

## DIAGNOSIS OF HUMAN CYSTIC ECHINOCOCCOSIS BY DETECTING ANTIGEN B IN SERUM AND URINE USING NANOMAGNETIC BEADS-ENZYME LINKED IMMUNOSORBENT ASSAY

By

AMIRA E. KHAYYAL<sup>1\*</sup>, MANAL S. E. MAHMOUD<sup>1</sup>, REHAN B. IBRAHIM<sup>1</sup>,  
IBRAHIM R. BAYOUMI<sup>2</sup> and ABEER F. BADAWEY<sup>1</sup>

Department of Parasitology<sup>1</sup>, Faculty of Medicine, Ain-Shams University, Cairo 11566,  
and Department of Parasitology<sup>2</sup>, Theodore Bilharz Research Institute, Imbaba 30,  
Giza, Egypt (\*Correspondence: amira\_elsaady12@yahoo.com)

### Abstract

Cystic echinococcosis (CE) is usually asymptomatic, and commonly presents by pressure symptoms according to its location. Diagnosis of CE is currently based on imaging techniques, which may not be available in some areas and antibody detection in serum, with its known several drawbacks. Conversely, antigen detection has proven its efficacy in serodiagnosis of CE. The study evaluated the efficacy of Nanomagnetic beads (NMB)-sandwich ELISA for diagnosis of CE by detection of *Echinococcus (E.) granulosus* Antigen B (AgB) in serum and urine samples as compared with sandwich ELISA. A total of 42 individuals were classified into 3 groups; CE infection group, other parasitic infections group and control group. *E. granulosus* AgB was prepared from human hydatid cysts to produce anti-*E. granulosus* AgB-immunoglobulin (Ig) G-poly-clonal Abs in rabbit, after purification, to detect AgB level in serum and urine samples by sandwich ELISA and NMB-sandwich ELISA. Using sandwich ELISA, AgB was detected in sera of CE patients with sensitivity and specificity of 88.9% & 91.7%, respectively. In urine samples, sandwich ELISA detected AgB in CE patients and the sensitivity and specificity were 83.3% and 87.5%, respectively. NMB-sandwich ELISA detected AgB in serum samples of CE patients with a sensitivity and specificity of 94.4% and 95.8%, respectively, whereas in urine samples it was detected in CE cases and the sensitivity and specificity were 88.9% and 95.8%, respectively, without significant statistical difference ( $p>0.05$ ) between serum and urine samples for detection of AgB when examined by either sandwich ELISA or NMB-sandwich ELISA. The best accuracy was obtained with NMB-sandwich ELISA detecting AgB in serum samples (95.2%), with a slightly lower accuracy of 92.9% when using urine samples. NMB-sandwich ELISA is efficient for detection of *E. granulosus* AgB in serum samples. Moreover, urine samples might be a good alternative to serum samples for the detection of AgB.

**Key words:** Antigen B, cystic echinococcosis, *E. granulosus*, hydatid cyst, Nanomagnetic beads-sandwich ELISA, sandwich ELISA, serum, Urine.

### Introduction

Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by the larval stage hydatid cyst (metacestode) of *Echinococcus (E.) granulosus* tapeworm (Brunetti and White, 2012). The diagnosis of CE remains challenging, as numerous cases stay asymptomatic for years and diagnosed only when complications develop (Manzano-Roman *et al*, 2015). CE diagnosis is mainly by imaging techniques and serodiagnosis (Tamarozzi *et al*, 2016). Different immunoassays are used as ELISA and IHAT (Mihmanli *et al*, 2016). Antibodies may persist in circulation for years, even after hydatid cyst removal or clinical cure by chemotherapy (Zhang *et al*,

2012). But, hydatid antigen can be detected in the serum, urine and in other body fluids during active or recent infection. Besides, their levels decrease gradually after successful treatment, so that antigen detection can help in monitoring the efficacy of treatment and follow up (Pakala *et al*, 2016). Antigen (Ag) B and Ag5 were identified as the most abundant immune reactive antigens in hydatid cyst fluid (Gonzalez *et al*, 2018).

Nanoparticles (NPs) such as quantum dot, liposomes and metal based inorganic NPs have been used for diagnosis and treatment of many parasitic infections like malaria, toxoplasmosis, leishmaniasis, giardiasis, and in vaccine trials (Abaza, 2016). Magnetic

NPs (MNPs) are NPs with magnetic properties used as biosensors to detect desired targets including deoxyribonucleic acid (DNA), proteins, enzymes, drugs and pathogens (Hau et al, 2010). They actively investigated for targeted cancer treatment, guided drug delivery, gene therapy and DNA analysis (Hasan, 2015). Moreover, the use of MNPs has been previously evaluated for detection of parasite antigens such as *Plasmodium falciparum* histidine-rich protein 2 (Castilho et al, 2011) and *Schistosoma haematobium* tegumental antigen (Aly, 2020).

The present study aimed to assess the use of nanomagnetic beads sandwich-enzyme linked immunosorbent assay (NMB-sandwich ELISA) for diagnosing of human CE by detecting *E. granulosus* AgB in sera and urine samples of CE patients, in comparison to sandwich ELISA.

### Subjects and Methods

Ethics approval and consent to participate: The study obtained approval from the Ethical Committee of the Faculty of Medicine Ain Shams University. Animal experiments were carried out according to the international guidelines, which agreed with the ethical guidelines of 1975 Declaration of Helsinki (6<sup>th</sup> Revision, 2008). Written informed consent was obtained from all participants.

