Original Article

Evaluation of the Diagnostic Performance of Nano Gold Beads-Based ELISA for Detection of Circulating Toxoplasma Surface Antigen 3 (SAG3) in Serum Samples of Infected Cases

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ABSTRACT

Objective: This study aimed to evaluate the diagnostic performance of Nanogold beads-based ELISA in serodiagnosis of human toxoplasmosis based on detection of circulating Toxoplasma surface antigen grade 3 (SAG3).

Material and Methods: This study was conducted on 100 serum samples. They were divided into 3 groups: group (I): 50 samples with positive IgG antibodies for Toxoplasma gondii &group (II):30 serum samples harboring other parasites than T. gondii which was divided as following, ten infected with Schistosoma mansoni, ten infected with Giardia lamblia and ten samples infected with Entamoeba histolytica while group (III): 20 free samples from toxoplasmosis and other parasitic infections. Preparation of Toxoplasma surface antigen 3 (SAG3) was done. After purification of the polyclonal antibodies it fractionated into three fractions. Conjugation of one fraction with Horseradish peroxidase (HRP) was carried out then conjugation of other fraction polyclonal antibodies with gold nanoparticles. The 3rd fraction remains without conjugation. Detection of SAG3 in the sera by Sandwich ELISA and Nano sandwich ELISA was done.

Results: Detection of Toxoplasma surface antigen grade 3 in the serum samples by Nano-sandwich ELISA was higher than sandwich ELISA regarding specificity (96% vs. 84%); PPV (95.7% vs. 85.2%) and diagnostic accuracy (93% vs.88%). Sandwich ELISA was higher than Nano-sandwich ELISA regarding sensitivity (92% vs.90%) and NPV (91.3 %vs.90.6 %).

Conclusion: The use of gold nanoparticles showed the potential advantage of improving the diagnostic methods of toxoplasmosis based on detection of circulating Toxoplasma surface antigen grade 3 (SAG3). The Nano diagnostic assay significantly increased specificity, PPV and diagnostic accuracy. We applied this new technique for the first trial for diagnosis of human toxoplasmosis.

Key Words: Nano sandwich ELISA, toxoplasma gondii, toxoplasma surface antigen grade III.

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INTRODUCTION

Toxoplasma gondii (T. gondii) is an obligate intracellular parasite with world-wide distribution[1]. It has been estimated that one third of the world population has been infected[2] Toxoplasmosis in immune deficiency especially, it is recognized as an important opportunist that can cause some clinical manifestations such as encephalitis, but in most of cases is asymptomatic. This infection causes the death of 10% of people who suffer from AIDS in Europe, and 30% of people who suffer from AIDS in America[3- 5].

In response to infection with T. gondii, the host provokes a cellular immune reaction through helper T lymphocytes[6]. Upon cytokine stimulation of cerebral endothelial cells, released adhesion molecules enhance the organism invasion into the deep tissues[7]. It is well documented that T. gondii crosses the BBB, thereby gaining access to tissues where it most likely causes marked pathologic lesions[8]. The infection is generally asymptomatic in the immunocompetent individual[9].

The surface antigen 3 SAG3 and SAG1 are very similar to each other in structure and function, these proteins participate in cellular invasion and attachment[10] Both tachyzoites and bradyzoites, it anchored to the membrane via glycosyl phosphatidyl inositol groups. SAG3 is found in tachyzoites and bradyzoites. Mice immunized with recombinant SAG3 secrete significantly more specific IgG2a antibodies[11]. Antigen detection can detect low titers found in early acute infection or chronic infection and may help in diagnosing the compromised patient whom antibody titers are low or absent[12].

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An immunomagnetic bead-ELISA technique utilizing T. gondii IgG polyclonal antibodies coated with magnetic NPs to capture circulating surface antigen 1 gave better results than sandwich ELISA for diagnosis of human toxoplasmosis using SAG1 antigen[13].

Gold's nanoparticles have been used against different parasites in several studies. Nano diagnostics involve the use of nanotechnology in clinical diagnosis to meet the demands for increased sensitivity, specificity and early detection in less time. The large surface area of nanomaterial enables attachment of large number of target specific molecules of interest for ultrasensitive detection[14].

