ORIGINAL ARTICLE

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Hypocholesterolemia in Patients with an Amebic Liver Abscess

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Background/Aims: Many parasites induce changes in the lipid profiles of the host. Cholesterol increases the virulence of *Entamoeba histolytica* in animal models and *in vitro* culture. This study aimed to determine, in patients with an amebic liver abscess, the correlation between cholesterol and other features, such as the size and number of abscesses, standard hematological and serum chemistry profiles, liver tests, and duration of hospital stay. **Methods:** A total of 108 patients with an amebic liver abscess and 140 clinically healthy volunteers were investigated. Cholesterol and triglycerides were measured in the sera. The data from medical observations and laboratory tests were obtained from the clinical records. **Results:** A total of 93% of patients with an amebic liver abscess showed hypocholesterolemia not related to any of the studied parameters. Liver function tests correlated with the size of the abscess. The most severe cases of amebic liver disease or death were found in patients whose cholesterol levels continued to decrease despite receiving antiamebic treatment and hospital care. **Conclusions:** Our results show that the hypocholesterolemia observed in patients with an amebic liver abscess is not related to any of the clinical and laboratory features analyzed. This is the first study relating hypocholesterolemia to severity of hepatic amebiasis. **(Gut Liver 2014;8:415-420)**

Key Words: Hypocholesterolemia; Cholesterol; *Entamoeba histolytica*; Amebic abscess; Invasive amebiasis

INTRODUCTION

The disease caused by *Entamoeba histolytica* constitutes the third cause of morbidity by parasite infection giving rise mortality with 70,000 deaths yearly worldwide, surpassed only by malaria and schistosomiasis. The incidence of amebiasis is higher in developing countries. Invasive intestinal amebiasis presents acute ulcerative colitis, toxic megacolon, ameboma, or amebic appendicitis. When trophozoites spreading via the bloodstream, invade other organs, causes extraintestinal amebiasis; mainly amebic liver abscesses (ALA).[1] Less than 10% of patients with invasive extraintestinal amebiasis release amoebas in stool.[2] There are cases of ALA in persons of all ages, but it is more common observed in men than women.[3,4]

Bansal *et al*.[5] reported that most of the parasites induce significant changes in lipid profiles in patients having active infections. In experimental models, animals cholesterol-fed are more susceptible to develop an ALA than animals under routine feeding.[6,7] The ameboma rarely occurs in guinea pigs, but if they are cholesterol-fed, they develop ameboma when infected intracecally with *E. histolytica* trophozoites.[8]

In vitro, *E. histolytica* became avirulent after more than 2 years of maintenance in axenic culture, but regains its virulence if cholesterol or phosphatidylcholine-cholesterol (PC-Chol) liposomes are added to culture.[9-14] Cholesterol-rich domain (lipid rafts) on the cell surface have been shown to control virulence in a variety of parasites, including *E. histolytica*.[15,16]

The aim of this study was to determine, in patients with ALA, the correlation between cholesterol and other features as size and number of abscesses, standard hematologic and serum chemistry profile, liver tests and hospital stay.

MATERIALS AND METHODS

1. Patients

This study included 108 patients with diagnosis of ALA admitted to "Jose Eleuterio González" University Hospital, Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, Mexico from 2002 to 2011. Serum samples from 140 clinically healthy volunteers were included. Levels of cholesterol and tri-

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glycerides were established from the control group, representing healthy population inhabiting an amebiasis endemic area.

2. Experimental design

The ALA was confirmed by ultrasound image or computed tomography. The sera of all patients included had positive two tests to diagnose invasive amebiasis; indirect hemagglutination assay (IHA Cellognost Amoebiasis; Behring Diagnostics GmbH, Marburg, Germany) and the western blot test patented by us.[17-21]

The standard treatment of liver abscess was the use of appropriate antibiotics and supportive care. Based on clinical unresponsive to therapy, some patients underwent needle aspiration of abscess to promote recovery. Sonographic examination of the upper abdomen was conducted in the patients. After a lesion was located and identified within the liver, percutaneous drainage was performed under ultrasound guidance and asepsis. To evacuate the abscess the fluid was aspirated as much as possible using Chiba needles, 18 to 22 gauge, and 15 to 20 cm long. The needle was selected according to the abscess cavity volume. During the aspiration, the needle was under screen control. The drained pus was examined microscopically for the presence of trophozoites and polymorphonuclear leukocytes. The aspirated pus underwent microbiologic examination to discard bacterial coinfection. *E. histolytica* culture was not made. Stool samples were not examined because only less than 10% of patients with liver abscess release amebas in stool.[2]