This study included 42 subjects divided into three groups: GI: 18 patients with CE, GII: 12 patients with other parasitic infections, and GIII: 12 healthy controls. All were selected from out and in patients, Department of Surgery Faculty of Medicine, Ain Shams University Hospitals, and Theodor Bilharz Research Institute (TBRI) from January 2020 to May 2022. CE was diagnosed by history, imaging techniques and IHAT, and then confirmed by microscopy of aspirated HCF from the removed hydatid cysts.

Sample collection: The HCF was collected from surgically removed hydatid cysts for AgB preparation. Blood and urine samples were collected from all enrolled individuals.

Preparation of AgB: HCF was centrifuged at 1,000 g for 15 minutes (min) then the su-

pernatant was dialyzed overnight against 0.1 M phosphate normal buffered saline (PBS) at 4°C (Nasrieh and Abdel-Hafez, 2004). After being transferred to a sterile tube, it was boiled in a water bath and maintained at 100°C for 15min, centrifuged at 15,000g for 20min at 4°C and the supernatant was transferred to a sterile tube to be stored at -20°C until used (Rogan et al, 1991). Protein content was 2000µg/ml as determined by Bio Rad protein assay (Bradford, 1976), followed by characterization of AgB by 12.5% Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and stained with Coomassie Blue (Laemmli, 1970).

Preparation and purification of anti-*E. granulosus* polyclonal antibodies (pAbs) (Sadjidi et al, 2009): A New Zealand white male rabbit (1.5 kg) was immunized by 3 intramuscular injections, as follows; one dose of AgB mixed with complete Freund's adjuvant then, 2 weeks later a booster dose mixed with incomplete Freund's adjuvant was given followed by a 2<sup>nd</sup> booster dose one week later. Rabbit immunization was confirmed by testing withdrawn blood samples for the presence of specific anti-*E. granulosus* AgB pAbs by indirect ELISA (Crowther, 1995), Serum of anti-*E. granulosus* AgB pAb was collected and stored at -20°C until used.

Preparation: Anti-*E. granulosus* AgB pAbs was purified by ammonium sulfate precipitation (Garvey et al, 1977) then by 7% caprylic acid precipitation (McKinney and Parkinson, 1987). Purified protein of anti-*E. granulosus* AgB-IgG-pAbs was determined (Bradford, 1976) and conjugated with horse-radish peroxidase (Tijssen and Kurstak, 1984). This was followed by loading the NMBs to IgG-pAb & HRP conjugated-IgG-pAb. Different concentrations of coating capturing antibodies and its HRP conjugate were tried and optimum concentrations were determined.

Detection of AgB by sandwich ELISA in sera and urine samples (Aly, 2016): Microtiterated ELISA plates (Costar, USA) were coated with 100µl/well of anti-*E. granulosus*

AgB-IgG-pAb (10µg/ml of 0.06M carbonate buffer) and incubated overnight at room temperature. Plates were washed 3 times with PBS & 0.05% Tween 20 (PBS-T) and blocked with PBS-T contained 1% bovine serum albumin for 30min. at 37°C and incubated for 2hrs at 37°C. Plates were then washed 3 times as before. One hundred µl of each sample in duplicates was added to wells, and incubated for 2hrs at 37°C. Plates were then washed 5 times, with PBS-T. One hundred µl/well of HRP-IgG-pAb, (25µg/ml PBS-T) was added to the wells and incubated for 1hr at room temperature. One hundred µl/well of substrate solution (O-phenylene diamine dissolved in 25ml of 0.05M phosphate citrate buffer, pH 5 with hydrogen peroxide urea (Sigma Chemicals Co., USA) was added to each well and plates were incubated in dark at room temperature for 30min. 50µl/well of stopping solution (8 N sulfuric acid) was added. Absorbance was measured at 492nm by ELISA reader (Bio-Rad, CA.). AgB was detected by NMB-sandwich ELISA in sera and urine samples using of NMBs-IgG-pAb as a coating capture antibody and conjugated NMBs-HRP-IgG-pAb at mentioned before.

Statistical analysis: Data were analyzed using Statistical Package for Social Sciences, version 23.0 (SPSS Inc., Illinois, USA). Mean ±SD, range and percentages were calculated. A one-way variance analysis (ANOVA) and receiver operating characteristic (ROC curve) analysis were used. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and test accuracy were determined. Significance was considered if *p*- value was less than 0.05.

### Results

GI: Patients (38.9%) were between 31 & 40 years old, with liver cysts in 88.9% and lung cysts in 11.1%. Clinical manifestations of liver cysts were abdominal pain, anorexia, obstructive jaundice and ascites with marked eosinophilia and in lung cysts, chest pain and/or chronic cough. GII: Patients were infected with *Hymenolepis nana* (3), *Entero-*

*bius vermicularis* (3), *Entamoeba histolytica* (2), *Giardia lamblia* (2) & *Blastocystis hominis* (2). 12.5% SDS-PAGE analysis of AgB showed 3 bands at molecular weights (8-12, 16 & 24 kDa), after rabbit immunization OD of serum anti-*E. granulosus* AgB was 1.7 by indirect ELISA. Protein purified t of anti-*E. granulosus* AgB-IgG-pAb content was 3.2µg/ml. Cut-off point for AgB detected by sandwich ELISA in sera was 0.58 and in urine samples was 0.97. Analysis showed that OD readings of GI were higher than those of GII and GIII with a highly significant difference (*p*<0.001) between GI & each of GII & GIII.

