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Evaluation of Nano Gold based ELISA for Detection of Hydatid Antigen in Serum Samples of Infected Subjects

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ABSTRACT

Background: Hydatid disease, or cystic echinococcosis (CE), is a significant zoonotic disease with a global distribution, posing a major public health concern in both human and veterinary fields.

Objective: This study aimed to evaluate the diagnostic efficacy of a Nanogold beads-based ELISA for detecting circulating protoscolex antigen (PS Ag) in human hydatidosis.

Material and Methods: The present study included 71 serum samples, categorized into three groups: group 1 (n=31) confirmed CE cases, group 2 (n=16) individuals with other parasitic infections, and group 3 (n=24) healthy controls. PS Ag was prepared, followed by purification of polyclonal antibodies, which were divided into three fractions. One of them was conjugated with horseradish peroxidase (HRP), and the second with gold nanoparticles, while the third remained unconjugated. PS Ag was detected using both Nano Sandwich ELISA (Nano-sELISA) and conventional Sandwich ELISA.

Results: Nano-sELISA demonstrated superior diagnostic performance compared to conventional Sandwich ELISA, with higher sensitivity (96.77% vs. 77.42%), specificity (93.75% vs. 75%), positive predictive value (PPV) (96.77% vs. 85.71%), negative predictive value (NPV) (93.75% vs. 63.16%), and diagnostic accuracy (95.75% vs. 76.6%).

Conclusion: The application of gold nanoparticles significantly enhanced the diagnostic accuracy of hydatid disease detection through improved sensitivity and specificity for circulating PS Ag. Nano-sELISA proved to be a promising and innovative diagnostic technique for human hydatidosis.

Keywords: Nano sandwich ELISA, hydatid disease, hydatidosis, protoscolex antigen.

INTRODUCTION

Cystic echinococcosis, also known as hydatidosis, is a parasitic disease induced by the larval form of the tapeworm *Echinococcus granulosus*. It is a major zoonotic disease and continues to be an endemic issue, posing a significant challenge to healthcare providers. It is engaged in a battle, with its influence deeply embedded in society [1].The disease is widespread in several regions of Egypt and poses a significant concern due to the rising morbidity and substantial economic impact [2]. Although there has been some progress in controlling echinococcosis, the disease is still main public health concern in several countries, where it is regarded as an emerging and re-emerging disease [3].

The disease is also endemic in Australia, Siberia, Indian subcontinent, South America, western China, Central Asia, eastern part of the Mediterranean region, sub-Saharan Africa, and northern and eastern Africa [4].

Hydatid cyst is a bladder-like cyst that develops in a variety of tissues and organs after oncospheres of *Echinococcus* grow[5]. Liver is the most affected organ, followed by spleen and lungs [1]. The disease can cause severe and fatal infections in humans [6].

Diagnosis of hydatid disease is based upon clinical suspicion [7]. Imaging is crucial in diagnosing, classifying, and assessing response to treatment of CE [8].

However, these methods have limitations, including the inability to distinguish abscesses from cysts, difficulties in identifying small-sized cysts, and challenges in diagnosing cysts located in the bone using ultrasonography. Therefore, early diagnosis should be confirmed by other tests that have to be of high specificity [9]. Serological tests such as indirect hemagglutination, double diffusion in agar and immunoelectrophoresis should be used to analyze the sera of patients. More recent sensitive tests are ELISA, direct immunofluorescence and immunoblotting test [10, 11].

The optimal serological test for diagnosis of hydatidosis is ELISA. The problem is that antibodies remain for years in patient's sera and it is not easy to differentiate between the acute and chronic phase of the disease or to follow up the patient after treatment or surgery [12] while some patients may fail to show a detectable immune response [11]. Systemic and local immunological profiles may indicate the parasite's ability to conceal its antigen from the host's immune system [13]. It should be noted that the asymptomatic presentation of CE is frequently more prevalent than expected**.** It is similar to the tip of iceberg, where only a small portion is visible, while the larger part remains hidden beneath the surface [1]. All these problems with serodiagnosis

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necessitate newly diagnostic immunological tests with a highly diagnostic performance.

