Altered lipid parameters in patients infected with *Entamoeba histolytica*, *Entamoeba dispar* and *Giardia lamblia*

D. BANSAL, H. S. BHATTI and R. SEHGAL
Department of Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

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ABSTRACT

Information on the effect of parasitic infections on lipid parameters is scarce. Certain parasites induce significant changes in lipid parameters, as demonstrated by the fact that substitution of lipid/cholesterol for serum in axenic culture medium (*in vitro*) and in experimental models (*in vivo*) supports vigorous growth of *Entamoeba histolytica*. Thus, significant changes in lipid parameters may be induced in an infected host. Blood samples are obtained from intestinal amoebiasis patients passing *E. histolytica* (n=8), *E. dispar* (n=15) or *Giardia lamblia* (n=9) cysts, or diagnosed with amoebic liver abscess (ALA; n=50) and from apparently normal healthy individuals (control group; n=30). Levels of total serum cholesterol, high-density lipoprotein and low-density lipoprotein are assessed using commercial kits. *E. histolytica* and *E. dispar* isolates are differentiated by hexokinase isoenzyme electrophoresis and by enzyme-linked immunosorbent assay (ELISA; Techlab) tests. Results show that *E. histolytica*, *E. dispar* and *G. lamblia* cyst passers had significantly lower levels of total serum cholesterol (73.42±2.24 mg/dL), compared to levels in ALA cases (101±2.85 mg/dL) and in controls (166.26±2.02 mg/dL). Further study of a greater number of cases is needed to explore the relevance of this finding.

KEY WORDS: Entamoeba dispar.
Entamoeba histolytica.
Giardia lamblia.
Lipids.

Introduction

Entamoeba histolytica and *Giardia lamblia* parasitise the gastrointestinal tract of humans and are a major cause of morbidity and mortality in tropical and subtropical countries.[1,2] In *G. lamblia* infection, excystation is accomplished by limiting the acidic conditions present in the stomach, and this is supported by the protease-rich and slightly alkaline environment of the small intestine. However, encystations can be induced *in vitro* by starving the trophozoites of cholesterol (a condition known to occur in the lower small intestine), either by using lipoprotein-deficient serum or augmenting the bile concentration in the culture medium.[3,4]

In *E. histolytica* infection, excystation occurs in the small intestine, where four amoebae are released from the mature quadrinucleate cyst. Trophozoites dwell in the colon, where they multiply and encyst, typically producing amoebae each containing four nuclei.[5] Cholesterol has been reported to be a growth promoter of *E. histolytica*.

Avirulent strains can be revived by adding cholesterol to the culture medium or by feeding cholesterol to experimental animals or the host.[6–8] Cholesterol is thought to act as an irritant on mucous membrane and thus helps the amoebae to colonise the injured site, enhancing the parasite's virulence.[9] *In vitro* study has shown that substitution of lipid/cholesterol for serum in axenic culture encourages the vigorous growth of *E. histolytica*.[10]

No data are available on the effect of serum cholesterol levels in patients infected with *E. histolytica*, *E. dispar* and *G. lamblia*, and the present study aims to assess the levels of total serum cholesterol in a group of individuals passing *E. histolytica*, *E. dispar* or *G. lamblia* cysts and in a group of patients with amoebic liver abscess (ALA). Sera from age-matched healthy individuals are used as controls.

Table 1. Age and gender of cyst passers, ALA cases and healthy controls.

	Age (range)	Sex	
		Male	Female
Cyst passers (n=32)	51.8 (17–68)	21	11
ALA patients (n=50)	48.7 (7–58)	38	12
Controls (n=30)	39.8 (22–55)	18	12
Total (n=112)	44.1 (7–68)	77 (68.7%)	35 (31.3%)

Materials and methods

Patients with a history of intestinal and/or extraintestinal symptoms were recruited from those attending the out-patients' department of Nehru Hospital, which is attached to the Postgraduate Institute of Medical Education and Research, Chandigarh, India (Table 1).

Correspondence to: Dr. Rakesh Sehgal
Central Research Institute, Kasauli, Himachal Pradesh, India
Email: rakesh.sehgal@rediffmail.com

Table 2. Lipid profiles of cyst passers versus those with amoebic liver abscess.

	Cyst passers	Amoebic liver abscess	Controls (normal values)
Total serum cholesterol (mg/dL)	73.4±2.2†a	101±2.8†c	166.2±2.0†b (150–240)
High-density lipoprotein (mg/dL)	29.2±1.4*a	31.6±1.6†c	46.3±1.5†b (41–58.7)
Low-density lipoprotein (mg/dL)	25.2±1.7†a	45.3±1.8†c	99.8±1.4†b (0–158)

Results are expressed as mean±SD from two experiments conducted in duplicate.
[a]cyst passers *vs*. ALA; [b]cyst passers *vs*. controls; [c]ALA *vs*. controls
*$P<0.05$, †$P<0.001$.

Subjects and samples
Group 1: Stool samples from 32 patients were included. Of these, 23 were positive for *E. histolytica* or *E. dispar* and nine were positive for *G. lamblia* microscopically and by culture.
Group 2: Fifty ALA patients diagnosed clinically, radiologically and serologically (antibody titre >1:1600 by enzyme-linked immunosorbent assay [ELISA]) were included in this group.
Group 3: Thirty healthy individuals without any history or symptoms of giardiasis and amoebiasis were included as controls.

