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A PIONEER USAGE OF GOLD NANOPARTICLES IN SANDWICH ELISA BASED ON CIRCULATING HYDATID ANTIGEN AS A TOOL FOR IMPROVEMENT OF SERODIAGNOSTIC TESTING

By
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Abstract

Human cystic echinococcosis is a zoonosis occurring due to dogs handling and exposure to Echinococcus granulosus ova in their stools being an accidental intermediate host. Serology remains the only sure and important tool to diagnose this disease. We aimed to improve the detection of circulating antigen by sandwich ELISA through its binding to gold nanoparticle and proving the role of minute nanoparticles in serodiagnosis. Gold nanoparticles (AuNPs) were employed for the capture of the antigens of protoscolices (pAg) in the patient's serum by sandwich ELISA. Cross-reactivity for antigens from *Hymenolepis nana*, *Entrobius vermicularis*, and *Fasciola gigantica* was ruled out by using anti-protoscolices polyclonal IgG antibodies (ppAb). Antigen of sonicated protoscolices which were removed from camel lung cysts, was purified and injected to a New-zealand white rabbit giving ppAb which then loaded on AuNPs being used as a diagnostic indicator for circulating pAg by both sandwich ELISA and nanogold sandwich ELISA techniques. Nanogold sandwich ELISA was able to give positive results with 96.3% of hydatid patients and 5% of non-hydatid patients while sandwich ELISA showed 81.4% and 20% positive cases of the same groups respectively. The sensitivity and specificity of nanogold sandwich ELISA were 96.3% & 95% against 81.5% & 80% for those of sandwich ELISA respectively. The conjugation of AuNPs to anti-Echinococcus IgG antibodies seems to be beneficial to increase PPV, NPV, and efficiency and reduce the overall cost of the assay as less antibody was needed.

Keywords: Hydatidosis, Protoscolices antigen, polyclonal IgG, Gold nanoparticles, Sandwich ELISA

Introduction

Cystic echinococcosis (CE) is endemic in many countries, including the Mediterranean basin, Latin America and Central Asia (Salama *et al*, 2014). In Egypt, CE is an endemic disease in animals (Omar *et al*, 2013) and man (Ibrahim and Morsy, 2020) Diagnosis of CE depended on clinical pictures and serodi agnosis and the development of reliable serodiagnostic technique was a must (Khanbabaie *et al*, 2019). Monitoring of hydatid fluid to react against IgG antibodies was used for hydatidosis diagnosis but of less value as the antibodies persist for long time in sera as well as of false positive and negative results (Hernández-González *et al*, 2018). Clinical hydatidosis diagnosis was vague and not accurate in diagnosis due to cross-clinical pictures with other hepatic parasites (El-Sayed *et al,* 2019), hence always supported by imaging and serodiagnosis but depending on immunodiagnostic tests to detect anti-hydatid antibodies gave less specificity and sensitivity in infected cases (Koura *et al*, 2015).

Due to lack of target antigen standardization in hydatid infections which is generally formed of crude antigen preparation of hydatid fluid inside cysts, there was an urgent need for the development of new tools among many immunodiagnostic tests which are commercially available to overcome this lack (Pagnozzi *et al*, 2016). The purified hydatid cyst fluid antigen was reliable in hydatidosis serodiagnosis compared to whole hy-

datid cyst fluid (Iraqi, 2016).

The use of nanotechnology in immunodiagnostic studies gave high sensitivity in detection of target analyzing specific to pathogens and micro-organisms with the advantages of nanomaterial properties allowed rapid detection even in small sample size (Aly *et al*, 2013). The nanoparticles as labels facilitated test proved useful to detect infectious agents with low costs than already used technologies (Abraham *et al*, 2008).

