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Research Article

Diagnostic Efficacy of IgG ELISA in Hydatid Disease: A Retrospective Study

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Keywords

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Abstract

Most serodiagnostic techniques have been evaluated for diagnosis of cystic hydatid disease caused by Echinococcus granulosus. Each, to varying degrees, has been shown to give false results, with considerable variation between laboratories. The comparative retrospective study was done concerning the sensitivity of the different commercially available IgG ELISA kits based on previous laboratory records of 55 patients diagnosed as hydatid disease either intraoperatively or post operative histopathology with different cyst locations. In house ELISA, DRG IgG kit, Nova Lisa kit and Ridascreen IgG kit were studied. Specific IgG ELISA AgB (antigen B-rich fraction) was the most sensitive test (100%) and the least sensitive tests were the In House ELISA (75%), DRG (75%), Nova Lisa (66.66%), these results coincide basically with the findings of most researchers.

ABBREVIATIONS

CE: Cystic Echinococcosis, ELISA: Enzyme-linked immunosorbent assay, IgG: Immunoglobulin G

INTRODUCTION

Cystic echinococcosis (CE), known as hydatid cyst or hydatid disease, is a parasitic zoonosis caused by the larval stage of *Echinococcus granulosus* (*E. granulosus*) which accounts for 95% of human echinococcosis. Dogs and other canids harbor the adults tape worm and herbivores act as intermediate host and become infected through ingestion of parasite's eggs. Human acquire the infection by accidental ingestion of *E. granulosus* eggs [1].

CE with its significant economic and medical impact constitutes an important public health problem in many developing countries [2,3]. Worldwide, echinococcosis causes an estimated annual loss of US $194,000,000. An estimated 1.2 million people worldwide are affected by CE and the disease accounts for annual estimate of 3.6 million DALYs (disability adjusted life years) through the world [4]. Early and proper diagnosis of CE can provide appropriate management and suitable treatment of the disease [5]. Diagnosis of CE is mainly confirmed through a combination of relevant history, serological testing, along with imaging approaches. A variety of serological methods have been developed and used for immunodiagnosis of CE in recent years, including indirect hemagglutination, immunoblotting, enzyme-linked immunosorbent assay, indirect fluorescent-antibody, latex agglutination test, and immunochromatography test [6,7]. For the development of these assays different antigens from adult worm, protoscolices, worm eggs or hydatid cyst fluid have been defined, purified and evaluated in the aforementioned serological tests [8].

Diagnosis of CE has drastically improved during the last two decades. Progress in methods for antigen purification, cloning expression and purification of *E. granulosus* recombinant antigens, and defining and synthesis of immunodominant peptides contributed to this development [9]. Nevertheless, immunodiagnosis of CE is still problematic. Commercially available serological tests show unsatisfactory performance. The lack of standardization of immunodiagnostic assays and also antigen preparation contribute to discrepancy in results reported in different laboratories. Cyst size, stage and location as well as patients characteristics may be accounted for the discrepancy of the same test performance in different clinical diagnostic laboratories [10-12].

Hence, serological assays still have a complementary role to imaging in the diagnosis of CE. Low sensitivity (up to 30% of false negativity) and also low specificity (up to 25% of false positivity) make serological results difficult to interpret. [13-15]. With this context, this study was conducted to evaluate and compare the diagnostic efficacy of four ELISA systems in our department.

MATERIALS AND METHODS

This retrospective study was done in Department of Microbiology; Sher-i-Kashmir Institute of Medical Sciences, North India on patients who underwent surgery for suspected hydatid cysts of liver, lung, bladder, 4th ventricle of brain from

December 1989 to December 2016. All these patients had undergone preoperative ELISA for IgG levels for Echinococcus. There were total of 55 patients in whom a diagnosis of hydatid disease was confirmed either by intraoperative findings or by postoperative histopathology .The data of all these patients was recorded routinely irrespective of their age, sex, clinical presentation or preoperative imaging. Two inclusion criterias taken into consideration were a preoperative estimation of IgG for hydatid disease and confirmation of hydatid disease during surgery or on postoperative histopathological examination.

The IgG levels had been estimated by the following In-house and standard commercially available kits.

In-house ELISA in which microtitration plate was coated with 2 µg/100 µl of crude hydatid sheep antigen [16].

The **DRG Echinococcus IgG ELISA Kit**, a solid phase enzyme-linked immunosorbent assay (ELISA), in which microtiter wells as a solid phase are coated with *Echinococcus* crude antigen [17].