Cholesterol and triglycerides were determined in sera by enzymatic-spectrophotometric test (Biosystems S.A., Barcelona, Spain). The inpatients medical records were reviewed to obtain the volume of the amebic abscesses, localization and numbers of the abscesses. From records also were acquired the number of punctures, the stay at the hospital, hematology and standard liver function test results including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (TBIL), direct bilirubin (conjugated bilirubin), gamma glutamyl transpeptidase (GGT).

The area of the abscess was measured directly during ultrasound examination. First, it was measured the x-axis and then the plane perpendicular to determine the y-axis. When it was possible, the plane z was measured. In case of multiple abscesses, it was taken into account the largest one. The area of the abscesses was calculated by multiplying the measures of x-axis by y-axis.

Healthy volunteers were living at Monterrey, an area that is endemic for amebiasis. The sera belonging to healthy group had negative the western blot and the commercial IHA test to diagnose invasive amebiasis. They had not detected parasites in feces, and they never had invasive amebiasis, nor recall an episode of bloody diarrhea within the previous year.

This research is part of a broader research protocol, and the consent forms were approved by the Ethics Committee of the Hospital and by the Ethics Committee of the Faculty of Biological Sciences.

3. Statistical analysis

The data were analyzed using the Statistical Package SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) employing correlation coefficients of Pearson or Spearman. Nominal variable was analyzed by contingency table. Results with p-values <0.05 were considered as statistically significant.

RESULTS

1. Demographic features

The demographic features of patients with ALA were 20 women with an average age of 53±14 years and 88 men with an average age of 35±16 years. The healthy volunteers were 60 women aged 30±18 years and 80 men aged 32±20 years. Differences between the groups were no significant (p>0.05).

2. Cholesterol values of ALA patients

Dispersion of cholesterol values of ALA patients shows a tendency of low values (Fig. 1). Hypocholesterolemia was found in 94 ALA patients (87%); 51 (47.2%) presented levels of <100 mg/dL. The minimum level found was 15 mg/dL. Fourteen samples had normal values and only one patient had hypercholesterolemia. The sera from healthy women showed a mean of 148±32 mg/dL and healthy men showed a mean of 170±38 mg/dL.

3. Size, number, and localization of the amebic abscesses

The size of the amebic abscesses was variable. Some of them had 3×2 cm, while other abscesses measured 17×13 cm or 11×13.6 cm. The mean of the abscess area was 73 cm^2 and the median was 51 cm^2. The measures of abscesses were taken from records; the reports of 60% abscesses had measures in the x-, y-, and z-axis, and 40% only in x- and y-axis; therefore, solely

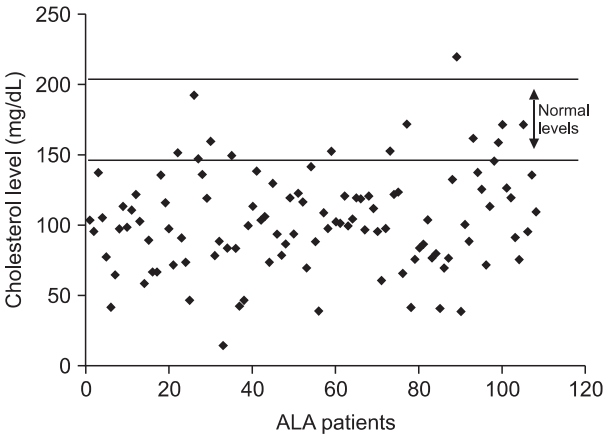

Fig. 1. Distribution of cholesterol levels in amebic liver abscess (ALA) patients. The serum cholesterol level was determined in 108 patients using the enzymatic-spectrophotometric test from ByosSystems. Normal level: 140 to 200 mg/dL.