Nano diagnostics refer to application systems, nanoscale materials, or devices, for diagnostic applications. It is an encouraging field, as enhanced techniques for clinical diagnostics are becoming available, offering greater sensitivity at reduced costs [14]. Biological tests that assess the presence or activity of specific analytes become faster, more sensitive, and more versatile when nanoscale particles are used as labels or tags, offering several benefits over traditional methods [15]. Nanoparticles have high surface areas and unique physicochemical properties that can be easily tuned, making them ideal candidates for developing biomarker platforms [15]**.** DNA-coated gold nanoparticles (NPs) have potential to create tests that offer higher sensitivity than traditional ELISA. When antigen is found in complex proteins, such as those in serum-containing culture media or crude body fluids, and analyzed through a sandwich assay, Nano conjugation generally detects antigen with 1–3 orders of magnitude higher sensitivity than traditional ELISA [16].

Gold nanoparticles have been employed in various studies targeting several parasites. Nano diagnostics apply nanotechnology in clinical diagnosis to meet demand for improved faster early detection specificity, and sensitivity. The vast surface area of nanomaterials allows for the binding of a high number of target-specific molecules, supporting ultrasensitive detection [17].

So, the present study aimed to evaluate the diagnostic efficacy of a Nanogold beads-based ELISA for detecting circulating protoscolex antigen (PS Ag) in human hydatidosis.

PATIENTS, MATERIAL AND METHODS

Study groups: The present analytical comparative study conducted during period from 2022 to 2024. The study included 71 cases that were divided into three groups:

Group 1(CE group): Included 31 cases of confirmed CE. They were collected from different provinces due to their rarity (Hepatogastroenterology and Infectious diseases department at Alzhraa University Hospital, Al-Azhar University, also, Tropical medicine, Surgery and Interventional Radiology departments at Kasr Al Aini hospital, Cairo University, Ain Shams University Hospitals, Zagazig University Hospitals, Clinical Pathology Department, Benha University Hospitals as well as Outpatient Clinics at Theodore Bilharz Research Institute (TBRI), Giza, Egypt). One case was obtained from Saudi Arabia. This group included 13 males and 18 females with age range from 10 to 70 years old.

Group 2 (infected control group): This group comprised 16 patients diagnosed with parasitic infections other than CE (*S. mansoni*, *Fasciola gigantica* and Hookworms). They were collected from the Outpatient Clinics at TBRI. The rationale for including patients infected with parasites other than *T. gondii* in our research was to prevent cross-reactivity in results.

Group 3 (Healthy controls group): This group included 24 healthy negative control adults who had not suffered from CE or any other disease in the recent past. Samples free from toxoplasmosis and other parasitic infections were used as negative controls to strengthen test's validity and reliability by offering a point of comparison.

Confirmation of hydatid cyst infection: Suspected cases of hydatid cyst infection were confirmed through:
1) PAIR technique and examination of hydatid sand.
2) Surgically removed cysts, and pathologically examined.
3) Radiological examination through ultrasonography and/or CT scan.

The present study was conducted at Department of Immunoparasitology and Immunology (TBRI) to assess the performance of Nano gold-based ELISA for detecting protoscolex antigen (PS Ag) in serum samples of infected individuals, comparing to sandwich ELISA method. Purified estimated protoscolex hydatid antigen was kindly supplied by the Department of Immunoparasitology and Immunology (TBRI). The origin of the antigen was from hydatid cysts removed from liver and lungs of infected slaughtered camels provided by an abattoir near Tokh Center [18].

Polyclonal antibodies (pAb) were produced following the method of **Guobadia and Fagbemi [19],** then purified from rabbit anti-*Echinococcus* IgG using the 50% ammonium sulfate precipitation technique [20], and further refined using the 7% caprylic acid method [21]. Once polyclonal antibodies were purified, the mixture was separated into three fractions. One fraction was conjugated with Horseradish peroxidase (HRP) was carried out [22], while another fraction of polyclonal antibodies was conjugated with gold nanoparticles [23]. Third fraction was left unconjugated. All 3 fractions were kept at -20°C until they were needed.