Antigen detection in hydatidosis became useful for serodiagnosis and also can be used for post-treatment follow up hence it improved the diagnostic abilities when used together with antibody detection tests. Latex agglutination test was used as a diagnostic test for CE and the revealed results were functi onalized as a simple and rapid diagnostic tool to confirm that a suspected cyst being hydatid one (Chandrakesan and Parija, 2003). Sandwich ELISA with paramagnetic nanoparticles provided an extraordinary tool for improving the sensitivity of hydatidosis antigen detection thus making benefits of the high binding capacity of magnetic beads in enhancing the antigen detection sensitivity (Koura *et al*, 2015). Lateral flow dipstick was developed to detect three types of hydatid antigen including: hydatid cyst fluid, native antigen B and recombinant antigen B, thus after a series of optimizations a combination of anti-native antigen B-IgG and gold-conjugated anti-hydatid cyst fluid IgG was used to develop lateral flow dipstick assay (Khanbabaie *et al*, 2019). A number of recombinant antigens mainly derived from hydatid fluid improved performances and standardizations of the serological tests, among them B2t & 2B2t antigens (Hernández-González *et al*, 2018). A new recombinant fusion protein was developed using coding sequence of antigen B2t and two sequences of EPC1 antigen evaluated by indirect ELISA test for sera from patients with hepatic CE (Fathi *et al*, 2018).

Covalent conjugation of antibodies to gold nanoparticles has more advantages over passive adsorption with higher stability, less co st, and reproduced conjugated antibodies reliable quantitation of analyses for best sensitivity (Hermanson, 2008).

The present work aimed to improve the capacity of sandwich ELISA in detection of hydatid protoscolices antigen in human sera by labeling anti-*Echinococcus* IgG polyclonal antibody on gold nanoparticles.

Materials and Methods

Protoscolices antigen production: Deposit of hydatid fluid from camel lung cysts containing protoscolices was exposed to freezing and thawing 3 times and use 10 times of its volume of 0.15 M phosphate buffer saline (PBS) to wash, pH 7.2 then sonicated by 150 W ultrasonic disintegrator and the sonicate was sedimented at 10000g for 30 minutes, the supernatant was split into aliquots and stored at -20^{0}C (Rafiei and Craig, 2002).

pAg purification by diethyl-amino-ethyl-sephadex (DEAE-Sephadex A50) chromategraphy: Removal of cross-reactive proteins and host components from *Echinococcus* antigens was purification (Jeyathilakan *et al*, 2014). DEAE Sephadex A 50 chromatography was used to purify the camel hydatid cysts antigens based on their charges, thus DEAE group maintained positive charges were neutralized by negative chloride charges (Smith *et al*, 1993). Anion exchange chromatography was done by adding 0.5gm DEAE Sephadex A 50 powder (Pharmacia, Uppsala, Sweden) to 200ml of 0.5M Tris-HCl buffer (Bio-Rad) adjusted at pH 7 slowly for 1-2days at 4^{o}C the Sephadex was swollen to 22ml beads. In a 30x2.5 column, swollen beads suspension was poured using a glass rod to avoid air bubbles trapping, and covered by binding buffer (20mM Tris-HCl). Hydatid antigen sample was dialyzed versus binding buffer allowed to penetrate beads, washed by eluting buffer (20mM Tris-HCl/150mM NaCl) and serum sample protein was calculated by collecting 10 fractions each in 2ml using spectrophotometer (Perkin Elmer Lambda 1 A). Absorbance at 280nm for each fraction was measured and

peak fractions of high absorbance were pooled together and the protein content was estimated by BioRad protein assay (Sadjjadi *et al*, 2009).

Indirect ELISA was used to assess the pAg reactivity (Engvall and Perlmann, 1971) against diluted human sera to confirm hydatidosis by C.T, Sonar, IHAT, and surgical biopsy (G1 individuals). Characterization of protoscolex antigen by SDS-PAGE showed three differ-rent protein bands represented purified protoscolex antigen. Anti-*E. granulosus* IgG ppAb was purified with 12.5% SDS-PAGE and antibody was other proteins free (Koura *et al*, 2015). Absorbance was measured at 492nm by ELISA reader (Bio-Rad microplate reader, Richmond Co., Wilmington, USA), and positivity cutoff point was the mean optical density reading.