MATERIAL AND METHODS

This study is an analytical comparative study. Preparation of Toxoplasma surface antigen 3 (SAG3) was done according to Brooks *et al*[15]. Toxoplasma gondii RH strain tachyzoites; (from the Faculty of Veterinary Medicine, Cairo University, was used for Preparation of Toxoplasma antigen.

Production of polyclonal antibodies (pAb) was obtained according to Guobadia *et al*[16] followed by Purification of the rabbit's anti- Toxoplasma serum by 50% ammonium sulfate precipitation method[17] then purification by 7% caprylic acid method[18]. After purification of the polyclonal antibodies it fractionated into three fractions. Conjugation of one fraction with Horseradish peroxidase (HRP) was carried out then conjugation of other fraction polyclonal antibodies with gold nanoparticles. The 3rd fraction remains without conjugation. The three fractions were stored at -20 °C until used[19].

The serum samples enrolled in the present study were collected from pregnant females attending for ante-natal care in outpatients' clinic of Gynaecology and Obstetrics Department of Al-Zahraa Hospital Faculty of Medicine for Girls Al-Azhar University, Clinical Pathology Department of Said Jalal Hospital Faculty of Medicine Al-Azhar University, outpatients' clinic of Theodor Bilharz Research Institute (TBRI). The serum samples were collected during the period from March to September 2015.

This study was carried out on on 100 individuals as follows

- Group I: 50 samples with positive IgG antibodies for Toxoplasma gondii.
- Group II: 30 samples having other parasites than T. gondii: ten infected with S. mansoni, ten infected with G. lamblia and ten infected with E. histolytica. The idea behind including patients harboring other parasites than T. gondii in our study was to avoid cross-reactivity in the results.
- Group III: 20 free samples from toxoplasmosis and other parasitic infections served as negative control to improve the validity and reliability of the test by providing a comparison and benchmark. Sera were separated and stored at -20oC till required.

Detection of SAG3 in the sera by Sandwich ELISA was done[20]. The cutoff point =0.203.

Detection of SAG3 in the sera by Nano sandwich ELISA was done. The cutoff point =0.16.

Statistical Analysis

The data (positive results of sandwich and nano-sandwich ELISA for each group) were presented as mean ± standard deviation (Mean ± SD). Student's t-test: is a test of significance used for comparison between two groups having quantitative variables. Results were presented in tables and charts.

$$t = \frac{x - \bar{\mu}}{s/\sqrt{n}}$$

The data were considered significant if p values were equal to or less than 0.05 Statistical analysis was performed with the aid of the SPSS (Statistical Package for the Social Sciences) computer program (Windows ver. 7).

The number of true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) test results was calculated. According to the following equations, sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (AC) was calculated and compared between both tests.

$$\textbf{Sensitivty (\%)} = \frac{\text{No. of true positive results (TP)}}{\text{No. of true +ve results (TP) + No. of false -ve results (FN)}} \times 100$$

$$\textbf{Specificity (\%)} = \frac{\text{No. of true negative results}}{\text{No. of true -ve results (TN) + No. of false +ve results (FP)}} \times 100$$

$$\textbf{PPV (\%)} = \frac{\text{No. of true positive results (TP)}}{\text{No. of true +ve results (TP) + No. of false +ve results (FP)}} \times 100$$

$$\textbf{NPV (\%)} = \frac{\text{No. of true negative results (TN)}}{\text{No. of true -ve results (TN) + No. of false -ve results (FN)}} \times 100$$

$$\textbf{Diagnostic accuracy (\%)} = \frac{\text{(TP) + (TN)}}{\text{(TP) + (TN) + (FP) +(FN)}} \times 100$$

RESULTS

On detecting Toxoplasma (SAG3) by sandwich ELISA, the results were positive in46 samples (92%) of group I, while 4 samples were negative (8%). In group II (patient with other parasites): 8 samples were detected as positive (2 with Schistosoma mansoni,4 with Giardia lamblia and 2 with Entamoeba histolytica), while other 22 samples were negative. All free samples (group III) were negative.

