peptides (14).

More studies are needed to better understand the mechanisms of immune escape from parasites. This will allow for a new approach in protecting, detecting and improving the host's inflammatory responses. In contrast, according to the 'hygiene hypothesis', clinical applications that reduce the incidence of infection in developed countries and, more recently, in developing countries are at the beginning of an increasing incidence of both allergic and autoimmune diseases.

CONCLUSIONS

1. Higher percentage of negative results in children with echinococcosis is aware of the fact that purulent-necrotic beams lead to beams in the molecule of this structure. The course of echinococcal fluid and tarnishes its antigenic properties.
2. The greater the diagnostic value of the combination Eliminate Immunological methods in Comparison with Self-Body which they use. The data we receive is statistically reliable ($p<0.005$).
3. Specific Immunological Investigations In our opinion, we will be able to do better early in the study. Diagnosis, post-surgery prognosis and opening wild according to other authors, however, their application is still in the process of being studied for they use them in their routine practice.
4. Changes in laboratory performance may not always be permanent, but may always be of use as assistance. our motivations for refining the diagnosis, adequate for our pre-drafting training and the prognosis of the affliction.
5. According to our clinical study, ELISA (86.60%) followed with greater sensitivity ImmunoCap and WesternBlot and RPHA bath (82.05%). The statistical analysis does not show statistically significant differences ($\chi 2$ = 0.05-0.55, $p <0.005$). mmunoCAP for the detection of serum anti-echinococcal IgE has parameters, characteristic of confirmatory tests.
6. According to the received data, a conclusion is drawn for high diagnostic sensitivity. The visibility and specificity of the RPHA, which are also witnessed by community co-ordinators authors,
7. The highest titles achieved at the ELISA cut to mileage are the highest bearing and specificity for proving the pain.
8. Our children's studies indicate that they are susceptible to discomfort with equal force before and after infestation. We believe that on the basis of the results obtained, the child's organs are immune. We realized that a single reaction alone could not always be relied upon and needed to be addressed several immuno-logical probes for complementarity
9. The high latent prevalence in the paediatric population was found (15.52% of all detected in screening cases) indicates the existence of a very active transmission of the parasite in our country.
10. Immunoscreen is an effective method of demonstrating latent infection by echinococcosis and should be used more widely in our country. Immune screening information obtained indicates the need for amplification of epidemiological surveillance and control of echinococcosis in our country can be implemented in the planning and implementation of organized control measures.

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