SUMMARY
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The salient findings of the present study are summarized below:

Axenic strains of *Giardia lamblia* namely, Portland-1 (P-1, ATCC-30888) was routinely maintained in Diamond's TYI-S-33 complete medium. Trophozoites were subcultured twice-a-week for further propagation. Antigen prepared from this culture was used in the biochemical and immunological studies.

- Antigenic reactivity of the 10,000xg supernatant and pellet fraction against antisera to *Giardia* whole cell (IRS-G1) and against the crude soluble fraction (10,000xg supernatant) was found to be immunologically more active than the sediment fraction (10,000xg pellet) in ELISA.

- Four fractions termed as F-I, F-II, F-III and F-IV were recovered when the crude soluble antigen of *G. lamblia* was subjected to gel filtration through Sephacryl S-300 column chromatography. The mol. wt. of the four fractions viz., F-I to F-IV were 150 k, 65 k, 50 k and 10 k dalton, respectively.

- The protein constituent of CSA and its four fractions was demonstrated in 10% SDS-PAGE. The protein profile of CSA showed about 28 discrete protein bands in the mol. wt. region of >125 kDa to 14 kDa. Similar banding pattern with less number of polypeptides (about 13) was observed with F-I. F-II, F-III and F-IV showed coomassie blue stained protein bands in the mol. wt. regions of >94 kDa to 43 kDa, <94 kDa to 21 kDa and 82 kDa to 10 kDa, respectively.
• It was observed that the antigenic activity was mostly restricted to the high mol. wt. fraction I, when the antigenic activity of CSA and its four fractions were compared in GDP, CIEP and ELISA against immunized sera. Furthermore, quantitative analysis of fraction I in ELISA showed that the antigenic activity was eight times more than the parent antigen (CSA) and other fractions.

• The immunoreactive polypeptides of fraction I was detected throughout the lane. However, a highly immunoreactive band at an apparent mol. wt. of 118 kDa was observed. The other notable immunoreactive bands were identified in the mol. wt. region of 170 kDa, 55 kDa and 30 kDa. A few less immunoreactive polypeptides were detected at the mol. wt. range of 30 kDa to <45 kDa.

• Antisera directed against F-I antigen, definitely suggested the expression/association of F-I antigen on the surface of live trophozoites as the F-I antibody when allowed to react with the trophozoite showed intensely high fluorescence on the outer periphery of the body, flagella and some extent of the ventral disc cytoskeleton.

• The prominent immunoreactive polypeptides found in F-I and on the surface of the live trophozoites were in the apparent mol. wt. range of 170 kDa, 118 kDa, 55 kDa, 38 kDa and 30 kDa. This was further confirmed by absorption of IRS-F-I with live trophozoites.

• Plasma membrane from axenic _G. lamblia_ trophozoites was successfully isolated and purified. The membrane preparation was of high purity as revealed by electronmicroscopy and marker enzyme studies. By electron microscopy the plasma membrane appeared as a vesicle with no recognizable intracellular organelles or intact nuclei. In marker enzyme studies, a
significant 17 fold enrichment of 5'-nucleotidase activity was observed in plasma membrane preparation than in the homogenate.

- Physico-chemical analysis revealed the glycoprotein nature of the plasma membrane as suggested by its sensitivity to heat, proteolytic digestion and periodate oxidation.

- Among the surface carbohydrates of _G. lamblia_ detected by lectin agglutination assay the N-acetyl-D-glucosamine and N-acetyl-D-neuraminic acid were found predominant over D-glucose and D-mannose sugars.

- In SDS-PAGE, plasma membrane proteins revealed 10 major and 15 minor polypeptides in the mol. wt. ranging from 225 kDa to 20 kDa. The most prominent protein bands had an apparent mol. wt. of 118 kDa, 84 kDa, 55 kDa and in a series in between 38 kDa and 32 kDa.

- Association of different saccharides linked with plasma membrane proteins of _Giardia_ in lectin blotting showed the presence of N-acetyl-D-glucosamine and N-acetyl-D-neuraminic acid residues (specific for WGA) in the apparent mol. wt. regions of 118 kDa, 84 kDa, 55 kDa and three bands in between 30 kDa and 20 kDa, respectively. However, D-glucose and D-mannose (specific for Con A) containing glycoproteins were identified at 60-70 kDa and 43 kDa regions respectively.