On detecting Toxoplasma (SAG3) in the serum by Nano sandwich ELISA in group I the results were positive in 45 samples (90%) ,while 5 samples were negative (10%).In group II (patient with other parasites) only 2 samples were detected as positive (2 samples of patient with Giardia lamblia), while the other 28 samples were negative. All free samples (group III) were negative.

Detection of Toxoplasma surface antigen 3 in the serum samples by Nano sandwich ELISA was higher than sandwich ELISA regarding specificity (96% vs. 84%); PPV (95.7% vs. 85.2%) and diagnostic accuracy (93% vs. 88%). Sandwich ELISA was higher than Nano sandwich ELISA regarding sensitivity (92% vs.90%) and NPV (91.3 %vs. 90.6 %) (Table 1 & 2) (Figures 1a &1b).

Table 1: Comparison between sandwich ELISA and Nano sandwich ELISA techniques regarding positivity for Toxoplasma surface antigen grade 3 (SAG3) in serum samples of different studied groups

Groups		Sandwich ELISA		Nano sandwich ELISA	
		Positive samples (n)	% of positivity	Positive samples (n)	% of positivity
I	Toxoplasma gondii(n=50)	46	92%	45	90%
II	Other parasites	8	26.6%	2	6.66%
	Schitosoma mansoni(n=10)	2	20%	0	0%
	Giardia lamblia(n=10)	4	40%	2	20%
	Entamoeba histolytica (n=10)	2	20%	0	0%
III	Healthy control(n=20)	0	0%	0	0%

n =number.

Table 2: Comparison between mean and standard deviation of positive results of both sandwich ELISA and Nano sandwich ELISA for detection of SAG 3 level in Toxoplasma infected groups.

	M ± SD	T test	*P value*
Sandwich ELISA	(0.618±0.501)	1.633	< 0.05
Nano sandwich ELISA	(0.843± 0.78)		

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

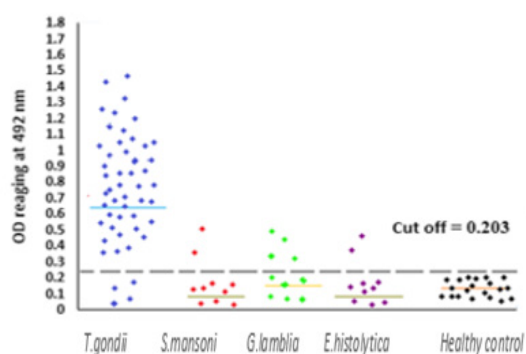

Fig. 1a: Scatter diagram of SAG3 in human sera by sandwich ELISA

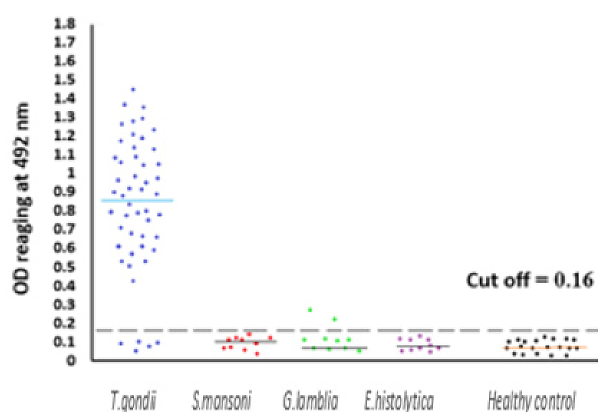

Fig. 1b: Scatter diagram of SAG3 in human sera by Nano sandwich ELISA

DISCUSSION

Toxoplasma gondii is a protozoan parasite that infects almost all warm-blooded animals, including humans, and is considered one of the most successful eukaryotic pathogens[1].

Different materials were used for nanoparticles synthesis and utilized in medical science and for treatment of some infections like silver, chitosan, gold, etc.[21]. The application of gold's nanoparticles nowadays turns into a popular option in industrial contexts, scientific and medical science. Gold's nanoparticles were used against different parasites. Nano diagnostics mean the use of nanotechnology in clinical diagnosis to solve the demands for increased sensitivity, specificity and early detection in less time. The nanomaterial enhances attachment of large number of target molecules for ultrasensitive detection due to their large surface area[14].