Sera were isolated and kept at -20°C until needed. Detection of PS Ag in the sera was performed using Sandwich ELISA, with a cutoff value of 0.36.
Detection of PS Ag in sera by Nano-sELISA was done. cutoff point = 0.302

Gold nanoparticles source: Gold nanoparticles were supplied by Nano Tech Egypt Company. (6 October City, Cairo, Egypt). The nanoparticles were delivered as

HAuCl4 solution (gold HCL solution) at concentration of 3.08x108 particles per milliliter. The particle size was 40 nanometers.

Reliability tests of obtained results [24]

1 Diagnostic sensitivity is determined as Sensitivity = TP / (TP + FN), where TP stands for true positives and FN stands for false negatives. A greater sensitivity percentage expresses greater number of positive results in individuals with the disease.

2. Diagnostic specificity is determined as Specificity = TN / (TN + FP), where TN stands for true negatives and FP stands for false positives. A higher specificity percentage reflects a greater number of negative results in healthy individuals.

3. percentage of positive results that are correctly identified as true positives, or the percentage of negative results that are accurately classified as true negatives.

PPV =TP/ (TP + FP), NPV =TN/ (TN + FN)

4. Diagnostic accuracy= TP + TN/ (TP + TN + FP+FN).

Ethical Considerations

This study was conducted following approval from the Research Ethics Committee, Faculty of Medicine, Al-Azhar University, Egypt. Written informed consent was obtained from all participants before their enrolment. The consent form clearly outlined their agreement to participate in the study and for the publication of anonymized data while ensuring the confidentiality and privacy of their personal information. This research was performed in accordance with the ethical principles of the World Medical Association's Declaration of Helsinki for studies involving human subjects.

Statistical Analysis: collected data were organized and statistically analyzed using Excel (Microsoft Office 2010) on a personal computer**.** Data were presented as mean ± standard deviation (Mean ± SD). mean values for each group were computed based on individual values of participants. Comparison between groups was conducted using the Student's t-test, with a significance level set at $P < 0.05$. The number of true-positive (TP) and true- negative (TN), and false-negative (FN) and false-positive (FP), test results were calculated. According to previous equations, specificity (SP), sensitivity (SN), diagnostic accuracy (AC), positive predictive value (PPV) and negative predictive value (NPV) Were determined and compared between the two tests.

RESULTS

When detecting PSAg using sandwich ELISA, group I revealed negative 7 samples (22.59 %), while 24 samples were positive (77.41%). In Infected control (group 2): 4 samples (25%) were identified as positive (2 cases infected with *Schistosoma mansoni*, one case with hook worm and one case with *Fasciola gigantica*), while the other 12 samples were negative. Samples from the healthy control group (Group 3) were negative.

Upon Recognition of PSAg in serum by Nano-sELISA, 30 samples in the first group were positive (96.8%), while one sample was negative (3.2%). In Infected control (group 2), only one sample was positive (infected with *Schistosoma mansoni*), while the other 16 samples were negative. All free samples Healthy control (Group3) were negative.

Upon identification of PS Ag in serum samples of CE cases in relation of infected control, Nano sandwich ELISA had the upper hand or attained Values greater than those obtained from the sandwich ELISA in relation to diagnostic accuracy (95.75% vs. 76.6%), specificity (93.75% vs. 75%); sensitivity (96.77% vs.77.42%); PPV (96.77% vs. 85.71%)and NPV (93.75 %vs. 63.16 %) In relation to the healthy control, Nano sandwich ELISA attained greater values than sandwich ELISA in relation to sensitivity (96.77% vs.77.42%); NPV (96 %vs. 77.42 %) and diagnostic accuracy (98.18% vs. 87.27%). Both techniques gave the same specificity and PPV (100%) **Table (2).**

Table 1: Mean Optical density reading for detection of PS Ag by sELISA and Nano-s ELISA among the studied groups

Groups	Total number	Sandwich ELISA	Nano sandwich ELISA	T test value	P- value
		OD (X ± SD)	OD (X ±SD)		
CE cases (Group 1)	31	0.661±0.350	1.381±0.951	2.3526	0.0001
Infected control (Group 2)	16	0.240±0.154	0.28275±0.124		
Healthy control (Group3)	24	0.237±0.041	0.203125±0.033		

X = mean, SD = standard deviation and p = probability value.