NovaLisa IgG ELISA Kit in which the microtiter wells as a solid phase are coated with *Echinococcus* crude antigen [18].

Ridascreen Echinococcus IgG Kit The micro test wells are coated with purified antigens i.e Specific IgG ELISA AgB [19].

RESULTS

The study was conducted on the data over a period of 16 years. There were 32 (58.18%) males (20-hydatid of liver ,10-hydatid of lungs,1-hydatid of bladder and 1 hydatid cyst 4th ventricle of brain) and 23(41.81%) females (15-hydatid of liver, 8-hydatid of lung) in the study group. Highest numbers of cases 24 (43.63%) were seen among age group of 40-59 years. Farmers constituted the major bulk of involved patients 27 (49.09%) followed by House wives 13(23.63 %) labourer 11(20 %) and 4 students (7.27 %). Majority of patients were from rural areas 43 (78.18%) and rest were from different urban areas 11(21.88%).

4 types ELISA kits used for estimation for IgG levels in these patients were

In house ELISA -20 patients, DRG IgG kit -16patients, Nova Lisa kit – 15 patients and Ridascreen IgG for 4 patients respectively.

Out of 35 patients of hydatid cysts of liver, 25 had a positive serology by IgG crude antigen (71.42%), 2 had positive serology by purified antigen (100%), giving the overall sensitivity of (77.14%)

Among 18 patients of hydatid lung 12 had positive serology for IgG by crude antigen (75%) and 2 had positive serology by purified antigen (100%) giving the overall sensitivity of (66.66%). Both the males having hydatid bladder and 4th ventricle of brain had positive serology by DRG IgG kit (100%).

DISCUSSION

This study was conducted on 55 patients of hydatid disease which included 58.18% (32) male patients most of them being farmers 27(49.09%) (Figure 1, Table 1). Our patient population was similar to most of the studies [20,21]. Among females 23(41.81%) the maximum numbers of cases were housewives 13(23.63%) (Figure 1, Table 1) slightly lower the researches done

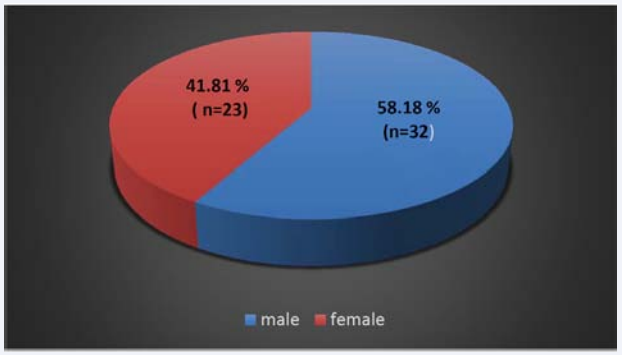

Figure 1 Sex distribution of hydatid cyst disease.

Table 1: Distribution of hydatid cyst disease based on occupation.

Farmer	Housewive	Labour	Students
27(49.09%)	13(23.63%)	11(20%)	4(7.27%)

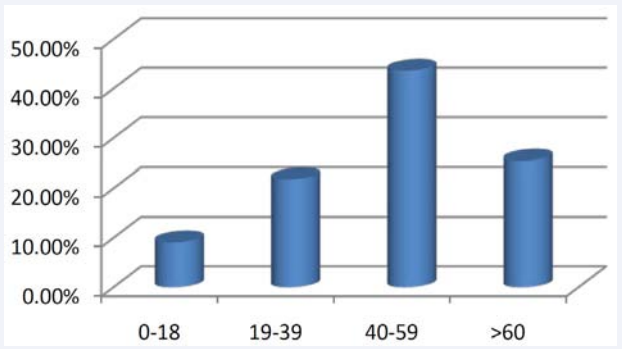

Figure 2 Age incidence of hydatid cyst disease.

by Al Barwari et al., and Jawed Akther et al. accounting around 37.90% and 39.32% respectively [22]. Farmers and housewives are more prone as they are involved in house hold activities related to animal breeding and agriculture in rural areas. In an interesting experimental study it was found that female gonadotrophins have an inhibitory action on parasitisation, while male hormones had no such effect or might even increase the susceptibility of host infection [23].

We found that the liver was the most common affected organ 35 (63.63%), followed by lung 18(32.72%), bladder1(1.81%) and 4th ventricle of brain1(1.81%) (Table 2). The higher rate of hepatic infection may be attributed to the fact that liver acts as a primary filter in the human body and lung is often thought to be the second filter [23]. There was a predominance of single organ involvement over the multiple organ involvement, which was a similar finding in most of the research works done on hydatid cysts [22].

