Acta Tropica 177 (2018) 19–24

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Advantages of bioconjugated silica-coated nanoparticles as an innovative diagnosis for human toxoplasmosis

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ARTICLE INFO

Keywords:
Human toxoplasmosis
Toxoplasma gondii
Nanotechnology
Diagnoses

ABSTRACT

Nanotechnology is a promising arena for generating new applications in Medicine. To successfully functionalised nanoparticles for a given biomedical application, a wide range of chemical, physical and biological factors have to be taken into account. Silica-coated nanoparticles, (SiO2NP) exhibit substantial diagnostic activity owing to their large surface to volume ratios and crystallographic surface structure. This work aimed to evaluate the advantage of bioconjugation of SiO2NP with PAb against *Toxoplasma* lyzate antigen (TLA) as an innovative diagnostic method for human toxoplasmosis. This cross-sectional study included 120 individuals, divided into Group I: 70 patients suspected for *Toxoplasma gondii* based on the presence of clinical manifestation. Group II: 30 patients harboring other parasites than *T. gondii* Group III: 20 apparently healthy individuals free from toxoplasmosis and other parasitic infections served as negative control. Detection of circulating Toxoplasma antigen was performed by Sandwich ELISA and Nano-sandwich ELISA on sera and pooled urine of human samples. Using Sandwich ELISA, 10 out of 70 suspected *Toxoplasma*-infected human serum samples showed false negative and 8 out of 30 of other parasites groups were false positive giving 85.7% sensitivity and 84.0% specificity, while the sensitivity and specificity were 78.6% and 70% respectively in urine samples. Using Nano-Sandwich ELISA, 7 out of 70 suspected *Toxoplasma*-infected human samples showed false negative results and the sensitivity of the assay was 90.0%, while 4 out of 30 of other parasites groups were false positive giving 92.0% specificity, while the sensitivity and specificity were 82.6% and 80% respectively in urine samples. In conclusion, our data demonstrated that loading SiO2 nanoparticles with pAb increased the sensitivity and specificity of Nano-sandwich ELISA for detection of *T.gondii* antigens in serum and urine samples, thus active (early) and light infections could be easily detected.

1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan pathogen that can infect any warm-blooded animal species and virtually any nucleated cell types, which makes toxoplasmosis a highly widespread parasitic infection with up to one-third of the world's human population has been estimated to be infected(Dubey et al., 2013). The infection is acquired by ingestion of *T.gondii* tissue cysts exist in the under-cooked meat or by ingestion of infectious oocysts passed into the environment by infected cat feces (Tenter, 2009).

Toxoplasmosis has a little clinical manifestation in immune-competent individuals. However, it can be severe or fatal in infected children during intra-uterine development and immunocompromised patients(Robert-Gangneux and Dardé, 2012).In pregnant women, early diagnosis of active toxoplasmosis can prevent severe congenital infection in the developing fetus(McLeod et al., 2009; Nimri et al., 2004).

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http://dx.doi.org/10.1016/j.actatropica.2017.09.024
Received 23 April 2017; Received in revised form 22 September 2017; Accepted 26 September 2017
Available online 28 September 2017
0001-706X/

Thus, there have been many studies to innovate a more reliable and efficient technique for rapid diagnosis of this parasitic infection (Ricciardi and Ndao, 2015; Rodrigues et al., 2015). The classical diagnosis of toxoplasmosis relies on serological methods and detecting specific immunoglobulin antibodies. However, these methods have poor efficiency, especially in neonates and immune-deficient patients (Béraud et al., 2009; Porter and Sande, 1992). Several investigations have emphasized on detecting *T.gondii* antigens in urine for diagnosis of the acute stage of toxoplasmosis(Garcia, 2007; Nimri et al., 2004; Rodrigues et al., 2015). In some investigations, demonstration of *T.gondii* antigens in serum samples of experimentally infected animals suggests that antigens are detectable in the acute phase of toxoplasmosis(Hafid et al., 2001; Liu et al., 2015). Recognition of *T.gondii* antigens in urine samples is a valuable and non-invasive procedure for the diagnosis of acute infection(Shojaee et al., 2007). Nanotechnology is of great use for medical diagnosis, and various nanoparticles have exhibited tremendous potential for detecting disease markers, precancerous cells, a fragment of viruses and other indicators. Various metal coating and metal nanoparticles functionalized with different biomolecules have been found useful in identifying specific proteins, antibodies and other disease indicators(Sato-Berrú et al., 2013). This work aimed to evaluate TLA (Toxoplasma Lyzate Antigen) as a diagnostic marker for human toxoplasmosis. A novel nano-diagnostic assay based on bioconjugation of polyclonal antibody (pAb) IgG with SiO2NP for detection of circulating TLA in sera and urine of patients suspected infected with *T. gondii*. Exploring an effective early diagnosis might be the keystone of the prevention and eradication of the disease. Our goal was achieved through detection of TLA in infected human sera and using the sandwich and Nano-sandwich ELISA techniques.