Differentiation of parasites
E. histolytica isolates were differentiated by hexokinase isoenzyme analysis[11] and antigen detection by ELISA kit (Techlab, Blacksburg, VA, USA).[12] Briefly, a water lysate of parasites was subjected to thin-layer starch gel electrophoresis and hexokinase was detected by specific enzymatic activity using formazan development. The Techlab kit is a monoclonal antibody-based ELISA, which detects *E. histolytica*-specific galactose inhibitable adherence lectin (Gal/Gal Nac lectin) in faecal specimens.

Cholesterol determination
Intravenous blood (2–3 mL) was collected in a plain tube. Serum was separated and stored at –20°C until used. Total serum cholesterol (TC) was estimated using an assay kit (Clonital, Carvico, Italy) in accordance with the manufacturer's instructions. Serum high-density lipoprotein (HDL) was measured using a commercial assay kit (Randox, Antrim, UK). Low-density lipoprotein (LDL) concentration was calculated according to the kit manufacturer's instructions.

Statistical analysis
Standard deviation (SD) was used to indicate the extent of variation in group mean values. The *P* value was calculated using Student's *t*-test.

Results

Of the 23 *Entamoeba* spp. cyst passers, eight were positive for *E. histolytica* and 15 were positive for *E. dispar* by both hexokinase isoenzyme analysis and Techlab ELISA. Significant differences were observed in serum TC, HDL and LDL levels in cyst passers and in ALA cases, compared with controls (Table 2). Levels of TC and LDL were significantly lower ($P<0.001$) in cyst passers compared to ALA cases, whereas the level of HDL was less significant ($P<0.05$). Levels of TC, HDL and LDL were significantly lower ($P<0.001$) in ALA cases and in cyst passers, compared to the controls. Lipid parameters were lower in *E. histolytica* cyst passers than in *E. dispar* cyst passers. Patients infected with *G. lamblia* showed lower lipid parameters than did *E. dispar* cyst passers (Table 3).

Table 3. Comparison of lipid profiles in cyst passers of *E. histolytica*, *E. dispar* and *G. lamblia*.

	E. histolytica	*E. dispar*	*G. lamblia*
Total cholesterol (mg/dL)	71.2±2.1†a	79.1±1.8†c	69.9±2.7*b
HDL (mg/dL)	27.1±1.4†a	31.1±1.2*c	29.5±1.5*b
LDL (mg/dL)	24.5±1.6†a	28.5±1.8†c	22.7±1.7*b

Results are expressed as mean±SD from two experiments conducted in duplicate.
[a]*E. histolytica vs*. *E. dispar*; [b]*E. histolytica vs*. *G. lamblia*; [c]*E. dispar vs*. *G. lamblia*.
*$P<0.05$, †$P<0.001$.

Discussion

In this study, the impact of *E. histolytica*, *E. dispar*, *G. lamblia* infection and ALA on lipid parameters *in vivo* was assessed. Previous *in vitro* studies have shown that cholesterol is a growth promoter for *E. histolytica* and that avirulent strains can be revived by adding cholesterol to culture media,[6,7] and replacing bovine serum with a lipoprotein cholesterol (LPC) solution and bovine serum albumin in pre-encystation and excystation media stimulates *G. lamblia* encystations and vesicle formation.[4] Similarly, using LPC in the culture medium can induce *in vitro* encystations.[2,3]

This is the first study to address the suggestion that *Entamoeba* spp. significantly affect lipid profile *in vivo*. A significantly lower lipid profile was apparent in *E. histolytica*, *E. dispar* and *G. lamblia* cyst passers and in ALA patients when compared to the control group. This suggests that the parasites utilise cholesterol for their growth in infected individuals; however, the mechanisms involved in lipid change remain unclear. Previously, it has been shown that *G. lamblia* inhibition of cholesterol-dependent activation-C_k results in the up-regulation of *CWP*-1 gene expression, leading to encystation.[13]

A review of the available literature shows that changes in lipid profile occur in patients harbouring most of the parasites and with active infections. Membrane proteins are probably involved as parasites may metabolise cholesterol, but all are not able to utilise its role in pathogenecity. In *E. histolytica* infection, changes in cholesterol and lipid level show a greater association with active infection that results in intestinal amoebiasis and liver abscess. In intestinal amoebiasis it is probable that cholesterol absorption from the intestine is reduced, while in liver abscess cases it may be depleted from serum.

Trophozoites in the intestine convert to cysts in response to the presence of host factors, such as bacterial flora, that might use up the cholesterol needed for encystation. Significantly lower amounts of cholesterol are utilised by *E. dispar* than by *E. histolytica*, which may be the reason why the former remains non-pathogenic. It is also possible that *E. histolytica* is more invasive in ALA patients, who have higher levels of cholesterol (Table 2), compared to cyst passers, who have lower levels of cholesterol (Table 3). This indicates, albeit indirectly, that cholesterol/lipids may have a role in the pathogenesis of *E. histolytica*, and is corroborated by studies that demonstrate that *E. histolytica* becomes more virulent in the presence of cholesterol.[6,7]

In conclusion, this study demonstrates that there is a significant impact on lipid levels in individuals infected with *Entamoeba* spp. and *G. lamblia*, and cholesterol level is lower in cyst passers than in ALA patients. These results suggest that there may be factors that allow the protozoa to break up and consume lipid/cholesterol. However, further study of a much larger cohort of patients, and of other parasites, is needed in order to better understand the mechanisms involved *in vivo*. □

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