Annals of Parasitology 2022, 68(3), 501–506
doi: 10.17420/ap6803.456

Original paper

Gene expression of RD5 and Psp5 in extra intestinal *Entamoeba histolytica* isolated from liver abscesses

Abdulla L. JIAD[1], Salwa S. MUHSIN[2], Montaha A. AL-SAFAR[2] , Tahreer Hadi SALEH[3]

[1]Department of Anesthesia Techniques, Medical Technical Institute, Baghdad-Middle Technical University, Iraq
[2]Department of Community Health, Medical Technical Institute, Baghdad-Middle Technical University, Iraq
[3]Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

Corresponding Author: Tahreer Hadi Saleh; email: tahreerhadi@gmail.com

ABSTRACT. Liver abscesses are known to be trophozoites of the amoeba parasite. They are devoured by the neutrophil cells in the liver and become large assemblies because these white cells do not eliminate the parasite and these white cells multiply. In this study, venous blood samples were taken from 61 patients have hepatic amoebosis and 61 healthy individuals as a control group. The patients attended Ghazi Al-Hariri Surgical Specialities Hospital, the Medical City, Baghdad, Iraq during the period from 15th January to 18th September 2021. The results showed that the mean age of patients was (41.84±15.88) years, while the mean age of the control group was (41.84±15.88) years with no significant difference (P>0.05). The prevalence rate of *Entamoeba histolytica* infection was 27 (44.2%) in males, and 34 (55.8%) in females with no significant difference. The mean anti-*Entamoeba* antibody IgA in urban areas was (1.95±1.25) and in the rural areas was (2.05±1.10), while the mean anti-*Entamoeba* antibody IgG in urban areas was (14.86±6.71), and in the rural areas was (13.55±7.43), with no significant differences (P>0.05). The mean anti-*Entamoeba* antibody IgA was (2.00±1.17) among the patient's group in comparison with the control group which was (0.09±0.17), while the mean anti-*Entamoeba* antibody IgG was (14.20±7.06) among the patients when compared with the control group which was (0.06±0.11) with highly significant differences (P<0.01). Expression of RD5 gene was investigated in *E. histolytica* in liver abscess patients and healthy controls by using qRT-PCR and the findings of amplification regarding atypical amplification plot. The amplification reaction had an early threshold cycle that was consistent with high levels of RD5 gene and the healthy controls. Psp5 gene was expression to investigated *E. histolytica* in liver abscess in 60 patients and (60) individuals as a control group by using qRT-PCR and the findings of amplification regarding atypical amplification plot. The amplification reaction had an early threshold cycle that was consistent with high levels of Psp5 gene and the healthy controls.

Keywords: gene expression, extra intestinal *Entamoeba histolytica*, liver abscess

Introduction

Amebosis is usually an intestinal infection that occurs relatively frequently, especially in young ages, and the trophozoites are inside the intestinal cavity and are asymptomatic in most cases [1]. They remain restricted to the intestinal cavity (not invasive luminal amebosis) [2]. When there are symptoms, which develop following incubation periods of 2–4 weeks and caused by trophozoite that invade mucosal cells of the intestine (this is the gaseous form) leading to acute colitis or associated amebic dysenteries, diarrhoea and mucous with blood [3]. In certain infected children, fever may occur due to the invasion of these intestinal parasites [4]. Adult amoebic colitis is limited and the symptom perhaps not appear in 4–7 days. Some people can have chronic infection which may last several month then disappear automatically [5]. The infection may develop into accompanied colitis and the appearance of ulcers in the mucous membrane of the intestine, which may include the muscular mucosa and lead to perforation of the intestine, and invade the intestinal tissues deeper layer leading to the departure of amebic activators to the portal circulations with the invasion of the hepatic tissue

Table 1. Distribution of study groups according to age

Parameter	Groups	Mean±SD	*P*-value (CS.)
Age	Cases (n=61)	41.84±15.88	*P*=0.730
	Control (n=61)	41.84±15.88	*P*>0.05 (NS)

Table 2. Distribution of *Entamoeba histolytica* infection according the gender

Gender	Patients group (61)	%
Male	27	44.2
Female	34	55.8
	χ_2 test (*P*-value)	*P*>0.05 (NS)

of the liver tissue and then progress to the occurrence of liver abscesses amoebosis [6]. Brain, pleura, pericardium, genitourinary tract or skin invasions occurs in very rare cases and symptoms vary from one affected organ to another [3]. Because of amoebic liver abscess extensions to abdominal walls, or existence of an invasive gastrointestinal fistula which may get access to the patient's abdominal walls [7]. The occurrence of cutaneous amoebosis in the genitals resulting from abnormal sexual practices, and the lesion may lead to the appearance of ulcers, inflammations, necrosis with severe damage of the genital regions [8]. *E. histolytica* has much potential to damage the intestinal mucosa and cause disease [9]. Trophozoites secrete several cysteine enzymes, which degrade myosin and extracellular lysate [10]. *E. histolytica* engulfs erythrocytes as well as nucleated cell in the host's body, and has the ability to induce virulence besides engulfing nucleated cells [11].