- In immunoreactive polypeptide analysis against IRS-PM several polypeptides were found antigenic in the mol. wt. range of >200 kDa to 20 kDa. However, prominent conserved antigens were detected at 160 kDa, 118 kDa, 55 kDa and 30 kDa regions. When the immunoreactivity of the same antigen was analysed against giardiasis patients sera, the most immunoreactive polypeptide was detected at 118 kDa mol. wt. The other antigenic
polypeptides were found in the mol. wt. range of 84 kDa, 55 kDa and in between 30 and 20 kDa.

- Antibody raised in rabbits against the 118 kDa polypeptide was tested in immobilization assay against live *G. lamblia* trophozoites. 60-84% immobilization of *G. lamblia* trophozoites was observed with the fresh antisera raised against different *G. lamblia* antigens. However, Only 5-20% lethal effect was observed with heat inactivated sera, thereby suggesting the positive role of complement in this regard. Antisera to 118 kDa polypeptide showed 70% immobilization effect. However, the maximum effect was recorded with antiserum raised against plasma membrane. The explanation could relates to a cumulative effect of different plasma membrane associated antigens including the 118 kDa.

- Further studies are needed to characterize the 118 kDa polypeptide to determine the orientation on the surface of the parasite, the involvement in the pathogenesis of the disease and the interaction with host-immune responses. Since the 118 kDa polypeptide was found in common with all the three strains studied from the same and from different geographic areas, it would be of great value to use this particular antigen in serodiagnosis of giardiasis.

- Level of the total circulating immunoglobulins of different classes (i.e., IgG, IgA and IgM) when assayed by radial agar gel diffusion test in various categories of giardiasis patients and *Giardia* free controls revealed no significant elevation or depression.

- It was found that anti-*Giardia* IgM antibody was always higher in symptomatic cases than in the asymptomatic group and control subjects.
However, the level of anti-\textit{Giardia} IgG antibody was more in asymptomatic carriers than in the acute \textit{giardiasis} patients. This study suggests that for current \textit{Giardia} infection one should search for anti-\textit{Giardia} IgM antibodies rather than the conventional IgG antibodies.

- A comparison of the two most sensitive tests namely IFAT and ELISA for serodiagnosis of \textit{giardiasis}, revealed that the ELISA was more specific and sensitive (96%) than IFAT. However, depending upon the need and facilities, one could use any of the tests for routine serodiagnosis of \textit{giardiasis} cases.

- Biochemical and immunological studies were conducted to find out if there are any differences between isolates within a community and that isolated from a different geographic area. PD-I and PD-2 were isolated from patients with symptoms (one from acute infection and other from chronic cases) attributed to \textit{giardiasis}. These strains were successfully axenized in the laboratory and maintained in Diamonds TYI-S-33 medium along with the reference P-1 strain. The heterogeneity among the two local isolates and the reference strain were investigated.

- In the isoenzyme study, the electrophoretic pattern of the six enzymes revealed three zymodemes among the three isolates of \textit{Giardia}. PD-I and PD-2 appeared to be quite different from P-1 in the electrophoretic patterns of the two enzymes \textit{viz.} PGM and ICDH. The two local isolates differed from each other in a number of bands and also in the nature of migration in enzymes ALP and ME. However, homogeneity among all the three isolates was observed in ACP and G6PDH enzymes.

- The SDS-PAGE protein patterns of the three \textit{G.lamblia} isolates showed 24 protein bands with mol. wts. ranging from 10,000 to 170,000
Daltons. No major differences were observed in the protein profiles of the three isolates, however, minor differences at 20,000 and 67,000 dalton mol. wt. regions could be detected.

- Immunological analysis of the three *Giardia* isolates revealed the sharing of major antigens in immunoblot technique against pooled giardiasis patient sera. The major conserved polypeptides were observed at 118,000; 94,000; 55,000 and a few less reactive antigens at 32,000 and a few between 45,000 and 55,000 dalton were detected. As such no major differences were observed except the higher intensity of antigen antibody reaction in the Indian isolates in comparison to the Portland isolate.

- These observations certainly suggest that heterogeneity exists among the *Giardia* isolates of different geographic areas and also within the same locality. Although observations on the protein profiles and antigenic analysis did not show marked differences but isoenzyme studies definitely suggest a remarkable variation. This could be associated with the variations in the clinical manifestation of giardiasis patients, differences in immune responses in host and a variable degree of virulence among the parasite. Correlation of genetic variation to pathogenicity and susceptibility to drugs of *G. lamblia* should be the future line of research in this regard.