2. Materials and methods

2.1. Preparation of silica nanoparticles

A modified method of Sato-Berrú et al. (2013) with different ethanol/water proportion was used for the synthesis of colloidal silica spheres. Tetraethyl orthosilicate (TEOS) (99.99%, Aldrich), ethanol (99.99%, Aldrich), ammonium hydroxide solution (28%, Aldrich) and tri-distilled water were used. The following procedure prepared the silica spheres nanoparticles: (50-x) ml of ethanol, x ml of water (x = 0, 1, 5, 10, 15, 20, 30, 40 and 45), 3.0 ml of ammonium hydroxide solution and 1.5 ml of TEOS were mixed. Thin stirred for 1 h to form a homogeneous solution. All the experiments were conducted at room temperature. The formation of SiO2 nanoparticles is evidenced by the appearance of a faint blue color in the reaction medium. The characterization was carried out by transmission electron microscopy and dynamic light scattering (DLS). TEM measurements have been conducted on a JEOL JEM-200CX transmission electron microscope at an acceleration voltage of 60 kV. Samples were moved onto carbon-coated copper grids and dried in air at room temperature for 24 h before observation. DLS measurements were carried out on a Nano- sizer system, Nano ZS90 model.

2.2. Preparation of antigen

2.2.1. Toxoplasma lyzate antigen (TLA)

Tachyzoites of the highly virulent RH strain of *T.gondii* were maintained by intraperitoneal passage in albino mice. Parasites were passed ten times through a 27-gauge needle to release the intracellular tachyzoites, harvested in RPMI-1640 medium, filtered on 3 μm polycarbonate membranes (Nucleopore, Pleasanton, Ca, USA), and washed twice in the same medium containing 100IU/ml penicillin and 100 μ g/ml streptomycin. The concentration of tachyzoites was determined after adequate dilution in RPMI-1640 medium by enumeration in a Neubauer counting chamber at 400X magnification(Daryani et al., 2003) [15]. The concentration of tachyzoites and contaminating host cells was calculated with an improved Neubauer counting chamber (Count numbers of tachyzoites at 1/1000 dilution and cellular contamination at 1/10). Carry out further washes as required to reduce cellular contamination to < 0.5% host mononuclear cells and < 0.25% for red blood cells. Centrifuge the fluid at 500 **g** for 15 min. The pellet was solubilized by adding distilled water, and then the solution was supplemented with a protease inhibitor, 5 mM phenyl methyl sulphonyl fluoride (PMSF). Rupture the tachyzoites by freezing and thawing five times, then sonicated for 20 s at 4 °C then centrifugation at 10,000 **g** for 30 min at 4 °C. The protein content of TLA was determined using Bradford method then stored at–20 °C (El-Malky et al., 2005; Wagner et al., 2015).

2.3. Preparation of polyclonal antibodies

Two New Zealand white male rabbits, weighing approximately 2 kg, were used for immunization. Prior clearance from the local ethics committee was obtained. Toxoplasma Lyzate Antigen (1 mg/0.1 ml) of *T. gondii* combined with 0.1 ml complete Freund's adjuvant were injected into each rabbit by intramuscular (i.m.) injection at multiple sites, followed by three subsequent boosts at 8-days intervals with antigens combined with incomplete Freund's adjuvant. The total dose of immunization for each rabbit was 3.5 mg. Then, antiserum was collected from an ear vein and checked once a week after the final booster dose. When the detected level of the antibody became stable by indirect ELISA, sera were collected from the hearts of the rabbits. The collected serum was treated with 50% saturated ammonium sulfate. The precipitate was dissolved in PBS (pH 7.2) and treated with 33% saturated ammonium sulfate three times. After centrifugation, the precipitate was dialyzed overnight against the same phosphate solution at 4 °C. The IgG fraction (PAb) was further purified by 7% caprylic acid method. PAb against TLA from rabbit serum was divided into three parts. The first part was conjugated with horseradish peroxidase (HRP) according to a standard NaIO4 conjugating method (Aly et al., 2013; Nakane and Kawaoi, 1974). The second part was conjugated with silica nanoparticles. The third part was unconjugated.

2.4. Characterization of PAb

The purified PAb against *Toxoplasma* Lysate antigen was characterized for determination of molecular weight range, to confirm purification and specification. Using discontinuous Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) in 12% slab gels (1 mm thick), under reducing conditions (+2- mercaptoethanol) and stained with Coomassie blue (Bio-Rad) as described by Laemmli (Laemmli, 1970).

2.5. Study population

The serum samples enrolled in the present study were selected from pregnant females attending the outpatient's clinic of Gynaecology and Obstetrics Department of Al-Zahraa Hospital, Faculty of Medicine for Girls, Al-Azhar University; outpatients clinic of Gynaecology and Obstetrics Department of Zagazig University. Hospital Faculty of Medicine Clinical Pathology − Department of Said Jalal Hospital, Faculty of Medicine Al-Azhar University, and also from different laboratories around Mahalla al-Kubra city.

Serum samples were collected during the period from April to September 2016. Ethical approval was obtained from the Committee of Research Publication, Ethics of Theodor Bilharz Research Institute. The objective of the work was explained to all individually participate in this work and informed consent was taken from each one.

This study was conducted on 120 individuals. Grouping of the participants was done as follows:

- Group I: 70 patients suspected for *Toxoplasma gondii* based on the