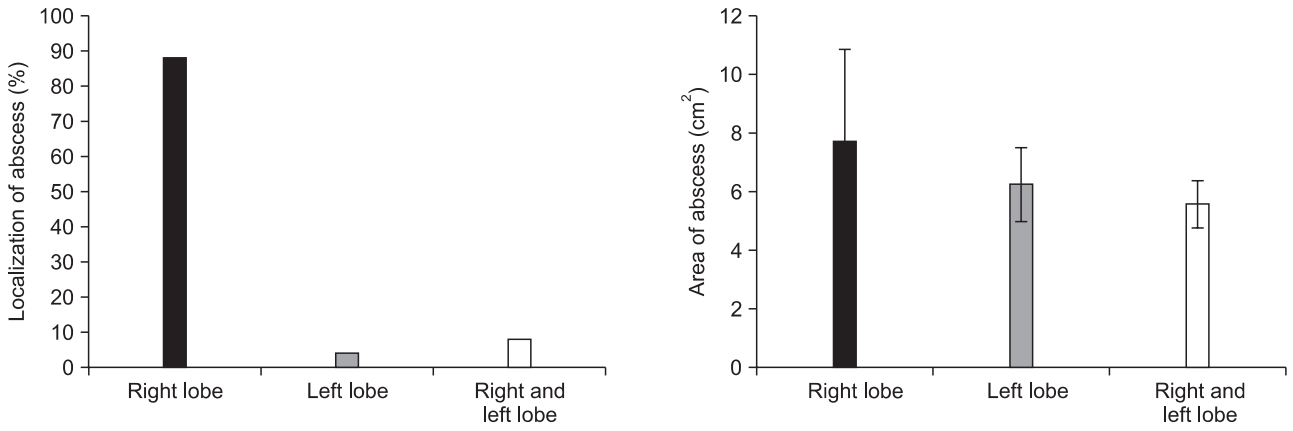

Fig. 2. Localization and area of amebic liver abscess in patients with invasive amebiasis.

Table 1. Correlation between Cholesterol and Some Variable Factors in Patients with an Amebic Liver Abscess

Variable factor	Cholesterol	
	Correlation coefficient	p-value
Age	0.326	>0.05
Gender, male/female	0.147	>0.05
Size of the abscess	-0.034	>0.05
Days of hospital stay	-0.073	>0.05
No. of the amebic abscesses	-0.236	>0.05
Localization of the amebic abscesses	0.173	>0.05

Pearson correlation was used for normally distributed variables, and for nonnormally distributed parameters, Spearman correlation coefficients were used. A contingency table was used for nominal variables. $p<0.05$ was considered to be statistically significant.

the area of the abscesses were calculated instead volume. Seventy percent ALA patients had a single abscess, and 30% had multiple abscesses. Ninety-four patients showed abscesses in the right lobe, nine had abscesses in both lobes, and four in the left lobe (Fig. 2). The size or localization of the amebic abscess did not present correlation with hypocholesterolemia, neither the numbers of the abscess ($p>0.05$). Thirteen patients underwent to needle aspiration, and four patients needed more than three functions (Table 1).

4. Hypocholesterolemia and days of hospital stay

Patients with ALA stay at the hospital an average of 10 days, with a minimum of 4 days and maximum 28 days. Hypocholesterolemia does not correlate with the hospital stay required by the patients ($p>0.05$). However, 84% patients whose cholesterol levels continue to decrease required more than 10 days at the hospital (Table 1).

5. Characteristics of patients with cholesterol values decreasing despite antiamebic treatment

The most common complaints among patients were abdominal pain, asthenia, and anorexia. Patients with cholesterol levels descending, despite receiving antiamebic treatment and hospital care, presented the most severe cases of amebiasis with rupture of the abscess or even death. These patients needed multiple percutaneous drainages and required 21 to 28 days at the hospital. All required multiple punctures despite antiamebic treatment. Three patients had multiple abscesses and the other three patients had a single abscess. The range of fluid drained in the punctures was 15 to 900 mL on each puncture. A patient died due that the abscess invaded the lung and had pleural effusion and liver dysfunction. His cholesterol level was 109 IU/L when he entered the hospital and the cholesterol levels continued decreasing until it reached a minimum level of 42 IU/L after 9 days at hospital.