Table 2: Percent of different reliability tests of sandwich ELISA and Nano sandwich ELISA for detection of PS Ag in serum samples of CE cases (group 1) in relation to infected control (group 2) and healthy control (group 3).

Techniques	Group	Reliability diagnostic tests (%)				
		Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy (DA)
Sandwich ELISA	Infected control	77.42	75	85.71	63.16	76.6
	Healthy control	77.42	100	100	77.42	87.27
Nano sandwich ELISA	Infected control	96.77	93.75	96.77	93.75	95.75
	Healthy control	96.77	100	100	96	98.18

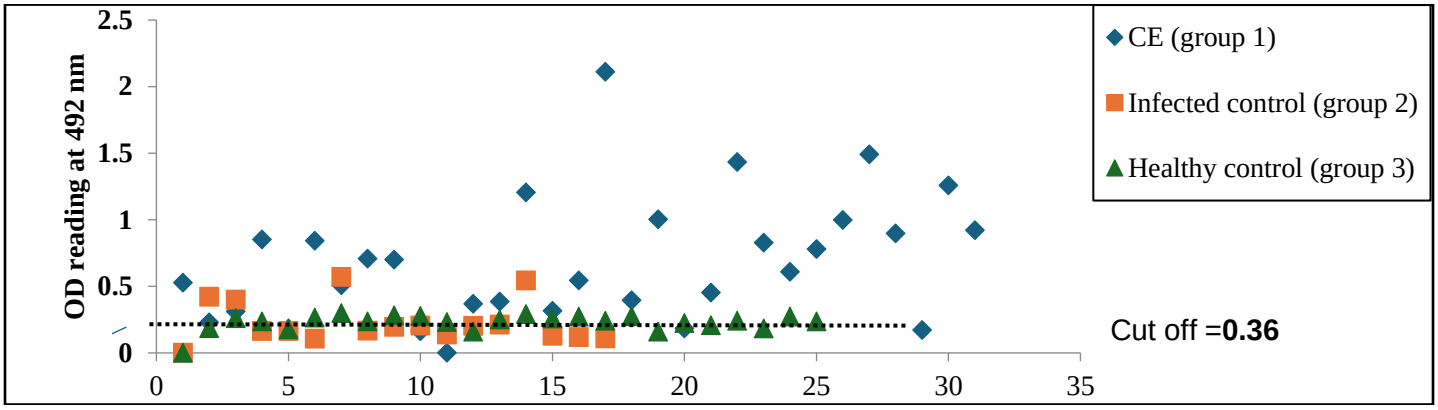

Figure (1): Optical density reading of individual data for members of different groups at 492nm for detection of PS Ag upon using sandwich ELISA

Figure 1 shows that upon using sandwich ELISA for detection of PS Ag in CE cases (group1), 24 out of 31 cases were +ve and 7 cases were negative. In group 2 (infected control), 4 cases were positive out of 16 (2 cases infected with *Schistosoma mansoni*, one case with hook worm and one case with *Fasciola gigantica*) while 12 cases were negative. In group 3 (healthy controls) all cases yielded negative results, with the determined cut-off value being 0.36.