Diagnosis by AgB detection in sera versus urine samples by sandwich ELISA showed that sera gave higher sensitivity (88.9%) & specificity (91.7%), than urine (83.3% sensitivity & 87.5% specificity). Cut-off point for AgB by NMB-sandwich ELISA in serum was 0.59 & in urine was 0.99. Analysis showed that GI OD was higher than each of GII & GIII with a highly significant difference (*p*<0.001) between GI and each of GII & GIII. AgB detected in sera versus urine samples by NMB-sandwich ELISA gave higher sensitivity (94.4%) than urine (88.9%), with specificity of (95.8%). NMB-sandwich ELISA & sandwich ELISA for AgB in sera by ROC curve showed sensitivity of 94.4% & 88.9% and specificity of 95.8% & 91.7% respectively.

NMB-sandwich ELISA gave higher diagnostic accuracy (95.2%) than sandwich ELISA (90.5%) in sera without significant difference (*p*>0.05). NMB-sandwich ELISA & sandwich ELISA for AgB in urine by ROC curve showed 83.3% sensitivity & 88.9% by NMB-sandwich ELISA. The specificity was 87.5% by sandwich ELISA & 95.8% by NMB-sandwich ELISA gave high accuracy (92.9%) than sandwich ELISA (85.7%) for AgB in urine without significant difference (*p*<0.05). NMB-sandwich ELISA gave best outcome results by using sera.

Details were given in tables (1, 2, 3, 4, 5 & 6) and in figures (1, 2, 3, 4 & 5).

Table 1: Data of participants

Groups	Age (years)		Sex	
	mean	Range	Male No. (%)	Female No. (%)
GI (n =18)	33.41	12 - 60	10 (55.6)	8 (44.4)
GII (n =12)	25.8	9 - 50	7 (58.3)	5 (41.7)
GIII (n =12)	30.2	15 - 54	6 (50)	6 (50)

Table 2: *E. granulosus* AgB detection by sandwich ELISA

Diagnostic method/ sample		GI		GII		GIII	
		No.	%	No.	%	No.	%
Sandwich ELISA / serum	Positive	16	88.9	2	16.7	0	0
	negative	2	11.1	10	83.3	12	100
Sandwich ELISA / urine	Positive	15	83.3	3	25	0	0
	negative	3	16.7	9	75	12	100
Total		18	100.0	12	100	12	100

Table 3: Analysis of *E. granulosus* AgB detected in sera & urine samples by sandwich ELISA among groups

Groups	Sandwich ELISA OD at 492 nm for sera		Sandwich ELISA OD at 492 nm for urine samples	
	M±SD	Range	M±SD	Range
GI	2.261±0.749	0.501-2.998	1.978±0.675	0.623-2.875
GII	0.418±0.125	0.285-0.675	0.493±0.334	0.245-1.120
GIII	0.536±0.022	0.512-0.581	0.783±0.093	0.617-0.874
ANOVA test	65.904		40.809	
p-value	<0.001*		<0.001*	

\*P-value &lt;0.001 highly significant

Table 4: *E. granulosus* AgB detection by NMB- sandwich ELISA.

Diagnostic method/ sample		GI		GII		GIII	
		No.	%	No.	%	No.	%
NMB- sandwich ELISA/ serum	Positive	17	94.4	1	8.3	0	0
	Negative	1	5.6	11	91.7	12	100
NMB- sandwich ELISA/ urine	Positive	16	88.9	1	8.3	0	0
	Negative	2	11.1	11	91.7	12	100
Total		18	100.0	12	100	12	100

Table 5: Statistical analysis of *E. granulosus* AgB detected in sera & urine samples by NMB-sandwich ELISA among groups.

Groups	NMB-sandwich ELISA OD at 492nm for sera		NMB-sandwich ELISA OD at 492nm for urine samples	
	M±SD	Range	M±SD	Range
GI	2.273±0.608	0.457-2.988	2.121±0.651	0.523-2.946
GII	0.432±0.100	0.387-0.749	0.423±0.240	0.253-1.113
GIII	0.580±0.007	0.569-0.591	0.830±0.081	0.710-0.899
ANOVA test	98.404		59.145	
p-value	<0.001*		<0.001*	

Table 6: Comparison between diagnostic performance of sandwich ELISA &amp; NMB- sandwich ELISA in serum &amp; urine samples.

Statistical analysis	Serum samples		Urine samples	
	Sandwich ELISA	NMB- sandwich ELISA	Sandwich ELISA	NMB- sandwich ELISA
Sensitivity	88.9%	94.4%	83.3%	88.9%
Specificity	91.7%	95.8%	87.5%	95.8%
PPV	88.9%	94.4%	83.3%	94.1%
NPV	91.7%	95.8%	87.5%	92.0%
Accuracy	90.5%	95.2%	85.7%	92.9%
p-value	0.310*		0.156*	

\*P-value &gt; 0.05= non-significant, PPV: positive predictive value, NPV: negative predictive value

## Discussion

Man is accidentally CE infected by ingesting the eggs in contaminated food or drink, or by contact with infected dogs, larvae develops in the affected organ into a three-layered cyst wall surrounding the HCF (Rouhi, 2022). Sero-diagnoses have a marked role in the CE diagnosis being helpful, least invasive and easy to perform. Moreover, MDCT

and MRI evaluated the CE more accurately (Cakir *et al*, 2021). Besides, ELISA & IHAT Reverse latex agglutination (RLA) test and IFAT proved valuable in the CE diagnosis (Mahmoud and Ezzat, 1999). Western immunoblotting (IB) detected serum anti-EgAP immunoglobulin (IgG) as compared to HCF (Mahmoud and Abou Gamra, 2004). Also, specific IgG antibodies subclasses detection

improved the efficacy of immunodiagnostic tests, as among IgG subclasses IgG2 & IgG4 proved to be good markers for diagnosis and IgG4 as a good marker for treatment follow-up (Sarkari and Rezaei, 2015).