6. Clinical laboratory and liver function tests from ALA patients

The median of triglycerides obtained from ALA patients levels were 89 mg/dL. High levels of triglycerides were observed in two ALA patients (253 and 505 mg/dL). The rest of these patients had normal levels. Healthy persons presented a median of 70 mg/dL. The differences in triglyceride levels among both groups were not significant ($p>0.05$) (Table 2).

All the ALA patients presented high white blood cell count 15,903±3,786 mm^3 (normal, 4,000 to 7,000 mm^3); high neutrophil count 82%±15% (normal, 35% to 70%) and low lymphocytes 13%±6% (normal, 20% to 50%). No correlation was found among hypocholesterolemia, and these features ($p>0.05$). The ALA patients showed results of other standard clinical laboratory tests within normal limits. Forty-three (49%) of the ALA patients had high AST, 40 (43%) ALT, 42 (45%) LDH, but no correlation was found with hypocholesterolemia neither the size of the abscess ($p>0.05$). Ninety-one (85%) of the ALA patients

Table 2. Correlation between Hypocholesterolemia and Standard Hematology Profile in Patients with an Amebic Liver Abscess

Test	Normal range	ALA patient (mean±SD)	Correlation coefficient	p-value
Hemoglobin, g/dL	12–16	12±4	0.239	>0.05
Hematocrit, %	37–47	33±6	0.308	>0.05
Leukocytes, /mm^3	4,800–10,800	15,903±3,786	0.038	>0.05
Lymphocytes, %	19–48	13±6	0.095	>0.05
PMN, %	40–74	82±15	0.209	>0.05
Triglycerides, mg/dL	<150	101±88	0.256	>0.05
Total bilirubin, mg/dL	0.1–1.0	2.4±2	0.158	>0.05
Direct bilirubin, mg/dL	0.0–0.4	1.5±2	0.009	>0.05
AST, IU/L	6–42	97.7±144	-0.057	>0.05
ALT, IU/L	10–56	67.5±70	-0.186	>0.05
LDH, IU/L	91–245	302.4±186	-0.034	>0.05
GGT, IU/L	7–64	145.5±123	0.010	>0.05
ALP, IU/L	30–121	207.8±109	0.083	>0.05
Total protein, g/dL	6.0–8.3	6.7±1	0.134	>0.05
A/G ratio	3.2–5.5	2.6±1	0.227	>0.05

Pearson correlation was used for normally distributed variables, and Spearman correlation coefficients were used for nonnormally distributed parameters. p<0.05 was considered to be statistically significant.
ALA, amebic liver abscess; PMN, polymorphonuclear; direct bilirubin, conjugated bilirubin; AST, aspartate aminotransaminase; ALT, alanine aminotransaminase; LDH, lactate dehydrogenase; GGT, gamma glutamyltranspeptidase; ALP, alkaline phosphatase; A/G, albumin/globulin.

Table 3. Correlation between Liver Function Tests and Abscess Size in Patients with an Amebic Liver Abscess

Test	Correlation coefficient	p-value
Triglycerides, mg/dL	0.229	>0.05
Total bilirubin, mg/dL	0.096	>0.05
Direct bilirubin, mg/dL	0.327	<0.05
AST, IU/L	0.282	>0.05
ALT, IU/L	0.150	>0.05
LDH, IU/L	0.021	>0.05
GGT, IU/L	0.388	<0.05
ALP, IU/L	0.419	<0.05
Total protein, g/dL	-0.213	>0.05
A/G ratio	-0.369	<0.05

Pearson correlation was used for normally distributed variables, and Spearman correlation coefficients were used for nonnormally distributed parameters. p<0.05 was considered to be statistically significant.
Direct bilirubin, conjugated bilirubin; AST, aspartate aminotransaminase; ALT, alanine aminotransaminase; LDH, lactate dehydrogenase; GGT, gamma glutamyltranspeptidase; ALP, alkaline phosphatase; A/G, albumin/globulin.

had low albumin to globulin (A/G) ratio, instead 74 (69%) had total and direct bilirubin elevated, 81 (76%) presented GGT elevated, 80 (75%) ALP. These features do not correlate with hypocholesterolemia (p>0.05) (Table 2); by contrast, these data are correlated to the size of the abscess (p<0.05). The larger the abscess is, more altered are the above test (Table 3).