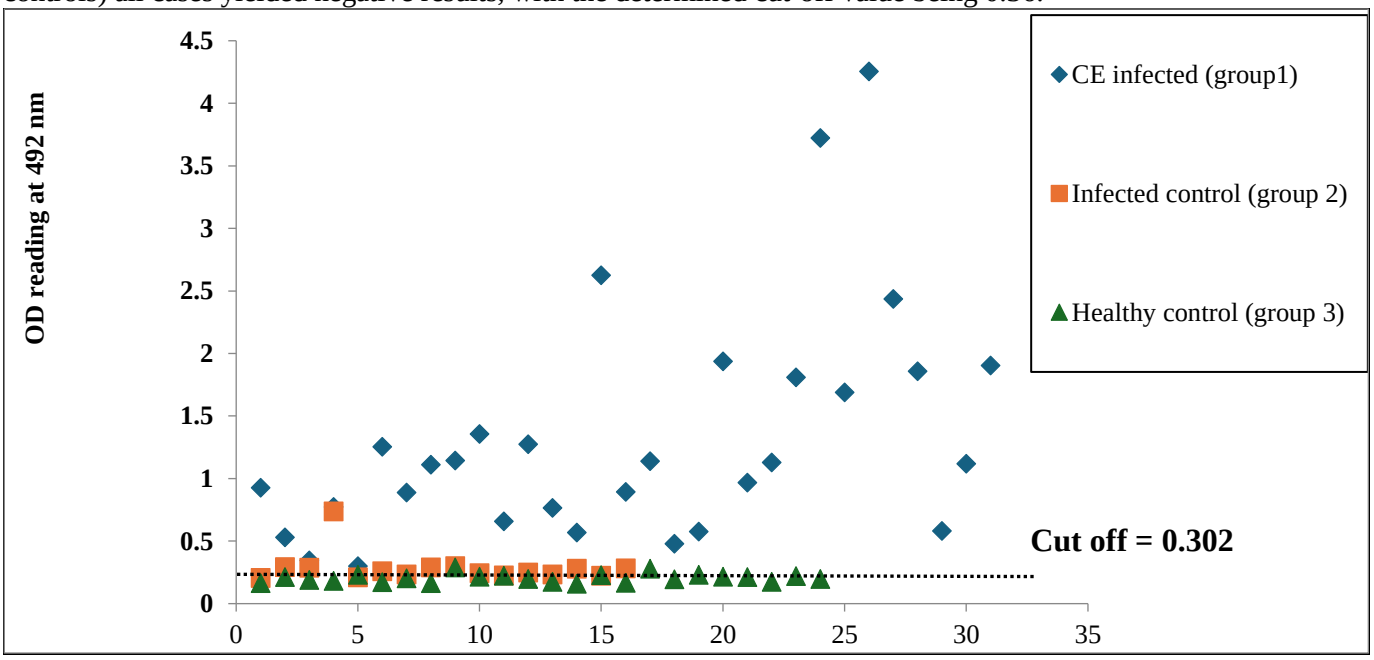

Figure (2): Optical density reading of individual data for members of different groups at 492nm for detection of PSAg after using Nano sandwich ELISA

Upon using Nano -sELISA for identification of PSAg, **Figure (2)**, revealed that 30 cases were positive out of 31 and one case was negative in group I while one case only was positive (infected with *Schistosoma mansoni*) in group 2. All cases in group 3, were negative. The calculated cut-off value was (0.302).

DISCUSSION

Cystic echinococcosis, also known as hydatid disease, is zoonotic disease of worldwide distribution that have a considerable impact on the economy and public health. The disease is caused by larval stage (metacestode) of parasite belonging to family Taeniidae and genus *Echinococcus* [25].

Various materials, such as silver, chitosan, and gold, have been employed in the synthesis of nanoparticles and are utilized in medical science for the treatment of certain infections [26].

The concept of conjugating nanoparticles (NPs) with parasite biomarkers is utilized in the diagnosis of numerous parasitic diseases [27].

Using of gold nanoparticles has become increasingly popular in scientific, industrial and medical fields. Nanoparticles have been employed in the fight against various parasites. Nano diagnostics refers to using of nanotechnology in clinical diagnosis aims to meet the demand for improved specificity, sensitivity, and faster early detection. Nanomaterials increase attachment of numerous target molecules for ultrasensitive detection because of their large surface area [17].

The aim of this study was to compare Nano-sELISA test with conventional sELISA in discovering PSAg in serum samples of confirmed CE cases. So results concerning the applications of both techniques on studied groups revealed that upon estimating the mean OD reading for detection of PS Ag in sera of confirmed CE (group 1), a higher significant value by Nano sandwich ELISA (1.381±0.951) was recorded compared to 0.661±0.350 by sandwich ELISA (Table 1) where $p < 0.005$.

The results of **Hassan** [28] match the current search results. The author compared diagnosis of hydatidosis in human sera using paramagnetic nanoparticles sandwich ELISA to conventional s ELISA for the identification of PS Ag antigen. OD values of *E. granulosus* infected group was 0.811 ± 0.113 while that of paramagnetic nanoparticles sandwich ELISA was 1.413±0.197.

