1. The objective of present study was to develop a sensitive and specific immunodiagnostic tool for the detection of specific antigen(s) in the serum samples of patients with hepatic amoebiasis by employing monoclonal antibodies.

2. Employing conventionally used cocktail crude amoebic extract as a source of antigen in micro-ELISA system, serum samples from all 16 (100%) of confirmed cases of ALA had detectable levels of anti-amoebic antibodies. In addition, 3 (27.2%) of the 11 clinically suspected cases of ALA were also positive for anti-amoebic antibodies. These results indicate that the demonstration of anti-amoebic antibodies has several limitations in confirming the diagnosis of amoebiasis.

3. The crude amoebic extract (CAE) from Indian axenic strain of *E. histolytica* after resolution on SDS-PAGE under reducing conditions revealed at least 30-35 discrete polypeptide bands. The serum samples, from 15 confirmed cases of amoebic liver abscess (ALA), recognised 7 major polypeptides of CAE. The polypeptides with molecular mass of 29, 38, 45, 58, 67, 89 and 95 KD were not recognised by the serum samples from patients with idiopathic ulcerative colitis or normal healthy subjects and these were always recognized by serum samples obtained from ALA cases. Thus an immunoassay based on the detection of either of these polypeptides or the antibodies against them may help in developing a good quality immunodiagnostic procedure for confirming on-going amoebic infection.

4. Employing anti CAE IgG antibodies as detector antibodies to detect PEG precipitated circulating immune complexes (CIC) in serum samples, 14 (87.5%) of the 16 ALA cases and 1 (9%) of the 11 clinically suspected ALA cases also had detectable levels of amoebic antigen specific CIC. Employing the same anti CAE IgG antibodies as detectors of amoebic antigen in glycine-HCl treated i.e.,
‘dissociated CIC’, from the serum samples, all the 16 confirmed ALA cases and 1(9%) of 11 suspected cases of ALA had amoebic antigen in CIC. Since CIC or antigen specific CIC do not remain in circulation for any significant length of period following eradication of infection, antigen detection based assay system appears to distinguish an ongoing amoebic infection from a past exposure.

5. The SDS-PAGE profile and western immunoblot of PEG precipitated CIC probed with anti CAE antibodies revealed three polypeptides with molecular masses 67, 58, and 29 KD to be amoebic specific. The polypeptide with molecular mass of 58 KD appeared to be the major one entrapped in CIC. The amoebic specific 58 KD molecule was eluted from the gel of SDS-PAGE of CAE and monospecific antibodies were raised in rabbits. The IgG fraction of anti 58 KD antibodies was accomplished by DEAE cellulose chromatography. Anti 58 KD IgG antibodies were coupled to CNBr activated sepharose-4B, so as to affinity purify 58 KD molecule. The purity of affinity purified amoebic antigen was indicated by the appearance of a single protein band at 58 KD molecular mass in SDS-PAGE. It retained its immunoreactivity and specificity as evidenced by a single band at 58 KD position upon reaction with anti CAE antibodies in western immunoblots.

6. Hybridization of amoebic antigen stimulated splenocytes (from inbred Balb/c mice) and mouse myeloma cells (from PAIOP3 cell line) resulted in development of several hybrids secreting anti-amoebic antibodies. The cloning by limiting dilution method led to the development of 9 clones secreting anti 58 KD amoebic antibodies as assessed by ELISA. These were sequentially labeled as C1-C9. The clone P1D11 (C6) which produced highest degree of anti-amoebic antibodies as assessed in ELISA was picked up for further study. The clone C6 was expanded in the peritoneal cavity of the Balb/c mice as ascites. The anti-amoebic antibodies in
ascitic fluid were labeled as MoAb C6. The monoclonality of antibodies produced by clone C6 was evidenced by immunoglobulin isotyping. Its specificity was confirmed by the recognition of amoebic antigen in western blots and ELISA. The monoclonal antibody was of IgG1 isotype and reacted with epitope(s) of polypeptide with molecular masses of 58 KD and weakly with 29 KD polypeptide in western immunoblot assay. The specificity of MoAb C6 in recognition of 58 KD amoebic antigen was evident by its failure to recognize any of the proteins in crude extract antigen of *Giardia lamblia* trophozoites or promastigotes of *Leishmania donovani* in an ELISA. Further the MoAb C6 did not cross react with components of spent TYI-S medium and horse serum which was used to grow axenic *E. histolytica* trophozoites.

7. Fluorescent probing by MoAb C6 indicated a strong patchy to uniform surface fluorescence of live *E. histolytica* trophozoites, while acetone fixed *E. histolytica* trophozoites revealed exclusive fluorescence of surface membrane and the cytoplasmic components got stained with counter stain (Evan's blue) only. A similar fluorescent pattern of *E. histolytica* trophozoites was obtained after probing with rabbit anti 58 KD monospecific IgG antibodies confirming the surface localization of 58 KD antigen.

8. Employing affinity purified 58 KD amoebic antigen in an ELISA system, serum samples from 15 (88.2%) of 17 ALA cases were found to contain anti 58 KD antibodies, while 1(5%) of 20 healthy controls, 2(8.3%) of the 24 cases with non-amoebic hepatic disorders and 8 (33.3%) of the 24 samples from clinically suspected cases of ALA also had anti 58 KD anti amoebic antibodies. These results indicate the limitation of antibody detection based assays to pin point a case with an active amoebic disease.
9. Employing anti 58 KD IgG monospecific antibodies (raised in rabbits) as detectors of 58 KD antigen in dissociated CIC, the serum samples from all the 17(100%) ALA cases contained detectable levels of 58 KD antigen, while 1(9.9%) of 15 samples from healthy controls and 1(6.14%) of 24 samples from suspected cases of ALA were also found to contain 58 KD amoebic antigen. Similarly, employing anti 58 KD monoclonal antibodies (from ascitic MoAb Q>) as detectors of 58 KD antigen in dissociated CIC, the serum samples from all the 17(100%) ALA and 1(9.9%) of 15 from healthy controls and 1(6.14%) of 24 from suspected cases of ALA were found to contain detectable levels of 58 KD amoebic antigen. The data generated in present study indicate that ELISA, for detection of amoebic antigen(s) in circulation using anti CAE antibodies as detector antibodies, is better than that using monoclonal antibodies.

10. Lastly, immunochemical characterization of affinity purified 58 KD amoebic antigen was carried out. The treatment of affinity purified 58 KD antigen with trypsin and pronase at increasing concentrations sequentially reduced its immunoreactivity. The immunoreactivity of affinity purified 58 KD molecule got abolished upon heat treatment at 80 and 100°C. The metaperiodate oxidation of purified 58 KD antigen again altered its immunoreactivity indicating the presence of a sodium metaperiodate sensitive carbohydrate epitope. These results suggested the 58 KD molecule of E. histolytica trophozoites to be a glycoprotein in nature.