This study was to evaluate the diagnostic performance of a novel Nano gold beads-based ELISA in serodiagnosis of human toxoplasmosis based on detection of circulating Toxoplasma surface antigen 3 (SAG3).

In the present work Toxoplasma gondii RH strain tachyzoites were used for preparation of SAG3. Purification of SAG3was done by ion exchange chromatography on Sephadex A-50 followed by gel filtration chromatography on Sephacryl-S-200 high resolution (HR) column. The eluted proteins from gel filtration column chromatography was analyzed by 12.5% SDS-PAGE under reducing condition and stained with Coomassie blue stain. It showed bands at 56, 43 and 37 KDa.

Ibrahim *et al* demonstrated that characterization of the purified Toxoplasma tachyzoites antigen using SDS-PAGE, revealed many bands ranged from110 to 12 KDa with 5 foremost proteins of 95, 49, 38, 27 &12 KDa[22].

As regards to the present work, the total protein content of crud antigen was 7.5 mg/ml, while it was 5.4 mg/ml after purification by Diethylaminoethyl DEAE Sephadex A-50 ion exchange chromatography and dropped to 2.9 mg/ml after purification by gel filtration chromatography on Sephacryl-S-200 column as measured by Bio-Rad protein assay. Testing the antigenicity of the target antigen using serum of T.gondii infected samples was carried out by indirect ELISA, gave reaction with OD 1.305 while gave no cross reaction with the sera of samples with other parasites.

While the total protein content in our study of crude rabbit serum was 11.5 mg/ml. By 50% ammonium sulfate precipitation method, the protein content was 5.8 mg/ml, while following 7% caprylic acid precipitation method the protein content dropped to 3.9 mg/ml.

In the diagnosis of other parasitic diseases, the same concept of nanoparticles conjugation with the parasite biomarkers was used. In toxoplasmosis, the use of specific agglutination of antigen-coated gold nanoparticles in the detection of the corresponding antibody gave satisfactory agreement with ELISA results[23].

In this study, we developed a novel antigen-capture immunoassay based on IgG pAb coated with gold nanoparticles for the detection of circulating SAG 3 of T. gondii in serum samples which was used as a first trial for diagnosis of human toxoplasmosis.

On detecting in the present work, Toxoplasma (SAG3) by sandwich ELISA, the results were positive in46 samples (92%) of group I, while 4 samples were negative (8%). In group II (patient with other parasites): 8 samples were detected as positive (2 with Schistosoma mansoni,4 with Giardia lamblia and 2 with Entamoeba histolytica), while other 22 samples were negative. All free samples (group III) were negative.

On detecting Toxoplasma (SAG3) in the serum by Nano sandwich ELISA in group I the results were positive in 45 samples (90%), while 5 samples

were negative (10%).In group II (patient with other parasites) only 2 samples were detected as positive (2 samples of patient with Giardia lamblia), while the other 28 samples were negative. All free samples (group III) were negative.

In the present work detection of Toxoplasma SAG3 in the serum samples by sandwich ELISA revealed that the sensitivity, specificity, PPV, NPP and diagnostic accuracy percentage of sandwich ELISA was 92%, 84%, 85.2%, 91.3 % and 88% respectively.

Khanaliha *et al* 2014 reported that the use of recombinant SAG3 in diagnosis of toxoplasmosis by ELISA, the sensitivity of the assay was 95.4%, the specificity was 91.2% and the diagnostic accuracy was 94%. While when the serum samples were tested by SAG3-ELISA for IgM, the sensitivity was 17.9% and the specificity was 76.7%. This indicates efficacy of this antigen for detecting specific IgG antibodies and low reactivity of this antigen with IgM Anti-Toxoplasma antibodies as was expected for this common tachyzoite and bradyzoite antigen[11].

Hegazi *et al* 2015 developed a novel antigen-capture immunoassay based on IgG polyclonal antibodies conjugated magnetic micro bead nanoparticles for the rapid detection of circulating surface antigen grade1 (SAG1) of Toxoplasma gondii in human serum samples. Sandwich ELISA elicited a sensitivity of 92%, a specificity of 92.7%, a positive predictive value (PPV) of 92%, and a negative predictive value (NPV) of 92.7%. Immunomagnetic bead-ELISA showed sensitivity (98%), specificity (96.4%), PPV (96%), and NPV (98.1%) which were higher than that of sandwich ELISA[13].