Nano diagnostic technology is widely used in diagnosis of numerous medical diseases. NPs' small size and large surface area permits attachment of large number of targeted diagnostic molecules, thereby, NPs use allows rapid, sensitive and specific detection of pathogens and molecules (Aly *et al.*, 2014). MNPs proved dependable in medical and immunoassay-based diagnosis as well as diagnostic therapy (Nguyen *et al.*, 2021), and can be used as biosensors to detect targets in antigens, antibodies, DNA and different biomarkers (Tran *et al.*, 2022).

In the present study, the CE patients' ages ranged from 12 to 60 years old, and 38.9% of them were between 31-40 years, which agreed with Moradi *et al.* (2019), and Heikal and El-lessy (2021). However, Sharafi *et al.* (2018) found that the highest rate of CE patients ranged between 10 to 20 years. Also, Gholami *et al.* (2018) reported that to determine the exact age of CE infection being difficult as CE might start in childhood and manifest in adulthood. The present CE infection was more in males than in females. This agreed with Elawamy *et al.* (2020), but Heikal and El-lessy (2021) found more infection in females than in males. This may be due to ecological conditions (Salama *et al.*, 2014).

In the present study, liver was the highly affected organ (88.9%) followed by lungs (11.1%) which agreed with the known concept that the liver is the first site followed by lung (Garcia, 2016). The present liver cysts clinical pictures were mainly abdominal pain and jaundice, but chest pain and cough in lung cysts. This agreed with El-Ghareeb *et al.* (2016) and Heikal and El-lessy (2021) who reported that CE liver was 75.5% & 83.87%, respectively with similar manifestations.

In the present study, the AgB was prepared from surgically removed human hydatid cyst (Nasrieh and Abdel-Hafez, 2004). Rahimi *et*

*al.* (2011) reported that AgB isolated from human hydatid cysts gave the best diagnostic results as compared to those from camel, cattle or goat. Abdi *et al.* (2010) evaluated the recombinant AgB (rAgB) subunit, native AgB & HCF by ELISA, found that they gave the same sensitivity and specificity (96% & 97%, respectively), but less than those of native AgB (98.6% & 100%, respectively). Jiang *et al.* (2015) compared reactivity of rAgB to native AgB for CE diagnosis found that rAgB was not a better candidate.

In the present study, AgB electrophoretic analysis by SDS-PAGE showed in 3 different protein bands at 8-12, 16 & 24 kDa by using a 12.5% gel concentration under reducing conditions and stained with Coomassie blue. This agreed with Mamuti *et al.* (2006) who found that AgB dissociated into 3 molecular bands 8-12, 16, & 24 kDa. Khalilpour *et al.* (2014) reported that 8-12 kDa subunits were of diagnostic value. Also, Shirazi *et al.* (2016) reported that SDS-PAGE of prepared AgB showed bands of 20 & 24 kDa, adding that differences in molecular weights may be a consequence of differences in polyacrylamide percentage in SDS-PAGE gels used or electrophoretic conditions.

In the present study, sandwich ELISA for AgB in sera showed 16/18 positive in CE patients, 2 /12 in patients with other parasites, and none in control, with sensitivity of 88.9% and specificity of 91.7%. Sandwich ELISA for AgB in urine showed 15/18 positive in CE patients, 3/12 in patients with other parasites, and none in control with sensitivity of 83.3% and specificity of 87.5%. Also, there was increase AgB levels in sera and urine samples of CE patients than in those with other parasites, and control. The reliability of immunoassays in detecting hydatid antigen in sera was reported (Sadjjadi *et al.*, 2009; Sarkari and Rezaei, 2015).

Hydatid antigen detection in urine was developed to diagnose human CE by counter current immunoelectrophoresis using hyper-immune antisera against crude HCF (Parija *et al.*, 1997). This agreed with Aly (2016), he

used sandwich ELISA to detect hydatid antigen; circulating protoscolices antigen in sera with sensitivity of 90.48% & specificity of 91.3%. Rashed *et al.* (2018) found that Dot-ELISA detected protoscolices antigen in CE sera of infected camels with 86.1% sensitivity and 90% specificity. Also, parasitic antigens in urine detected *Wuchereria bancrofti* filarial antigen by SDS-PAGE & immunoblotting (Chenth-amarakshan *et al.*, 1993), *Onchocerca volvulus* larval antigen by dipstick assay (Ayong *et al.*, 2005), *S. haematobium* & *S. mansoni* circulating cathodic antigen by ELISA (Peralta and Cavalcanti, 2018), *Taenia solium* antigen by capture ELISA (Paredes *et al.*, 2016), and human visceral leishmaniasis by the repressor differentiated kinase 2 antigen in urine by ELISA & immunoblot techniques (Bhattacharyya *et al.*, 2022).

