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Nano-Immunoassay for Diagnosis of Active Schistosomal Infection

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Abstract : There is a constant need to improve the performance of current diagnostic assays of schistosomiasis as well as develop innovative testing strategies to meet new testing challenges. This study aims at increasing the diagnostic efficiency of monoclonal antibody (MAb)-based antigen detection assays through gold nanoparticles conjugated with specific anti-Schistosoma mansoni monoclonal antibodies. In this study, several hybidoma cell lines secreting MAbs against adult worm tegumental Schistosoma antigen (AWTA) were produced at Immunology Department of Theodor Bilharz Research Institute and preserved in liquid nitrogen. One MAb (6D/6F) was chosen for this study due to its high reactivity to schistosome antigens with highest optical density (OD) values. Gold nanoparticles (AuNPs) were functionalized and conjugated with MAb (6D/6F). The study was conducted on serum samples of 116 subjects: 71 patients with S. mansoni eggs in their stool samples group (gp 1), 25 with other parasites (gp2) and 20 negative healthy controls (gp3). Patients in gp1 were further subdivided according to egg count in their stool samples into Light infection {≤ 50 egg per gram(epg) (n= 17)}, moderate {51-100 epg (n= 33)} and severe infection {>100 epg(n= 21)}. Sandwich ELISA was performed using (AuNPs -MAb) for detection of circulating schistosomal antigen (CSA) levels in serum samples of all groups and the results were compared with that after using MAb/ sandwich ELISA system. Results Gold- MAb/ ELISA system reached a lower detection limit of 10 ng/ml compared to 85 ng/ml on using MAb/ ELISA and the optimal concentrations of AuNPs -MAb were found to be 12 folds less than that of MAb/ ELISA system for detection of CSA. The sensitivity and specificity of sandwich ELISA for detection of CSA levels using AuNPs -MAb were 100% & 97.8 % respectively compared to 87.3% &93.38% respectively on using MAb/ ELISA system. It was found that CSA was detected in 9 out of 71 S.mansoni infected patients on using AuNPs - MAb/ ELISA system and was not detected by MAb/ ELISA system. All those patients (9) was found to have an egg count below 50 epg feces (patients with light infections). ROC curve analyses revealed that sandwich ELISA using gold-MAb was an excellent diagnostic investigator that could differentiate Schistosoma patients from healthy controls, on the other hand it revealed that sandwich ELISA using MAb was not accurate enough as it could not recognize nine out of 71 patients with light infections. Conclusion Our data demonstrated that: Loading gold nanoparticles with MAb (6D/6F) increases the sensitivity and specificity of sandwich ELISA for detection of CSA, thus active (early) and light infections could be easily detected. Moreover this binding will decrease the amount of MAb consumed in the assay and lower the coast. The significant positive correlation that was detected between ova count (intensity of infection) and OD reading in sandwich ELISA using gold- MAb enables its use to detect the severity of infections and follow up patients after treatment for monitoring of cure.

Keywords : Schistosomiasis, nanoparticles, gold, monoclonal antibodies, ELISA
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