

Summary

Immunodiagnosis of urinary schistosomiasis has proved to be very useful and sensitive. Antigen detection assays are considered of prime importance for immunodiagnosis, as the detection of circulating antigens can indicate an active infection.

The present study described the immuno-affinity purification of cysteine proteases (CP) from ES products of living *S. haematobium* eggs after being cultured in RPMI 1640 medium. Purification was done by gel filtration chromatography on sephadex A-50 (DEAE column chromatography method) followed by gel filtration chromatography on sephacryl-S-200 HR column. The eluted proteins, from the gel filtration column chromatography analyzed by 12.5% SDS PAGE under reducing condition, showed only one band at 27.5 kDa which was CP. Testing the antigenicity of the target antigen was carried out by indirect ELISA and gave strong reaction.

Rabbit polyclonal anti-cysteine protease antibodies were generated by immunization of New Zealand white rabbits with the prepared CP antigen. The procedure was followed by three purification steps; ammonium sulfate precipitation, caprylic acid treatment and finally ion exchange chromatography method. The purity of IgG pAb was assayed by 12% SDS-PAGE under reducing condition. The purified pAb IgG was represented by H- and L-chain bands at 53 and 31 kDa, respectively being free from other proteins. Reactivity of the purified pAb to *S. haematobium* CP was determined by indirect ELISA and gave a strong reactivity. Conjugation of the purified pAb with horse reddish peroxidase (HRP) was carried out. Then loading of nano-magnetic beads (NMBs) to fraction of the pAb and to another fraction of the HRP conjugated-PAb was done to be applied in the different antigen detection ELISA techniques evaluated in the study.

The current study was held in different rural communities of El-Fayoum Governorate in summer months (June to August 2013).

This study was conducted on 120 individuals. By parasitological examination, they were divided into 3 groups, 60 individuals were positive for *S. haematobium* ova in urine (group A), 60 individuals were positive for intestinal parasites ova and were negative for *S. haematobium* ova in urine (group B) and 60 control individuals with negative urine and stool examination for *Schistosoma* ova or other intestinal parasites (group C). The *S. haematobium* infected group was classified into 3 subgroups, according to Nuclepore filtration results, which included 25 light infection patients, 28 moderate infection patients and 7 high infection patients. Frequency of infection was higher in males (75%) more than females (25%) and was mainly in the age group of 11-20 years old (30%).

Standardization of sandwich ELISA used for detection of CP antigen was carried out before the application of the technique on human samples. The purified IgG fraction of the rabbit sera was employed as both antigen capture and peroxidase conjugated detecting antibody.

In this study, using the nanomagnetic beads increased the sensitivity and specificity of the applied technique. The sensitivity of the traditional sandwich ELISA was 85% in serum and 83.3% in urine and it increased by using the sandwich IMB-ELISA to be 95% in serum and 91.7% in urine. The specificity of sandwich ELISA was 88.3% in serum and 85% in urine and it increased by using the sandwich IMB-ELISA to be 93.3% in serum and 91.7% in urine.

The same was noticed in the dot-ELISA techniques. The sensitivity of the traditional sandwich dot-ELISA was 91.6% in serum and 88.3% in

urine and it increased by using the sandwich IMB-dot-ELISA to be 96.6% in serum and 93.3% in urine. The specificity of sandwich dot-ELISA was 90% in serum and 91.7% in urine and it increased by using the sandwich IMB- dot-ELISA to be 93.3% in serum and 96.7% in urine.

For all techniques, detection of CP in urine recorded slightly lower results than that detected in serum. The other parasites-infected groups mainly with *Fasciola* showed mild degree of cross reactivity with CP, it was higher in urine by using the traditional sandwich ELISA technique and minimal with using sandwich IMB-dot-ELISA.

Finally, the employment of rabbit anti *S. haematobium* CP IgG-pAb in sandwich ELISA techniques for the detection of CP circulating antigen in serum and in urine provided a sensitive and specific tool for immunodiagnosis for *S. haematobium* infection. CP antigen detection was shown to be a good correlate to the intensity of infection. The IMB-ELISA appears to be a sufficiently sensitive and feasible assay for detection of schistosomal antigenemia. The IMB-dot-ELISA assay was highly sensitive and specific and of a technical value as an applicable, fast, cheap, accurate and promising diagnostic technique for schistosomiasis in the field of endemic regions.