The present results were higher than those reported by Chaya and Parija (2013), using sandwich ELISA for detection of a 24 kDa fragment of hydatid antigen in sera sensitivity of 80%, but sensitivity & specificity were 72% & 96%, respectively in urine.. Sadjjadi *et al.* (2009) reported much less sensitivity of 25.7% by sandwich-ELISA in detecting circulating hydatid antigen in sera by anti-hydatid IgG raised against HCF from hydatid infected sheep. Sunita *et al.* (2011) by ELISA to detect antigen in sera and urines, reported a low sensitivity of 40% and 52%, respectively. Elawamy *et al.* (2020) reported a lower sensitivity and specificity of 81.5% & 80%, respectively, by sandwich ELISA to detect circulating protoscolices antigen in patients' sera. Differences may be due to the use of hyperimmune sera raised against AgB prepared from human hydatid cysts, whereas the others used non-human hydatid cysts. Also, difference in sensitivities may be due to different circulating antigens concentrations or formation of immune complexes from antigen-free sera (Siavashi *et al.*, 2005).

In the present study, IgG-pAb loaded to MNPs in NMB-sandwich ELISA to detect AgB in sera and urines may be the first trial. NMB-sandwich ELISA for AgB in sera was

positive in 17/18 CE patients, in 1/12 control, but none with other parasites with a sensitivity of 94.4% and a specificity of 95.8%. Also, using NMB-sandwich ELISA for AgB in urine samples was positive in CE patients (16/18), in control (1/12), but non in patients with other parasites with a sensitivity of 88.9% and a specificity 95.8%. AgB levels increased in sera and urines of CE patients than in those with other parasites and /or in healthy controls by NMB-sandwich ELISA. This agreed with Aly (2016) who by NMB-sandwich ELISA detect circulating protoscolices antigen in sera with 95.2% sensitivity and 95.5% specificity. Rashed *et al.* (2018) used nanogold dot-ELISA to detect protoscolices antigen in camels' sera got 94.5% sensitivity & 90% specificity. But, when they (2019) used nanogold dot-ELISA for human sera found 96.3% sensitivity & 94.4% specificity. Khanbabaie *et al.* (2019) by gold NPs conjugated anti-HCF-IgG for circulating antigen in human CE by lateral flow dipstick assay found 77.14% sensitivity and 82.35% specificity

The present NMB-sandwich ELISA gave better diagnostic performance in AgB detection in sera and urine samples of CE cases than sandwich ELISA. With sera diagnostic accuracy was 95.2% versus 90.5%, sensitivity was 94.4% versus 88.9% and specificity 95.8% versus 91.7%. But, sandwich ELISA for urines, diagnostic accuracy was 92.9 vs. 85.7%, sensitivity was 88.9% vs. 83.3% and specificity was 95.8% vs. 87.5%. Nanotechnology is more valuable in diagnosis human CE. This agreed with Elawamy *et al.* (2020), who reported that in detecting protoscolices antigen in human sera, nanogold sandwich ELISA was better than sandwich ELISA in sensitivity (96.3% versus 81.5%) & specificity (95% versus 80%). Besides, Jafari *et al.* (2022) reported that gold NP based nanobiosensor method for CE was 2.4 times high in diagnosis than in ELISA. Tran *et al.* (2022) added that NPs to ELISA enhanced sensitivity and time saving in CE diagnosis.

Besides, Nanogold ELISA for circulating

*Toxoplasma gondii* surface antigen in pregnant females' sera showed more sensitivity & specificity than sandwich ELISA (El-Kholy *et al*, 2020). Gold NPs to diagnose toxoplasmosis by rapid immunochromatographic test for antigen in sera gave sensitivity and specificity of 100% & 96.7%, respectively (Khamisi *et al*, 2020). Aly (2020) found that NMB-ELISA was a better assay than traditional sandwich ELISA in diagnosing *S. haematobium* antigen in patients with increased sensitivity from 85 % to 95%, & specificity from 88.2% to 92.6%, respectively. Castro-Sesquen *et al*. (2014) reported that gold NPs as better diagnostic test for many parasites in detecting their excreted antigen in urine. Alnasser *et al*. (2016) detected *Plasmodium vivax* DNA in urine by using gold and silica NPs colorimetrically with 84% & 97% sensitivity and specificity, respectively. Aly *et al*. (2018) with Silica NPs in nano-sandwich ELISA diagnosed *T. gondii* antigen in urine

In the current work, the AgB detection was more satisfactory in sera than urine samples by ELISA. This agreed with Sunita *et al*. (2011), reporting better specificity. Also, the present sensitivities agreed with Chaya and Parija (2013), who reported a high sensitivity for hydatid antigen in sera than urine, and added that lower antigenic quantity excreted in urine decreased sensitivity.

The present study recorded false positive AgB in sera and urine of patients with other parasites by sandwich ELISA and NMB-sandwich ELISA, cross reactivity was with *H. nana* and *E. vermicularis*. Also, Elawamy *et al*. (2020) reported cross-reactivity in sera of patients infected with *H. nana*, *E. vermicularis* and *Fasciola gigantica*. Others reported cross reactivity between echinococcal antigens, *Taenia solium* and *Ascaris lumbricoides* (Sunita *et al*, 2011), *Toxocara* species (Khalilpour *et al*, 2014) and *Taenia saginata* (Khanbabaie *et al*, 2019). Cross reactivity may be attributed to common antigens shared between *Hymenolepis* and *Echinococcus* (Sadjjadi *et al*, 2009), and/or undiagnosed hydatid cyst, in patient suffered from mixed

infection with either *H. nana* or *E. vermicularis*.

### Conclusion

NMB-sandwich ELISA gave a highly sensitive and specificity to detect *E. granulosus* AgB in patients' sera and urine samples, improving MNPs in sandwich ELISA.

Antigen detection in urine is recommended for CE diagnosis especially in areas without imaging techniques.

*Authors' contribution:* All authors equally contributed in both the practical and theoretical study

*Authors' declaration:* Authors stated that neither have conflict of interest nor received fund.