Finally, the differences between the results of the present work and the others may be due to the difference in the method of antigen preparation, the strain of T. gondii, the number of positive samples, other parasites and control groups.

CONCLUSIONS

This new technique was used as for a first trial for diagnosis of human toxoplasmosis. The use of gold nanoparticles showed a potential advantage of improving the diagnostic methods of toxoplasmosis based on detection of circulating Toxoplasma surface antigen 3 (SAG3). The Nano diagnostic assay significantly increased specificity, PPV and diagnostic accuracy. Further studies are recommended for confirmation of the potential advantage of gold nanoparticles.

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CONFLICTS OF INTEREST

There are no conflicts of interest

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الملخص العربى

تقييم القدرة التشخيصية لجزيئات النانومن الذهب المضافة الى اليسا لتحديد المستضد السطحى من النوع ٣ لتوكسوبلازما في عينات السيرم للحالات المريضة

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الأهداف: تهدف هذه الدراسه الي تقييم القدره التشخيصيه لجزيئات النانومن الذهب كاضافه لـ اليسا المستخدمه في تشخيص مرض التوكسوبلازما في السيرم عن طريق البحث عن المستضد السطحى من النوع٣

المرضي والطرق: أجريت هذه الدراسه علي ١٠٠عينة من السيرم وتنقسم الي ٣ مجموعات المجموعه الأولى: ٥٠عينه ايجابيه من حيث الاجسام المضاده للتوكسوبلازما جوندى، المجموعه الثانيه: ٣٠ عينه من السيرم لمرضى بطفيليات اخري وكانت مقسمه علي النحو التالي ١٠ عينات لمصابين بمرض بلهارسيا مانسوني، ١٠ عينات لمصابين بطفيل جيارديااللمبيه و١٠ عينات لمصابين بطفيل انتاميبا هستولوتيكا بينما المجموعه الثالثه تتضمن ٢٠ عينه من السيرم لاشخاص لا يعانون من التوكسوبلازما او اى طفيل اخر. وقد تم اعداد المستضد السطحى للتوكسوبلازما من النوع٣. ثم بعد تنقية الاجسام المضاده للتوكسوبلازما تم تقسيمها الى ثلاثة أجزاء. تم اقران الجزء الأول مع انزيم بيروكسيداس المعلم واقران الجزء الثانى من الاجسام المضاده متعددة الأوجه مع جزيئات النانو من الذهب بينما الجزء الثالث لم يتم اقرانه بشئ. تم الكشف عن المستضد السطحى للتوكسوبلازما من النوع٣ في عينات السيرم المختلفة بواسطه نانوساندويتش مقايسة الممتز المناعى المرتبط بالانزيم (ELISA) وساندويتش مقايسة الممتز المناعى المرتبط بالانزيم (ELISA)

النتائج: أظهرت النتائج ان نانوساندويتش(ELISA)كانت الأفضل من حيث الخصوصية (٩٦% مقابل ٨٤%) وعدد الحالات الايجابيه الصحيحة(٩٥.٧% مقابل ٨٥.٢%) ودقة التشخيص(٩٣% مقابل ٨٨%).بينما كان ساندويتش (ELISA) الأفضل من حيث الحساسيه(٩٢% مقابل ٩٠%) وعدد الحالات السلبيه الصحيحة (٩١.٣% مقابل ٩٠.٦%).

الاستنتاجات: اظهراستخدام جزيئات النانو من الذهب الميزة المحتملة لتحسين أساليب التشخيص لمرض التكسوبلازما بالكشف عن المستضد السطحى من النوع٣ حيث ان التشخيص باستخدام جزيئات النانو يزيد بطريقة واضحة في الخصوصية وعدد الحالات الإيجابية الصحيحة والدقة التشخيصية.قد تم تطبيق هذه التقنية الجديدة عبر هذه الدراسة لتشخيص مرض التوكسوبلازما.