DISCUSSION

The ALA patients studied in our series presented hypocholesterolemia in 93% of cases. Other authors reported abnormal cephalin-cholesterol flocculation test in patients with ALA and patients with intestinal amebiasis.[22,23] Bansal *et al*.[24] found significantly lower levels of lipid profile in ALA patients and cyst passers of *E. histolytica* or *E. dispar*, compared with healthy controls.

The laboratory features of ALA patients recorded in this study agreed with previous reports.[1,3,22] No correlation was found among hypocholesterolemia and data of laboratory standard tests or the liver function tests. Low A/G ratio and high total bilirubin, direct bilirubin, GGT, and ALP had a correlation with the size of the abscess as has been reported previously,[25] but not with hypocholesterolemia. Liver function tests in some ALA patients studied by us, suggests cholestasis, as has been reported previously.[26,27]

We did not find correlation between the hypocholesterolemia and the size of the abscess, nor their location or the numbers of the abscess. Patients with small abscesses show hypocholesterolemia as well as patients with big abscesses. We detected no correlation between hypocholesterolemia, and the hospital stay required by the patients. However, patients whose cholesterol levels continue to decrease after hospital care, and antiamebic treatment initiation, required multiple punctures and more days of hospitalization. Some of them presented the most serious cases of amebiasis with rupture of the abscess and pleural effu-

sion, or even death. In the contrary, cholesterol levels were rising in patients with initial low cholesterol values that responded to treatment. Gujral *et al*.[28] found triglycerides, cholesterol increased in liver of hamsters infected with *E. histolytica* trophozoites while these animals showed hyperlipidemia and hypocholesterolemia in serum. Our data from ALA patients does not agree with the above animal model, because we have detected normal values of triglycerides in sera; only two patients presented high values. Perhaps the difference in the data obtained by our group could be explained by differences in lipid processing between hamsters and humans. The hypocholesterolemia reported in hamsters models, tended to normalize in relation to the metronidazole treatment period.[28] It still remains to be determined the period required by ALA patients to recover the normal levels of cholesterol after antiamebic treatment.

The role of lipids is essential to maintaining the structure of membranes, participate in protein folding, in transport, signal transduction pathways, growth, differentiation, and the maintenance of cellular physiology.[29] *E. histolytica* lack mitochondria. However, they survive and multiply by scavenging nutrients from the host. It has been reported that *E. histolytica* is unable to synthesize the majority of their own lipids and cholesterol *de novo* and scrounge them on the host or the growth medium.[30] Apparently, *E. histolytica* take exogenous phospholipids to undergo fatty acid remodeling by deacylation/reacylation reactions, bypassing the synthesis of entirely new phospholipid molecules.[30] Phospholipases are considered key enzymes of the deacylation/reacylation of lipid molecules, and *E. histolytica* are rich in phospholipases and lysophospholipase important for cytolysis of target cells.[31] *E. histolytica* are capable of elongating/desaturating long-chain fatty acids, and assembling novel glycophospholipid molecules.[32,33] Lipid molecules stimulate the kinases expression. It has been proposed that trans membrane kinases may be important molecules involved in cellular proliferation, virulence and erythrophagocytosis of *E. histolytica*.[34] Important *E. histolytica* virulence factors are composed by lipids as the surface antigens Gal/GalNAc lectin and the lipophosphoglycanlike.[35-38] Antilipophosphoglycan antibodies reduce the ability of the parasite to invade host mammalian cells, and prevented ALAs.[39] Recently, Seifert *et al*.[40] reported that analogs of alkylphosphocholines possess antiamebic activity.

The above information indicates the importance of lipids in vital processes of amebas, so probably they consume cholesterol from the host to obtain and metabolize lipids. Most of the cholesterol moves through the enterohepatic circulation as *E. histolytica* do, therefore, we propose that protozoa mainly invade organs where cholesterol may be available in larger quantities like liver and intestine. Further studies are needed for better understanding how *E. histolytica* is involved in hypocholesterolemia.