Immunization of a rabbit and construction of ppAb: A New Zealand white male rabbit parasites free was purchased from Rabbit Research Unit, Faculty of Agriculture, Cairo University. Experiment duration was 4 weeks under standard laboratory care at 21°C, 16% moisture, filtered drinking water with salt (1cm/5liter) & vitamins (1cm/10liter), and diet contained 15% protein, 3% fat & 22% fiber. A rabbit was immunized by I.M. injection of a priming dose of 1 mg purified pAg mixed with a similar volume of complete Freund's adjuvant (*Sigma*), then a first booster dose of 0.5mg pAg was given two weeks after priming dose and two booster doses of 0.5mg pAg at weekly intervals (Fagbemi, 1995). Three days after last immunization, the blood sample was taken and centrifuged at 4000g for 15min. and serum IgG ppAb was fractionated and kept at -20^{o}C. Protein content was estimated by Bradford dye-binding procedure using color change of Coomassie Brilliant Blue G-250 dye by reference to a standard curve consisting of known concentrations of purified protein by using a protein assay kit (Bradford, 1976). Rabbit was exposed to euthanasia by I.V. injection of phenobarbital sodium at experimental end it suffered from severe pain during experimentation and not re-used again.

Isolation of rabbit ppAb: As a result of polar and ionic groups of proteins, their solutions form hydrogen bonds in water and adding highly charged ions such as ammonium sulfate, to compete with proteins to bind water and precipitated water molecules from proteins (Nowotny, 1979). Saturated ammonium sulfate solution $(NH_4)_2SO_4$ was mixed with rabbit serum, centrifuged at 3500g for 20min. at 4^{o}C in cooling centrifuge (Heraues), supernatant was discarded and repeated precipitations, final precipitate was dissolved in 0.01M PBS, pH 7.2 & $(NH_4)_2SO_4$ was dialyzed against 0.01M PBS, pH 7.2 for 72 hrs to get pure protein (Nowotny, 1979). The protein content in isolated IgG ppAb after ammonium sulfate treatment was determined by dye-binding procedure (Bradford, 1976).

Labeling of ppAb with Horse Radish Peroxidase (HRP): IgG ppAb molecules were conjugated to HRP (*Sigma*) enzyme exploiting its glycoprotein nature, and enzyme saccharide residues were oxidized by sodium periodate to give aldehyde groups that reacted with IgG molecule amino groups and led to Schiff bases production reduced to high molecular weight conjugate (Wisdom, 2009). Labelling was done on two successive days (Tijssen and Kurstak, 1984) on 1st day, 5mg of HRP was dissolved in 1.2 ml distilled water (dist. H_2O) then 0.2ml sodium periodate was added for 20min. at room temperature and dialysis against 1 mM sodium acetate buffer, pH 4 at 4^{o}C overnight to give solution (A). 5mg/ml of IgG ppAb was dialyzed against 0.02M carbonate buffer, pH 9.6 at 4^{o}C overnight to give solution (B). On 2nd day, solution (A) was mixed with solution (B) at room temperature for 2hrs and 0.1ml of sodium borohydride was added to the mixture, finally dialyzed against 0.01M of PBS, pH 7.2 at 4^{o}C overnight and the resulting conjugate was stored at -20^{o}C till used.

Synthesis of antibody-AuNPs conjugates: AuNPs (40nm/particle) were purchased in form of gold HCl solution with $3.08x10^{8}$ pa-

