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Evaluation of a Pre-Selected Epitope of *Fasciola hepatica* Cathepsin-L1 for the Diagnosis of Human Fasciolosis by IgG-ELISA Test.

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ABSTRACT

Coprological analysis is still commonly employed to diagnose human fasciolosis despite the fact that this method is not utterly reliable. By far serologic methods in this case hold more validity and reliability. The purpose of the present study was to evaluate a 35-mer peptide covering a preliminary epitope of Cathepsin L1, a new purified antigen of *Fasciola hepatica,* by IgG-ELISA method for diagnosis of human fasciolosis. The advantage of this method is that identical batches of a synthetic peptide could be generated immediately without any variation in prepared batches and can be applied to diagnose thousands of infection cases assertively.

Totally 90 individuals coprologically positive for fasciolosis and 15 control negative serum samples were included in the test. The cut-off value ($O.D._{450\ nm;}$ 1:1250 serum dilutions) with this peptide was 0.16. Considering this value 27 cases of false negative were detected; hence the calculation of the sensitivity of the IgG-ELISA was identified as 77%. No cases of false positive were spotted. It is concluded that this peptide is not capable to detect antibody in patients' sera. With reference to other peptides some measurements are being put into operation.

Key Words: Fasciolosis, Peptide, ELISA.

Introduction:

Fasciolosis, or liver fluke disease, caused by parasites of the genus Fasciola is emerging as an important disease in man and in animal, particularly in northern parts of Iran. It is now recognised by the World Health Organisation (WHO) as a serious public health problem in man and in animals (13). Several outbreaks of this disease recently occurred in the Gilan province of Northern Iran and in 1999 alone over 10,000 individuals were infected (1, 7).

Coprological analysis is still commonly employed to diagnose human fasciolosis despite the fact that this method is not utterly reliable (5). Using this method eggs are not detected until the latent period of infection when much of the liver damage has already occurred. In addition, eggs are released sporadically from the bile ducts and hence stool samples of infected patients can contain no eggs (8). Serological diagnosis is preferred particularly since anti-fasciola antibodies can be detected as early as two weeks post infection and can thus facilitate early chemotherapeutic intervention (5). Recently more highlighting is on the antigenic product of cysteine proteinase, chiefly cathepsin L1 (CL1) (2, 9, 10, 11). Cysteine proteinase is a major component of the excretory-secretory products and because it is immunogenic at all stages of liver fluke development in the definitive host it can be used to diagnose both the acute and chronic stages of infections. Nevertheless regarding the variations of prepared antigen batches and some cross-reactivity observed using the aforementioned antigen, preparing and evaluating of a purer antigen sounds a significant idea.

The purpose of the present study was to evaluate of a 35 –mer peptide covering a preliminary epitope of CL1 by IgG-ELISA method for diagnosis of human fasciolosis. The advantage of this method is that identical batches of a synthetic peptide could be generated immediately without any variation in prepared batches and can be applied to diagnose thousands cases of infection confidently.

Materials and Methods:

Clinical samples:

Blood samples from fasciola-infected individuals were collected during the fasciolosis outbreak in 1999 in the Central Health Clinic of Rasht, a major city in the Gilan province of Northern Iran. Coprological analysis for *Fasciola* eggs was performed on faecal samples obtained from all individuals as previously described (6). Only individuals that were coprologically positive (90 individuals) and presented with a history of the disease were included in the present study. Control serum samples were obtained from 15 volunteers at Tehran University of Medical Sciences, Iran.

Peptide derived from *Fasciola hepatica* Cathepsin L1:

A synthetic peptide of Cathepsin L1 of about 35 aa in length called JDDCU, 02 with a molecular weight of 40993,3 Dalton was synthesised by PEPCEUTICALS, Ltd Company. This peptide was derived from the preliminary epitope of CL1 isolated from adult *Fasciola hepatica* (12) and was labelled with Biotin. The amino acid sequence of this peptide is as follows:

Biotin:
GLSWGERGYIRMVRNRGNMCGIASLASLPMVARFP: acid

Indirect ELISA:

Indirect ELISA was performed as described by EXIQON Company instruction manual. The plates (Streptoavidin