This is the first report that correlates hypocholesterolemia with the severity of hepatic amebiasis. We propose that it is important to survey cholesterol levels to predict the outcome of patients with invasive amebiasis.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Espinosa-Cantellano M, Martínez-Palomo A. Pathogenesis of intestinal amebiasis: from molecules to disease. Clin Microbiol Rev 2000;13:318-331.
2. Katzenstein D, Rickerson V, Braude A. New concepts of amebic liver abscess derived from hepatic imaging, serodiagnosis, and hepatic enzymes in 67 consecutive cases in San Diego. Medicine (Baltimore) 1982;61:237-246.
3. Kapoor OP. Ameobic liver abscess [Internet] Bombay: S.S. Publishers; 1979 [cited 2011 Feb 2]. Available from: http://www.bhj.org.in/books/liver/s4c02.htm.
4. Lodhi S, Sarwari AR, Muzammil M, Salam A, Smego RA. Features distinguishing amoebic from pyogenic liver abscess: a review of 577 adult cases. Trop Med Int Health 2004;9:718-723.
5. Bansal D, Bhatti HS, Sehgal R. Role of cholesterol in parasitic infections. Lipids Health Dis 2005;4:10.
6. Das SR, Ghoshal S. Restoration of virulence to rat of axenically grown Entamoeba histolytica by cholesterol and hamster liver passage. Ann Trop Med Parasitol 1976;70:439-443.
7. Gill NJ, Ganguly NK, Mahajan RC, Bhusnurmath SR, Dilawari JB. Model of amoebic liver abscess in cholesterol-fed guinea pigs through intracaecal infection route. Indian J Med Res 1983;78:489-496.
8. Bhatti HS, Bhushnurmath S, Mahajan RC, Ganguly NK, Sehgal R. An experimental model of ameboma in guinea pig. Exp Parasitol 1992;74:283-289.
9. Mattern CF, Keister DB, Natovitz PC. Virulence of Entamoeba histolytica upon continuous axenic cultivation. Arch Invest Med (Mex) 1982;13 Suppl 3:185-190.
10. Phillips BP. Entamoeba histolytica: concurrent irreversible loss of infectivity-pathogenicity and encystment potential after prolonged maintenance in axenic culture in vitro. Exp Parasitol 1973;34:163-167.
11. Meerovitch E, Ghadirian E. Restoration of virulence of axenically cultivated Entamoeba histolytica by cholesterol. Can J Microbiol 1978;24:63-65.