On applying reliability tests on both techniques, Nano-sELISA and sELISA, the same superiority of Nano sandwich ELISA was recorded in the present study (Table 2). First, in relation to infected control, Nano sandwich ELISA had the upper hand as it attained greater values than sandwich ELISA in relation to specificity (93.75% vs. 75%); sensitivity (96.77% vs. 77.42%); diagnostic accuracy (95.75% vs. 76.6%); PPV (96.77% vs. 85.71%) and NPV (93.75 %vs. 63.16 %). In relation to the healthy control, Nano sandwich ELISA attained greater values than sandwich ELISA in relation to sensitivity (96.77% vs.77.42%); NPV (96 %vs. 77.42 %) and diagnostic accuracy (98.18% vs. 87.27%). Both techniques gave the same specificity and PPV (100%).

In accordance with the present results is **Hassan** [28] who compared diagnosis of hydatidosis in human sera using paramagnetic nanoparticles sandwich ELISA to conventional sELISA for the identification of PS Ag antigen. The author recorded 90.48% Sensitivity, 91.3% Specificity, 95% PPV and 91.3% NPV by using traditional sandwich ELISA while the corresponding values by paramagnetic nanoparticles sandwich ELISA were 95.2%, 95.5%, 97.6%, 95.5 respectively. This strongly confirms the superiority of nanotechnology.

El-Kholy *et al.* [29] study detected *Toxoplasma* **sAg** grade Three in human serum samples using both techniques, revealing that the sandwich ELISA exhibited a sensitivity of 92%. sensitivity, 84%. Specificity, 85.2% PPV, 91.3 % NPP and 88% diagnostic accuracy. By Nano sandwich ELISA the corresponding percentages were 90%, 96%, 95.7%, 90.6 % and 93% respectively. The difference in the sensitivity of Nano based technique versus that of **Hegazy *et al.*** [30] may be explained by the type of used antigen. For diagnosis of *Giardia lamblia* antigen in stool sample,

Moharam *et al.* [31] used conjugated anti-purified *G. lamblia* cyst antigen (PGA) with nanoparticles-s ELISA and compared technique with conventional sELISA. sELISA accomplished 132 positive predictive value (PPV) of 95.7% negative predictive value (NPV) of 88%, specificity of 92.5 % and sensitivity of 93%, corresponding values by Nano-sandwich ELISA were 95.8%, 95%, 97.2% and 92.6%, respectively showing higher values.

Comparison of application of Nano - ELISA to conventional ELISA in detection of infection of parasite is a goal of several researchers as well as current research. Among them is **Aly *et al.*** [32], who reported Nano diagnostic assay, using magnetic nanoparticles for detection of circulating microsomal fraction Ag for swift diagnosis of human schistosomiasis haematobium infection by magnetic microbeads based- sandwich ELISA. The sensitivity of assay was 96.5% and specificity was 96.3% but detection of the same antigen by traditional sandwich ELISA, sensitivity and specificity of was 88.2% & 87.3 % respectively.

Referring to the diagnosis of human toxoplasmosis gondii in serum samples, **Hegazy *et al.*** [30] offered a new antigen-capture immunoassay was developed, employing IgG polyclonal antibody-coated magnetic microbead nanoparticles for swift detection of circulating sAg 1. The authors found that Sandwich ELISA elicited NPV of 92.7%, PPV of 92%, a specificity of 92.7% and sensitivity of 92%, while Immunomagnetic bead-ELISA showed PPV (96%), NPV (98.1%),

specificity (96.4%) and sensitivity (98%) greater than that of **sELISA.**

Despite the promising diagnostic performance of Nano-sELISA, this study has some limitations. The sample size was relatively small, which may impact the generalizability of the findings. Additionally, cases were collected from multiple centers, potentially introducing variability in sample handling. Cross-reactivity with other parasitic infections, though minimized, remains a consideration. Further large-scale studies with diverse populations are needed to validate these findings and explore the clinical utility of Nano-sELISA in routine diagnostics.

CONCLUSION

The Nano sandwich ELISA test is more specific and sensitive than traditional sandwich ELISA in detecting PS Ag in serum samples of CE cases. For the identification CE disease, this study showed the excellent superiority of sandwich ELISA based on gold nanoparticles technique over the traditional sandwich ELISA in diagnosis of human echinococcosis.

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