### References

- Abaza, SM, 2016:** Applications of nanomedicine in parasitic diseases. PUJ 9, 1:1-9.
- Abdi, J, Kazemi, B, Mohebbali, M, Bandehpour, M, Rahimi, MT, Rokni, MB, 2010:** Gene cloning, expression and serological evaluation of the 12-kDa antigen-B subunit from *Echinococcus granulosus*. Ann. Trop. Med. Parasitol. 104, 5:399-407.
- Alnasser, Y, Ferradas, C, Clark, T, Calderon, M, Gurbillon, A, Gamboa, D, et al, 2016:** Colorimetric detection of *Plasmodium vivax* in urine using MSP10 oligonucleotides and gold nanoparticles. PLoS Negl. Trop. Dis. 10, 10:0005029. doi.org/10.1371/journal.pntd.0005029
- Aly, IR, 2016:** Effective diagnosis of hydatidosis (*Echinococcus granulosus*) by immunomagnetic bead ELISA technique using paramagnetic nanoparticles. Int. J. Infect. 45:150. doi.org/10.1016/j.ijid.2016.02.362.
- Aly, IR, 2020:** Efficacy of iron oxide nanoparticles in diagnosis of schistosomiasis. AIMJ 2: 219-24
- Aly, IR, Zalat, R, El Aswad, BE, Moharm, I M, Masoud, BM, Diab, T, 2014:** Novel nanomagnetic beads based-latex agglutination assay for rapid diagnosis of human schistosomiasis *haematobium*. Trop. Med. Int. Hlth. 7, 12:977-83.
- Aly, IR, Taher, EE, Gehan, EL, Sayed, HE, Mohammed, FA, Hamad, RS, et al, 2018:** Advantages of bioconjugated silica-coated nanoparticles as an innovative diagnosis for human toxoplasmosis. Acta Trop. 177:19-24.
- Ayong, LS, Tume, CB, Wembe, FE, Simo, G, Asonganyi, T, Lando, G, et al. 2005:** Development and evaluation of an antigen detection dip-

- stick assay for the diagnosis of human onchocerciasis. *Trop. Med. Int. Hlth.* 10, 3:228-33.
- Bhattacharyya, A, Kamran, M, Ejazi, A, Das, S, Didwania, N, et al, 2022:** Revealing a novel antigen repressor of differentiation kinase 2 for diagnosis of human visceral leishmaniasis in India. *Pathogens* 11, 2:120-30.
- Bradford, MM, 1976:** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72(1-2):248-54.
- Brunetti, E, White, AC, 2012:** Cestode infestations: Hydatid disease and cysticercosis. *Infect. Dis. Clin. North Am.* 26, 2:421-35.
- Cakir, O, Sade, R, Alper, F, 2021:** Radiological imaging of pericardial hydatid cyst. *Rev. Soc. Bras. Med. Trop.* 54:e0753-2020. Online 2021
- Castilho, M, Laube, T, Yamanaka, H, Alegr-et, S, Pividori, MI, 2011:** Magneto Immunoassays for *Plasmodium falciparum* histidine-rich protein 2 related to malaria based on magnetic nanoparticles. *Analyt. Chem.* 83, 14:5570-7.
- Castro-Sesquen, YE, Gilman, RH, Galdos-Cardenas, G, Ferruffino, L, Sanchez, G, et al, 2014:** Use of a novel Chagas urine nanoparticle test (Chunap) for diagnosis of congenital *Chagas* disease. *PLoS Negl. Trop. Dis.* 8, 10:e3211. doi: org/10.1371/journal.pntd.0003211
- Chaya, DR, Parija, SC, 2013:** Evaluation of a newly designed sandwich enzyme linked immunosorbent assay for the detection of hydatid antigen in serum, urine and cyst fluid for diagnosis of cystic echinococcosis. *Trop. Parasitol.* 3, 2: 125-8.
- Chenthamarakshan, V, Padigel, UM, Ramaprasad, P, Reddy, MVR, Harinath, BC, 1993:** Diagnostic utility of fractionated urinary filarial antigen. *J. Biosci.* 18, 3:319-26.
- Crowther, JR, 1995:** Indirect ELISA. In: *ELISA Theory and Practice*. MIMB V. 42: Humana Press, New Jersey, USA.
- Elawamy, WE, Rashed, SM, Nassr, ME, Shalash, IR, Ali, NS, et al, 2020:** A pioneer usage of nanogold sandwich ELISA in the detection of hydatid antigen in humans as a tool for improvement of serodiagnostic testing. *Res. Squa.* Article ID: PPR149265 doi:10.21203/rs.2.21283/v2.
- El-Ghareeb, AS, Waked, NM, Al-Feky, HM, 2016:** Clinical and parasitological studies on pulmonary and hepatic hydatid cysts in hospitalized children and adults. *JESP* 46, 1:9-18.
- El-Kholy, WA, Shaheen, HA, Baiuomy, IR, El-Ahl, SA, 2020:** Evaluation of the diagnostic performance of Nano gold beads-based ELISA for detection of circulating *Toxoplasma* surface antigen grade III (SAG3) in serum samples of infected cases. *J. Adv. Med. Res.* 1, 1:32-8.
- Garcia, LS, 2016:** Tissue cestodes: Larval forms. In: *Diagnostic Medical Parasitology*. 6<sup>th</sup> ed.: ASM Press, Washington DC.
- Garvey, JS, Cremer, NE, Sussdor, DH, 1977:** Ammonium sulfate precipitation. In: *Methods in Immunology*, 3<sup>rd</sup> ed., Benjamin Reading, USA.
- Gholami, S, Tanzifi, A, Sharif, M, Daryani A, Rahimi, MT, et al, 2018:** Demographic aspects of human hydatidosis in Iranian general population based on serology: A systematic review and meta-analysis. *Vet. World* 11, 10:1385-96.
- González, A, Sánchez-Ovejero, C, Manzano-Román, R, González, M, Delgado, JM, et al, 2018:** Evaluation of the recombinant antigens B2t and 2B2t compared with hydatid fluid in IgG-ELISA and immunostrips for diagnosis and follow up of CE patients. *PLoS Negl. Trop. Dis.* 12, 9:e0006741. doi.org/10.1371/journal.pntd.
- Hasan, SA, 2015:** Review on nanoparticles: Their synthesis and types. *Res. J. Recent Sci.* 4:1-3.
- Haun, JB, Yoon, T, Lee, H, Wielder, R, 2010:** Magnetic nanoparticle biosensors: Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 22, 3:291-304.
- Heikal, EA, El-Lessy, FM, 2021:** Demographic aspects of human hydatidosis in Egypt. *Egypt. J. Hosp. Med.* 85, 2:3706-11.
- Jafari, F, Maghsood, AH, Fallah, M, Jalilvand, A, Matini, M, et al, 2022:** Design of highly sensitive nano-biosensor for diagnosis of hydatid cyst based on gold nanoparticles. *Photodiagnosis Photodyn. Ther.* 38: 102786.
- Jiang, L, Zhang, YG, Liu, MX, Feng, Z, 2012:** Analysis on the reactivity of five subunits of antigen B family in serodiagnosis of echinococcosis. *Exp. Parasitol.* 131, 1:85-91.
- Khalilpour, A, Sadjjadi, SM, Moghadam, ZK, Yunus, MH, Zakaria, ND, et al, 2014:** Lateral flow test using *Echinococcus granulosus* native antigen B and comparison of IgG & IgG4 dipsticks for detection of human cystic echinococcosis. *Am. J. Trop. Med. Hyg.* 91, 5:994-9.
- Khamsi, HM, Javad-Seyyed, S, Gholamzad, M, 2020:** Evaluation of a newly designed immunochromatographic test using gold nanoparticles and recombinant antigen gra7 for rapid diagnosis of human toxoplasmosis. *Iran. J. Med. Microbiol.* 14, 1:101-15.
- Khanbabaie, S, Riazi, M, Chang, CH, Yunus,**