rticles/ml (Nano Tech. Co, Egypt). Loading of IgG ppAb on AuNPs (Hermanson 2008), all glass wares were soaked with Aqua Regia (PanReac, Germany) for overnight and on next day rinsed with tap water, the hydrogen tetrachloroaurate III ($HAuCl_4$) solution was vortexed and 250ml of it was added in a round bottom flask, condensed for 1hr, refluxed using a sand bath, then 25ml of 38.8mM sodium citrate were added and the flask was left to reflux again for 15 min. and stored in a dark place. Functionalization of 30ml of AuNPs solution with 45µl of 1mM mercaptoundecanoic acid (MUA) in ethanol (Sigma, Aldrich, Germany) overnight at 4°C and AuNPs concentration was determined before and after functionalization by UV/vis spectrophotometry using Beer's law (Zhou *et al*, 2009). Covalent Conjugation of ppAb with AuNPs-MUA was done by adding 5ml of AuNPs-MUA to 2ml of ppAb in presence of N-hydroxy Succinimide/1-Ethyl 3-Dimethylaminopropyl Carbodiimide (NHS/EDC) as covalent cross-linkers (Fluka, Germany) to obtain powerfully built AuNPs-MUA conjugates, by adding 5 ml of ppAb-AuNPs conjugates to 5ml of a mixture (5mM sodium phosphate buffer= pH 7, 1.2mM NHS & 2.8mM EDC), left overnight at 4°C to elicit electrostatic binding between ppAb and AuNPs-MUA (Mukher- jee *et al*, 2005).

Quantitation of protein after conjugation of ppAb with AuNPs: Based on Bradford dye-binding procedure, quantitation of protein content in ppAb-AuNPs conjugates was done by preparing standards of protein samples in form of serial dilutions of bovine serum albumin (BSA) with dist. H_2O as (BSA: 1, 5, 10, 15, 25, 50 & 80µl against dist. H_2O: 99, 95, 90, 74, 50 & 20µl respectively). The preparing standards ppAb-AuNPs conjugated samples in form of conjugates with dist. H_2O (conjugates 3, 10, 15 & 20µl against dist. H_2O: 97, 90, 85 & 80µl respectively) and 5ml of diluted dye (phosphoric acid and methanol) was mixed with both preparations and the color absorbance was measured at 595nm for each standard tube being planned on standard curve for protein content measurement calculation.

Patients sampling: Blood samples were kindly collected from human cases, Benha University Hospital, Benha Teaching Hospital, Kasr Eleni University Hospital, Aboelresh Pediatrics Hospital, Ain Shams University Hospital, and Theodore Bilharz Institute Hospital from March 2016 to October 2017 and manipulated in immuno-parasitology department of TBRI, Giza.

Samples 5ml were divided into 3 groups: G1: 27 blood samples from proved hydatid osis patients by US, CT, IHAT and cyst surgical excision. G2: 20 blood samples from *H. nana*, *E. vermicularis*, and/or *F. gigantica* infected patients and hydatidosis-free (cross-reactivity group). G3: 20 blood samples from apparently normal individuals; workers and official hospital personnel (control group). Samples were allowed to clot for 2hrs at room temperature to separate serum and kept at-20°C until used.

Demographic data: Medical sheets were collected about age (from 11 to 60 years), sex (male or female), residence (rural or urban), and occupation. Clinical presentation (symptoms, signs and organs affected) and personal hygiene (washing hands, vegetables & contact with dogs) data were recorded in addition to data from patients' sheets as cyst size, treatment, IHAT laboratory result, a titer above 1/160 was considered positive.

Surgically removed cysts from different organs (liver, spleen, and lung) of patients attended the above-mentioned hospitals, were sent to the pathology department of TBRI for histopathological examination of the cyst wall and hydatid fluid.

Detection of pAg in serum samples by sandwich ELISA: Coating of polystyrene microtiter plates wells (96-flat bottom wells, M 129A Dynatehc, Telangana, India) with 100 µl of a 20µg/ml concentration of purified IgG ppAb and incubated at room temperature for overnight, then washing 3 times with 0.1 M PBS/T, and pH was maintained at 7.4. Free sites in wells were blocked with 200µl/

well of BSA, pH at 7.4 for 2hrs at 37°C, and washed 3 times with buffer. Sera, each of 100µl/well were added to wells, incubated for 2hrs at 37°C, and then wells were washed 3 times. IgG ppAb conjugated with HRP in PBS/T (1/10µg/ml) was added by 100µl/ well for 1hr at room temperature, then wells were washed 3 times with buffer. Substrate solution (one O-phenylene diamine-dihydrochloride OPD tablet dissolved in 25ml of 0.05M phosphate citrate buffer) was added by 100µl/well for 30min. in dark at room temperature, 50µl/ well of 8N H_2SO_4 was added to stop enzyme-substrate reaction and absorbance was measured at 492nm by the ELISA reader (Richmond, CA, USA). According to the manufacture's protocol, antigen titer below 0.26 was negative and above 0.26 was positive; cut-off value was mean OD of negative controls + 2SD.