Immunodiagnosis is an important tool for diagnosis of CE infection. Thus, in addition to imaging techniques, a reliable serodiagnosis improves prognosis for patients with cystic *Echinococcus* [20]. The definitive diagnosis of cystic *Echinococcus* is by combination of CT or MRI with serological testing. USG is indicative of the disease but not diagnostic [24].

In serology, the detection of circulating *E. Granulosus* antigens in sera is less sensitive than antibody detection, which remains the method of choice [25]. Insensitive and non-specific tests like Casoni intradermal test, complement fixation test, indirect haemagglutination test and latex agglutination test have been replaced by ELISA, indirect immunofluorescence antibody test, immunoelectrophoresis and immunoblotting [26]. Among these, ELISA for detection of IgG antibodies is most commonly used. It is considered to be highly sensitive and specific in detecting anti-Echinococcus antibodies irrespective of the site of cyst localization [16].

The intensity of the serological response to hydatid antigens varies considerably, depending on the host and the location of the parasitic cysts. In this sense, ever since the beginning of serological diagnosis of hydatidosis, lung cysts have given very low responses, similar to as shown in our study (66.66%) [27] (Table 4). Nevertheless, other locations such as the liver offer good or acceptable serological responses (77.14%) [28] (Table 3). In our study the most sensitive test was ELISA AgB (100%) from all the kits together (Table 3,4,6), followed by the In House (75% sensitivity), DRG(75%), Nova Lisa (66.66%), these results coincide basically with the findings of most researchers [29,30] (Table 6).

In a similar study by Wattal et al., among purified antigens, ELISA for IgG was the most sensitive (96.5%) in comparison to our study which showed almost sensitivity of 100% [16].

The ELISA technique using purified antigen, performed in our laboratory, gave good diagnostic values (100% sensitivity) (Table 6). These results are similar to those obtained by Kaddah et al.

Table 2: Distribution of hydatid cyst disease based on organs involved.

Organs involved	Number of cases
Liver	35(63.63%)
Lungs	18(32.72%)
Bladder	1(1.81%)
4th ventricle of brain	1(1.81%)

Table 3: Serology tests in patients of hydatid cysts of liver.

	Positive	Sensitivity
In House (15)	11	73.33%
DRG (10)	8	80%
Nova Lisa (8)	6	75%
Ridascreen (2)	2	100%
35	27	77.14%

Table 4: Serology tests in patients of hydatid cysts of lung.

	Positive	Sensitivity
In House (5)	4	80%
DRG (4)	2	50%
NovaLisa (7)	4	57.14%
Ridascreen (2)	2	100%
18	12	66.66%

Table 5: Serology tests in patients of hydatid cysts of Bladder and 4th ventricle of brain.

	Positive	Sensitivity
DRG(2)	2	100%

Table 6: Combined results of serology comparing the four kit.

	Positive	Sensitivity
In House (20)	15	75%
DRG (16)	12	75%
Nova Lisa (15)	10	66.66%
Ridascreen (4)	4	100%
55	27	74.54%

[31], who used antigens obtained by affinity chromatography, and to the results of Ito et al. [32] and Poretti et al. [33].

The sensitivity and specificity of the diagnosis of most of the tests varied considerably according to the nature, purity, and quality of the antigen, according to the nature of the immunoglobulins (e.g., isotypes), and according to the sensitivity methodology chosen [34].

The reported high sensitivity of tests using purified antigens was reproduced in this study as well. Patients subjected to ELISA testing with a kit, using crude antigens, showed overall sensitivity of 72.54 %, while patients subjected to ELISA testing using Ridascreen kit, which uses purified antigens, had sensitivity of 100% but the later was not being widely used and this could be one reason for the overall decreased sensitivity of ELISA by the crude antigen kit as being shown in this study (Table 6).

CONCLUSION

The performances of currently available immunodiagnostic test in diagnosis of CE are not satisfactory and the best serological test for diagnosis of CE is still the subject of debate. ELISA IgG test using crude antigens are the most commonly employed tests for diagnosis of hydatid disease but the reported high sensitivity of the test could not be reproduced in comparison to the Kit using purified antigen, but they were not being as widely used as the ones using crude antigens. Sole dependence on IgG ELISA using crude antigen for diagnosis of the Hydatid disease should be avoided and kits using purified antigens should be considered. The results obtained in the present work confirm that the use of purified antigens is crucial in the immunodiagnosis of the Hydatid disease.

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