- MH, Noordin, R, 2019:** Lateral flow dipstick antigen assay for human cystic echinococcosis. *Acta Trop.* 190:171-6.
- Laemli UK, 1970:** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 5259:680-5.
- Mahmoud, MS, Abou Gamra, MM, 2004:** Alkaline phosphatase from *Echinococcus granulosus* metacestodes for immunodiagnosis of human cystic echinococcosis. *J. Egypt. Soc. Parasitol.* 34, 3:865-79.
- Mahmoud, MS, Ezzat, HM, 1999:** Detection of hydatid antigen in human cyst fluid by reverse latex agglutination test. *J. Egypt. Soc. Parasitol.* 29, 3:805-15.
- Mamuti, W, Sako, Y, Nakao, M, Xiao, N, Nakaya, K, et al, 2006:** Recent advances in characterization of *Echinococcus* antigen B. *Parasitol. Int.* 55:S57-62.
- Manzano-Roman, R, Sanchez, C, Hernandez-Gonzalez, A, Casulli, A, Siles-Lucas, M, 2015:** Serological diagnosis and follow-up of human cystic echinococcosis: A new hope for future? *Biomed. Res. Int.* Article ID 428205. doi.org/10.1155/2015/428205.
- McKinney, MM, Parkinson, A, 1987:** A simple non-chromatographic procedure to purify immunoglobulins from serum and ascites fluid. *J. Immunol. Meth.* 96, 2:271-8.
- Mihmanli, M, Idiz, UO, Kaya, C, Demir, U, Bostanci, O, et al, 2016:** Current status of diagnosis and treatment of hepatic echinococcosis. *World J. Hepatol.* 8, 28:1169-81.
- Moradi, M, Meamar, AR, Akhlaghi, L, Roozbehani, M, Razmjou, E, 2019:** Detection & genetic characterization of *Echinococcus granulosus* mitochondrial DNA in serum and formalin-fixed paraffin embedded cyst tissue samples of cystic echinococcosis patients. *PLoS One* 14, 10:e0224501. doi.org/10.1371/journal.pone.022451
- Nasrieh, MA, Abdel-Hafez, SK, 2004:** *Echinococcus granulosus* in Jordan: Assessment of various antigenic preparations for use in the serodiagnosis of surgically confirmed cases using enzyme immuno assays and the indirect haemagglutination test. *Diagn. Microbiol. Infect. Dis.* 48, 2:117-23.
- Nguyen, MD, Tran, HV, Xu, S, Lee, TR, 2021:** Fe<sub>3</sub>O<sub>4</sub> Nanoparticles: Structures synthesis magnetic properties surface functionalization and emerging applications. *Appl. Sci.* 11, 23: 11301. doi.org/10.3390/app112311301.
- Pakala, T, Molina, M, Wu, GY, 2016:** Hepatic echinococcal cysts: A review. *J. Clin. Transl. Hepatol.* 4:39-46.
- Paredes, A, Sáenz, P, Marzal, MW, Orrego, MA, Castillo, Y, et al, 2016:** Anti-*Taenia solium* monoclonal antibodies for the detection of parasite antigens in body fluids from patients with neurocysticercosis. *Exp. Parasitol.* 166:37-43.
- Parija, SC, Ravinder, PT, Rao, KS, 1997:** Detection of hydatid antigen in urine by counter-current immunoelectrophoresis. *J. Clin. Microbiol.* 35, 6:1571-4.
- Peralta, JM, Cavalcanti, MG, 2018:** Is POCC-CA a truly reliable test for schistosomiasis diagnosis in low endemic areas? Trace results controversy. *PLoS Negl. Trop. Dis.* 12, 11:0006813. doi.org/10.1371/journal.pntd.0006813
- Rahimi, H, Sadjjadi, S, Sarkari, B, 2011:** Performance of antigen B isolated from different hosts and cyst locations in diagnosis of cystic echinococcosis. *Iran. J. Parasitol.* 6:12-9.
- Rashed, SM, Nasr, ME, Bayoumi, IR, Ali, NS, Mohamed, WE, et al, 2018:** Evaluation of nanogold particles-based enzyme-linked immunosorbent assay for detection of hydatidosis. *Benha Med. J.* 35, 2: 134-8.
- Rashed, SM, Nasr, ME, Shalash, I, Ali, N, Kishik, S, et al, 2019:** *Echinococcus granulosus* protoscolex antigen used in serodiagnosis of hydatidosis by nano-gold dot-ELISA. *PUJ* 12, 2: 110-5.
- Rogan, MT, Craig, PS, Zeyhle, E, Romig, T, Lubano, GM, et al, 1991:** Evaluation of a rapid dot-ELISA as a field test for the diagnosis of cystic hydatid disease. *Trans. R. Soc. Trop. Med. Hyg.* 85, 6: 773-7.
- Rouhi, S, Nakhli, R, Tarmidi, M, Moutaj, R, Elmezzouari, E, 2022:** Diagnosis efficacy of serological test in echinococcosis cysts: A retrospective study. *Saudi J. Med.* 7, 2:109-12.
- Sadjjadi, SM, Sedaghat, F, Hosseini, SV, Sarkari, B, 2009:** Serum antigen and antibody detection in echinococcosis: Application in serodiagnosis of human hydatidosis. *Korean J. Parasitol.* 47, 2:153-7.
- Salama, AA, Othman, AA, Zayed, HA, 2014:** Cystic echinococcosis in the middle region of the Nile Delta, Egypt: Clinical and radiological characteristics. *Egypt. J. Radiol. Nucl. Med.* 45, 3:641-9.
- Sarkari, B, Rezaei, Z, 2015:** Immunodiagnosis of human hydatid disease: Where do we stand? *World J. Methodol.* 5, 4:185-95.
- Sharafi, AC, Kheirandish, F, Valipour, SM,**