Detection of circulating pAg in sera by a nanogold sandwich ELISA: Coating of polystyrene microtiter plates wells colored white for optical absorbance, was done with 100 µl of 20µg/ml concentration of purified IgG ppAb conjugated with AuNPs representing a capture antibody, diluted in 0.1M carbonate buffer, pH 9.6 for an overnight at room temperature, and then washed 3 times with 0.1 M PBS/T, pH 7.4. Blocking of wells was done by 100µl/ well 0.1 BSA for 2hrs at 37°C, and washed 3 times with PBS/T. Tested sera (100µl) were pipetted into wells, incubated for 2hrs at 37°C, and washed 3 times and continued as above.

Statistical analysis: Data were tabulated and analyzed using SPSS (statistical package for social science) version 16. Significance was adopted at $P < 0.05$. Chi-square test (X^2) and fisher exact test (FET) were used for groups comparing categorical data. ROC curve assessed validity of diagnostic sensitivity, specificity and predictive values (Gonzalez-Sapienza *et al*, 2000). Probability: if $P > 0.05$ (non-significant), if $P \leq 0.05$ (significant) and if $P < 0.01$ (highly significant). Accuracy was detected by agreement percentage between sensitivity and specificity, positive and negative predictive values.

Ethics approval: The Scientific Research Ethical Committee, Faculty of Medicine, Benha University (2016) approved the work. Before taking samples, a written informed consent was obtained from all patients. The animal involved was in line with the Ethical Committee of Theodor Bilharz Research Institute approval (TBRI) and under the ethical guidelines by the Ethical Committee of the Federal Legislation and NIH Guidelines.

Results

Hydatid cyst wall showed protoscolices, germinal layer and laminated layer while the fluid showed scattered hooks, multiple invaginated protoscolices with hooks and evaginated protoscolices appearing with the suckers, rostellum with its hooks and body region (Fig. 1).

Hydatid infection in males (51.9%) was higher than in females (48.1%), with highest ages were 21-30 years with a total of 37.03% in both sexes and lowest infected ages were 11-20 years and 51-60 years with a total of 3.7% (Tab. 1).

Rural areas cases were higher (70.37%) than urban ones (29.63%), as to animal contact the patients were 59.26%, and those eating unwashed raw vegetables were 55.56%. Infected farmers were (48.15%). IHAT showed positivity (1/160 or more) in 51.9% of 25 infected cases and 2 cases were IHAT negative (Tab. 2).

Main symptom was right hypochondriac pain with (77.78%) liver affection followed by (14.81%) spleen affection, and (7.41%) lung affection (Tab. 3).

Sandwich ELISA in G1 showed positivity in 22 sera (81.48%) with significant difference by using the nanogold sandwich ELISA technique the positivity in hydatidosis group reached to 26 sera (96.3%) with significant difference. In G2 (cross-reactivity group) sandwich ELISA was positive (20%), but nanogold sandwich ELISA was (5%) positive (Tab. 4). Sensitivity of nano-gold sandwich ELISA was higher than sandwich ELISA in detection of protoscolices antigen

(96.3% vs. 81.5%), validity tests were higher in nanogold sandwich ELISA than in sandwich ELISA; specificity (95% vs. 80%), positive predictive value (96.3% vs. 84.6%), negative predictive value (95% vs. 76.2%) and accuracy (95% vs. 80.9%), as (Fig. 2).

