THESIS TITLE : STUDIES ON CHARACTERIZATION OF *Fasciola gigantica* ANTIGENS BY SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS AND IMMUNOBLOTTING TECHNIQUE FOR SERODIAGNOSIS OF HUMAN FASCIOLIASIS

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ABSTRACT

Infection with the liver fluke *Fasciola gigantica* affects most common in cattle, water buffaloes and sheep. Human infection can occur accidentally by eating the water plants that contain the infected metacercariae. The diagnosis of human infection is currently based on the demonstration of eggs in the stool however this method is the inefficient diagnostic technique. Other advanced methods, such as ultrasonography, Computed Tomography and endoscopic retrograde cholangio pancreatography require the expensive equipments and these methods are reliable only in the hands of experienced personnel. Thus, serodiagnosis is a useful alternative method. However, identification and characterization of antigens appear to be important for establishing the specific diagnostic technique. For these reasons, we have characterized *Fasciola gigantica* antigens by SDS-PAGE and immunoblotting in order to improve the sensitive and specific diagnostic serological test.

Each of excretory-secretory (ES) and somatic antigens of *Fasciola gigantica* were revealed by SDS-PAGE and immunoblot analysis of sera from patients with *Fasciola*

gigantica infection, from patients with clinical fascioliasis, from patients with other illness and from healthy adults. By SDS-PAGE , it was found that the ES products comprised more than 6 polypeptides. The immunoblot analysis revealed 12 components ranging from less than 14.4 to 38 kDa which were strongly recognized by fascioliasis sera. One antigenic band at approximate molecular mass of 27 kDa was found to give consistent reaction with fascioliasis sera (100% sensitivity). The specificity, positive and negative predictive values of the test using this antigenic band were 97.9%, 66.7% and 100% , respectively. These ES products were also separated by two-dimensional electrophoresis and subsequent immunoblotting technique to estimate the pI of the 27 kDa antigenic components. The 27 kDa components were differentiated into 3 antigenic spots with approximate pH at 4.6, 5.3 and 6.0, respectively. The adult somatic antigen revealed by SDS-PAGE comprised more than 22 polypeptides. And immunoblotting revealed 13 polypeptides ranging from less than 14.4 kDa to more than 94 kDa which were strongly recognized by fascioliasis sera. The band at approximate molecular mass of 38 kDa was found to give consistent reaction with fascioliasis sera (100% sensitivity). The specificity, positive and negative predictive value using this antigenic band were 96.7%, 55.6% and 100%, respectively. In conclusion, the present finding suggest that the 27 kDa component of adult ES product and the 38 kDa component of adult somatic antigen are sensitive and specific for the diagnosis of human *Fasciola gigantica* infection.