12. Vinayak VK, Chakravarti RN, Agarwal KC, Naik SR, Chhuttani PN. Pathogenicity of Entamoeba histolytica: effect of cholesterol on the virulence of strains of amoebae. Indian J Med Res 1978;67:545-552.
13. Katiyar SK, Ghoshal S, Gupta AK, Das SR, Pandey VC, Sagar P. Action of cholesterol on virulence related biochemical functions of Entamoeba histolytica. Indian J Exp Biol 1988;26:848-850.
14. Serrano-Luna J, Gutiérrez-Meza M, Mejía-Zepeda R, Galindo-Gómez S, Tsutsumi V, Shibayama M. Effect of phosphatidylcholine-cholesterol liposomes on Entamoeba histolytica virulence. Can J Microbiol 2010;56:987-995.
15. Goldston AM, Powell RR, Temesvari LA. Sink or swim: lipid rafts in parasite pathogenesis. Trends Parasitol 2012;28:417-426.
16. Welter BH, Goldston AM, Temesvari LA. Localisation to lipid rafts correlates with increased function of the Gal/GalNAc lectin in the human protozoan parasite, Entamoeba histolytica. Int J Parasitol 2011;41:1409-1419.
17. Flores MS, Tamez-Treviño E, Castañeda F, Tijerina-Menchaca R, Galan-Wong L, Rangel R. Preparation of Entamoeba histolytica antigens without enzymatic inhibitors. Parasitology 2005;131(Pt 2):231-236.
18. Flores-de-Castañeda MS, inventor. Procedure to preserve antigens of Entamoeba histolytica without enzymatic inhibitors. United States patent US 5,459,042. 1995 Oct 17.
19. Flores-de-Castañeda MS, inventor; Universidad Autonoma De Nuevo Leon, assignee. Procedure to preserve antigens of Entamoeba histolytica without enzymatic inhibitors and their use in immunological methods. United States patent 5,861,263. 1999 Jan 19.
20. Flores-de-Castañeda MS, inventor. Procedimiento para la preservación de moléculas antigénicas sin el uso de inhibidores enzimáticos. Mexican patent 209646. 2002.
21. Flores-de-Castañeda MS, inventor; Universidad Autonoma De Nuevo Leon, assignee. Procedimiento para la preservación de moléculas antigénicas sin el uso de inhibidores enzimáticos y su aplicación en métodos inmunológicos. Mexican patent 209648. 2002.
22. Viranuvatti V, Harinasuta T, Plengvanit U, Choungchareon P, Viranuvatti V. Liver function test in hepatic amebiasis, based on 274 clinical cases. Am J Gastroenterol 1963;39:345-361.
23. Magill GB, Killough JH. Plasma cholinesterase and other liver function tests in hepatic amebiasis. J Lab Clin Med 1958;51:333-344.
24. Bansal D, Bhatti HS, Sehgal R. Altered lipid parameters in patients infected with Entamoeba histolytica, Entamoeba dispar and Giardia lamblia. Br J Biomed Sci 2005;62:63-65.
25. Salako LA. Liver function tests in the diagnosis of hepatic amoebiasis. J Trop Med Hyg 1967;70:19-22.
26. Nigam P, Gupta AK, Kapoor KK, Sharan GR, Goyal BM, Joshi LD. Cholestasis in amoebic liver abscess. Gut 1985;26:140-145.
27. Salles JM, Moraes LA, Salles MC. Hepatic amebiasis. Braz J Infect Dis 2003;7:96-110.
28. Gujral S, Patel N, Chaudhuri SK, Seth D. Altered lipid profile in liver amoebiasis and its emendation with metronidazole treatment. Indian J Physiol Pharmacol 1982;26:240-245.
29. Das S, Stevens T, Castillo C, Villasenõr A, Arredondo H, Reddy K. Lipid metabolism in mucous-dwelling a mitochondriate protozoa. Int J Parasitol 2002;32:655-675.
30. Das S, Castillo C, Stevens T. Phospholipid remodeling/generation in Giardia: the role of the Lands cycle. Trends Parasitol 2001;17:316-319.
31. Long-Krug SA, Fischer KJ, Hysmith RM, Ravdin JI. Phospholipase A enzymes of Entamoeba histolytica: description and subcellular localization. J Infect Dis 1985;152:536-541.
32. Sawyer MK, Bischoff JM, Guidry MA, Reeves RE. Lipids from Entamoeba histolytica. Exp Parasitol 1967;20:295-302.
33. Moody S, Becker S, Nuchamowitz Y, Mirelman D. Virulent and a virulent Entamoeba histolytica and E. dispar differ in their cell surface phosphorylated Glycolipids. Parasitology 1997;114(Pt 2):95-104.
34. Shrimal S, Saha A, Bhattacharya S, Bhattacharya A. Lipids induce expression of serum-responsive transmembrane kinase EhTMKB1-9 in an early branching eukaryote Entamoeba histolytica. Sci Rep 2012;2:333.
35. Petri Wa Jr. Amebiasis and the Entamoeba histolytica Gal/GalNAc lectin: from lab bench to bedside. J Investig Med 1996;44:24-36.
36. Moody S, Becker S, Nuchamowitz Y, Mirelman D. Identification of significant variation in the composition of lipophosphoglycan-like molecules of *E. histolytica* and E. dispar. J Eukaryot Microbiol 1998;45:9S-12S.
37. Srivastava G, Anand MT, Bhattacharya S, Bhattacharya A. Lipophosphoglycan is present in distinctly different form in different Entamoeba histolytica strains and absent in Entamoeba moshkovskii and Entamoeba invadens. J Eukaryot Microbiol 1995;42:617-622.
38. Bhattacharya A, Satish S, Bagchi A, Bhattacharya S. The genome of Entamoeba histolytica. Int J Parasitol 2000;30:401-410.
39. Marinets A, Zhang T, Guillén N, et al. Protection against invasive amebiasis by a single monoclonal antibody directed against a lipophosphoglycan antigen localized on the surface of Entamoeba histolytica. J Exp Med 1997;186:1557-1565.
40. Seifert K, Duchêne M, Wernsdorfer WH, et al. A new approach for chemotherapy against Entamoeba histolytica. Arch Med Res 2000;31(4 Suppl):S6-S7.