Table 1: Age and sex distributions of hydatid infected human cases:

Ages in years	Male		Female		Total No.	Total Percentage	FET test	P-value
	No.	Percentage	No.	Percentage				
11-20	1	3.70%	0	0%	1	3.7%	3.05	0.68
21-30	6	22.2%	4	14.8%	10	37%		
31-40	2	7.4%	4	14.8%	6	22.2%		
41-50	4	14.8%	5	18.51%	9	33.3%		
51-60	1	3.7%	0	0%	1	3.7%		
Total	14	51.85%	13	48.15%	27	100		

Table 2: Personal history and IHA test results of hydatid infected human cases:

Personal data		No.	%	Total hydatidosis cases
Residence	Urban	8	29.63	
	Rural	19	70.37	27
Animal contact (dogs & cats)	Yes	16	59.26	
	No	11	40.74	27
Consumption of unwashed raw vegetables	Yes	15	55.56	
	No	12	44.44	27
Occupation	Farmer	13	48.15	
	House wife	8	29.63	
	Employee	5	18.52	27
	Student	1	3.70	
IHA test	Positive	14	51.9	
	Negative	11	40.74	27
	Not requested	2	7.41	

Table 3: Clinical data of different affected organs in hydatid infected human cases:

Affected organ	No.	Percent	Symptoms	No
Liver	21	77.78	Asymptomatic	1
			Right hypochondriac pain	14
			Abdominal colic	3
			Epigastric pain	3
Spleen	4	14.81	Asymptomatic	3
			Left hypochondriac pain	1
Lung	2	7.41	Cough, expectoration & wheezes, chest pain	2
Total	27	100		27

Table 4: Sandwich ELISA and nanogold sandwich ELISA in detection of protoscolices antigen:

Groups	Sandwich-ELISA				Nano-gold sandwich-ELISA			
	+ve cases		-ve cases		+ve cases		-ve cases	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Hydatidosis (n=27)	22	81.48	5	18.52	26	96.3	1	3.7
Cross-reactivity (n=20)	4	20	16	80	1	5	19	95
Control group (n=20)	0	0	20	100	0	0	20	100
X2 test, P-value	36.37, <0.001**				59.05, <0.001**			
Sensitivity	81.5%				96.3%			
Specificity	80%				95%			
PPV	84.6%				96.3%			
NPV	76.2%				95%			
Accuracy	80.9%				95%			

Discussion

In the present study, infected males were higher than females. This agreed with Salama *et al.* (2014) who found that hydatidosis was higher in males (72.03%) than in females (27.96%), due to many exposure to infective sources as dogs, soil (Rakhshanpour *et al.* (2012) and/or eating raw vegetables or consumption of contaminated water (Khazaei *et al*, 2016),

In the present study, hydatidosis was high among age group 21-30 years. Salama *et al.* (2014) found that 4th decade (46.7%) was the highest; Chalechale *et al*. (2016) found

that the age group 21-40 years (41.2%) was the most affected, and Abdulhameed *et al.* (2018) showed that the 3rd decade or youth group was highest (26.9%) CE infected one.

In the present study, high hydatidosis was in rural areas. This agreed with El-Shazly *et al.* (2007) in Egypt, Acosta-Jamett *et al.* (2014) in Chile, and Chalechale *et al.* (2016) in Iran.

In the present study, the liver was the commonest affected site. This agreed with Salama et al. (2014) in Egypt, Chalechale *et al.* (2016) in Iran, Also Abdulhameed *et al.* (2018) in Iraq particularly the right upper quadrant causing abdomen pain. El-Ghareeb *et al*. (2016) reported asymptomatic hepatic hydatidosis patients (20.5%) as well as pulmonary ones (21.7%), but El-Sayed et al. (2020) reported symptomatic lung patients presented with chronic cough (sometimes with accompanied with hemoptysis or evacuation of cyst material), chest pain, pleuritis or dyspnea, with more than one sign and symptoms in same patient mainly with lung infection. The clinical variations could due to variable sizes and sites of hydatid cysts exerting different mass effects on surrounding organs and complications (WHO, 2019).