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immobiliser, EXIQON) were washed 3 times using PBS/0.05% Tween 20 (PBS/T), afterwards the wells were coated with 100-µl antigen (1 µg/ml) in DMSO. Optimal antigen concentration and serum dilutions were determined by checkerboard titration. The plates were incubated for 1 h at room temperature while shaking leisurely, and then washed three times with PBS/T. After coating and without further blocking stop the wells were filled with 100 µl serum prediluted 1:1250 in PBS/T. The plates were incubated for anothere1 h at room temperature, washed as before. 100 µl of 1:6000 dilution of Anti-bovine IgG conjugated with horseradish peroxidase in PBS/T was added to each well and the plates were incubated again for 1 h at room temperature. Following a final washing step 100 µl of O-phenylendiamine dihydrochloride (OPD) substrate (from Sigma Chemical Co., Poole, Dorset, United Kingdom) was added to each well and the reaction stopped after 5 minutes by adding 50 µl of 12.5% H_2S0_4. The optical density (OD) of the samples was measured at 492 nm using a Titerteck (Helsinki, Finland) multiscan ELISA plate reader. All assays were tested in triplicate and repeated twice. The cut-off value was the O.D. 450 nm of sera from healthy control cases at 1:1250 dilutions, plus 3.09 times the S.D. of these sera.

Statistical analysis:

Statistical analysis was carried out using SPSS for windows version 10. The parameter of sensitivity was determined according to the formula as: SE=TP/ (TP+FN) × 100 (4) where "FN" stands for false negative (number of proven fasciolosis samples that were negative by ELISA and "TP" for true positive (number of patients infected with fasciolosis).

Results

The cut-off value ($O.D._{450\ nm;}$ 1:1250 serum dilutions) with this peptide was 0.16. This value discriminates between the seropositive and seronegative groups. The distribution of O.D. absorbances of fasciolosis and healthy control cases comparing the reference line, i.e., the cut-off value is presented in Fig. 1. As it is palpable in this figure totally 27 cases of false negative were detected, hence the calculation of the sensitivity of the IgG-ELISA using this antigen was identified as 77%.

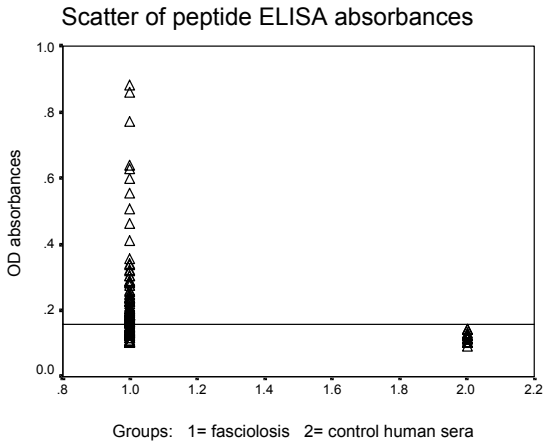

Fig. 1: Scatter of peptide ELISA OD absorbances

Discussion

No cases of false positive were spotted, nonetheless it couldn't be judged that the specificity was 100% because when we came across with low sensitivity, we arrived at a conclusion not to evaluate the specificity further and consequently other parasitic infection sera were not included in the test. Cornelissen et al (3) has used a 16-mer aa portion of this peptide (aa 296-311) in his study besides the other peptides to diagnose fasciolosis in ELISA test as to cattle sera and couldn't get a reliable result, but he has reported a sensitivity and specificity of 100% and 99.8% of the test using another 20-mer peptide covering aa 110 - 129 from CL1. He hasn't used of human sera to detect the validity of test and there is no report in this case up to date.

Using of CL1 as antigen shows high sensitivity and specificity in ELISA test (9, 10, 11) but as it was mentioned earlier to reduce the number of cross-reactivity and because the peptides are relatively cheap and easy to produce in a reproducible manner implementation of an ELISA test based on peptide as antigens is highly recommended.

It is concluded that this peptide is not capable of detecting antibody in patients' sera and should be replaced with another aa sequence. With reference to other peptides some measurements are being put into operation.

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