**Saki, M, Nasiri, E, et al, 2018:** Sero-epidemiology of human cystic echinococcosis among nomads of Lorestan province. Iran. Arch. Clin. Infect. Dis. 13, 3: 8-11.

**Shirazi, S, Madani, R, Hoghooghi, RN, Ranjbar-Bahadori, S, 2016:** Isolation and purification of *Echinococcus granulosus* antigen B from hydatid cyst fluid using three different methods. Arch. Razi. Inst. 71, 2:103-8.

**Siavashi, MR, Taherkhani, H, Rezaei, K, Razavi-Deligani, MR, Assmar, M, 2005:** Comparison of dot-ELISA and sandwich ELISA diagnostic tests in detection of human hydatidosis. Iran. Biomed. J. 9, 2:91-4.

**Sunita, T, Dubey, ML, Khurana, S, Malla, N, 2011:** Specific antibody detection in serum, urine and saliva samples for the diagnosis of cystic echinococcosis. Acta Trop. 101, 3:187-91.

**Tamarozzi, F, Covini, I, Mariconti, M, Narra,**

**R, Tinelli, C, et al, 2016:** Comparison of the diagnostic accuracy of three rapid tests for the serodiagnosis of hepatic cystic echinococcosis in humans. PLoS Negl. Trop. Dis. 10, 2: Article ID: 0004444. doi.org/10.1371/journal.pntd.

**Tijssen, P, Kurstak, E, 1984:** Highly efficient and simple methods to prepare peroxidase & active peroxidase-antibody conjugates for enzyme immunoassays. Anal. Biochem. 136, 2:451-7.

**Tran, HV, Ngo, NM, Medhi, R, Srinoi, P, Liu, T, et al, 2022:** Multifunctional iron oxide magnetic nanoparticles for biomedical applications: A review. Materials 15, 2:Article ID: 503. doi: 10.3390/ma15020503.

**Zhang, W, Wen, H, Li, J, Lin, R, McManus, P, 2012:** Immunology and immunodiagnosis of cystic echinococcosis: An update. J. Immunol. Res. Art. ID: 101895. doi.org/10.1155/101895

### Explanation of figures

Fig. 1: SDS-PAGE analysis of AgB (stained with Coomassie blue) showed AgB bands at molecular weights of 8-12, 16 & 24 kDa

Fig. 2: Plot diagram showing OD readings (a) for serum samples (b) for urine samples sandwich ELISA

Fig. 3: Pilot diagram showed OD readings (a) of serum samples and (b) of urine samples by NMB-sandwich ELISA

Fig. 4: ROC curve for detection of AgB by sandwich ELISA (red line) & NMB-sandwich ELISA (green line) in serum samples.

Fig. 5: ROC curve for detection of AgB by sandwich ELISA (red line) & NMB-sandwich ELISA (blue line) in urine samples.