The nanogold particles to detect ELISA antibodies increased sensitivity and specificity (Ciaurriz *et al*, 2017).

In the present study, ppAb were loaded on nanogold particles in sandwich ELISA as a new combination to diagnose hydatidosis circulating antigens, but lateral flow dipstick assay was used (Khanbabaie *et al,* 2019) to conjugate IgG fractions on gold nanoparticles. Dot-immunogold staining method detected antibodies against sheep hydatidosis by antigen B labeled with gold nanoparticles and analyzed by SDS-PAGE, nanoparticles gave a typical purple color by binding at immunoreaction site strip (Jahan *et al*, 2014).

In the present study, nanogold sandwich ELISA gave sensitivity & specificity; 96.3% & 95% versus 81.5% & 80% by sandwich ELISA. This agreed with Koura *et al.* (2015) who used paramagnetic nanoparticles to detect human protoscolices antigen and found that paramagnetic sandwich ELISA showed 95.2% sensitivity & specificity 95.5%, and ordinary sandwich ELISA showed sensitivity 90.48% & specificity 91.3%. The present result disagreed with Bauomi *et al.* (2015) who found 52.5% sensitivity by ELISA for human circulating protoscolices antigen.

In the present study, nanogold sandwich ELISA positive predictive value (96.3%) was higher that sandwich ELISA (84.6%). Predictive value in immunodiagnosis proved effective in epidemiology as low value indicated confidence lack (Allsopp *et al*, 1987).

In the present study, two *Hymenolepis nana* cases, one *Entrobius vermicularis* case & one *Fasciola gigantica* gave false-positive results by sandwich ELISA (20%), but one *H. nana* case gave false-positivity by nanogold sandwich ELISA (5%). This was due to the common antigens shared between *Hymenolepis* and *Echinococcus* protoscolices antigens (Song *et al*, 2017). Sero-diagnosis hydatidosis gave poor clinical support due to cross-reactivity with other helminthes (Tawfeek *et al*, 2011). Cross-reaction was in few hydatidosis-free patients by indirect ELISA using antigen B (Sadjjadi *et al*, 2009). False-positivity was in taeniasis co-infected with hydatidosis (Mohammadzadeh *et al*, 2012). (Hernández-González *et al*, 2018) reported low cross-reactivity with alveolar hydatidosis patients with recombinant ELISA antigens, for antigen B2t, it gave (9.5%) least one & for 2B2t antigen was (16.7%) but highest one was with hydatid fluid immunochromatography (64.3%). But, Hadj Rabia *et al.* (2018) did not find cross-reaction among patients with toxoplasmosis and hydatidosis.

Conclusion

Labeling of gold nanoparticles to sandwich ELISA test improved its sensitivity and specificity to detect human hydatid protoscolices antigen in sera and raised clinical diagnostic confirmation. Nanogold sandwich ELISA improved the test predictive value and recommended in epidemiological study.

Competing interest: Authors declared that

they neither have conflict of interests nor received fund.

Author's contributions: All authors contributed to the design and organized the study. Amira SG Elghannam and Ibrahim RA Shalash collected specimens and contributed to the practical work. Samia M Rashed, Mona E Nasr, and Nagwa SM Ali shared in data analysis. Waleed E Elawamy shared in writing manuscript, experimental work and data analysis and contributed to the editing of manuscript for publication. All authors read and approved this manuscript.

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Explanation of figures

Fig. 1: Surgical removed: Associated keys/annotations: A) Multiple invaginated protoscolices with hooks. B) An invaginated protoscolex with hooks (red arrow). C) *E. granulosus* hooks. D) Hydatid cyst wall: laminated layer (red arrow), germinal layer (blue arrow) and scolices (green arrow). E) Evaginated protoscolex showing the suckers, rostellum with its hooks and body region (posterior part).

Fig. 2: ROC curve: Sensitivity and specificity of sandwich ELISA vs. nanogold sandwich ELISA for hydatidosis antigen in human cases.