Materials and Methods

In the current study, venous blood samples were taken from 61 patients with liver abscess and from 61 healthy individuals as a control group. The patients attended Ghazi Al-Hariri Surgical Specialities Hospital, the Medical City, Baghdad, Iraq during the period from 15th January to 18th September 2021. For detection of the specific anti-*Entamoeba histolytica* IgG and IgA in human sera, the *Entamoeba* test (R-B, AG-Germany) has been used. The procedure uses a microtiter plate coated with purified antigen. Serum samples are loaded as 1:50 in sample diluents and incubated at room temperature for 15 minutes. The freehand method is used since it has no restriction of the attached needle guide, which requires that the needle be passed in fixed or specific angles relative to the transducers. The freehand method permits changing of the needle's path. Local anesthesia has been applied using 10 ml of 2% lidocaine hydrochloride. During the freehand procedure, patients have been asked about any pains and if they required conscious sedations. Then the form is withdrawn from the patients to be examined. To determine the gene expression in RD5 and Psp5 genes, the primers were used:
RD5 SS rRNA GGAAGCTTATCTGGTTGATCCT GCCAGTA 1950;
Psp5 SS rRNA GGCCAATTCATTCAATGAATTG AG 876

Statistical analysis

Data analysis was done by using the SPSS Vr.24 program and t-test with Mont Carlo test (MCP) were applied at 5% and1% levels of significance [12].

Results

Table 1 showed that the patients' mean **(**mean±SD) age was (41.84±15.88) years, and the control group's mean age was (41.84±15.88), with no significant difference (*P*>0.05).

According to gender, the prevalence rate of *Entamoeba histolytica* infection was shown to be 27 (44.2%) in males and 34 (55.8%) in females with no significant difference as shown in table 2, (*P*>0.05, NS).

Regarding residency, the mean serum anti-

Table 3. Distribution of mean serum *Entamoeba* IgA and IgG according to residency

Parameter			Mean±SD	t-test	P-value
	Residency	N			(CS.)
Entamoeba IgA	Urban	30	1.95±1.25	0.350	*P*=0.728
	Rural	31	2.05±1.10		*P*>0.05 (NS)
Entamoeba IgG	Urban	30	14.86±6.71	0.723	*P*=0.473
	Rural	31	13.55±7.43		*P*>0.05 (NS)

Table 4. Comparison between the studied groups according to mean serum *Entamoeba* IgA and IgG

Parameter	Groups	Mean±SD	*P*-value (CS.)
Entamoeba IgA	Cases (n=61)	2.00±1.17	*P*=0.000
	Control (n=61)	0.09±0.17	*P*<0.01 (HS)
Entamoeba IgG	Cases (n=61)	14.20±7.06	*P*=0.000
	Control (n=61)	0.06±0.11	*P*<0.01 (HS)

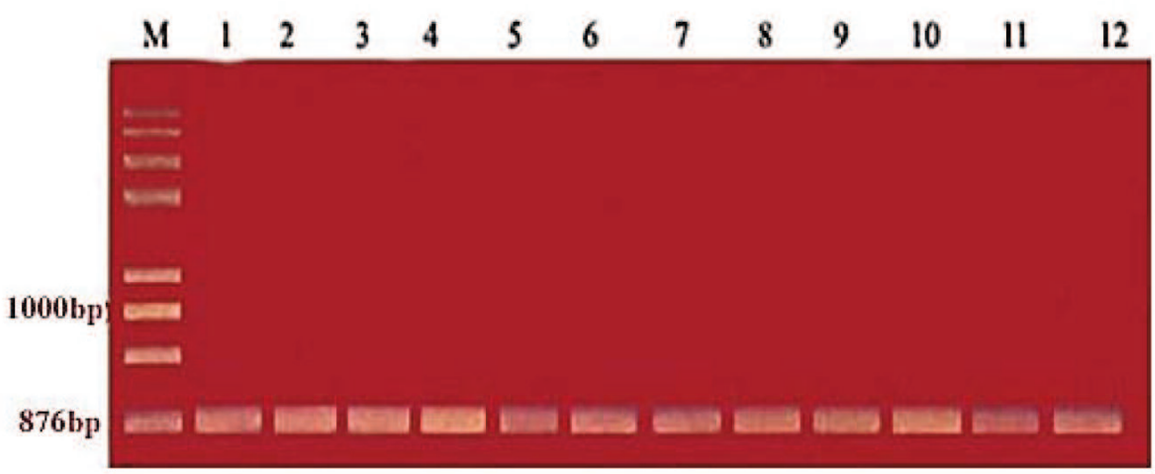

Figure 1. In 1000 bp DNA ladders: (1) Positive control for RD5 genes of *E. histolytica* strains; (2) H20 sample; (3–12) DNA obtained through drainage from amoebic liver abscess patients. The product was amplified and stained using ethidium bromide. The product's size was 876 bp

Entamoeba antibody IgA was (1.95±1.25) in urbans and (2.05±1.10) in rural residents, while the mean serum anti-*Entamoeba* antibody IgG in urbans was (14.86±6.71) and in rural residents was (13.55±7.43) with no significant differences (*P*>0.05) as shown in table 3.

The mean anti-*Entamoeba* antibody IgA was (2.00±1.17) among the patient's group in comparison with the control group which was (0.09±0.17), while the mean anti-*Entamoeba* antibody IgG was (14.20±7.06) among the patients when compared with the control group which was (0.06±0.11) with highly significant differences (*P*<0.01) as shown in table 4.

In amplification using nested PCR test to inflate the piece 98bp of RD5 gene of the DNA extracted by ready kit for all categories. Following the reaction stage completion within the (Thermo-cycler), the reaction solution was deported electrically using the 2.5% agarose gel and using the volumetric guide DNA ladder (M) of 1000 bp size.

Expression of RD5 gene was investigated in *E.*

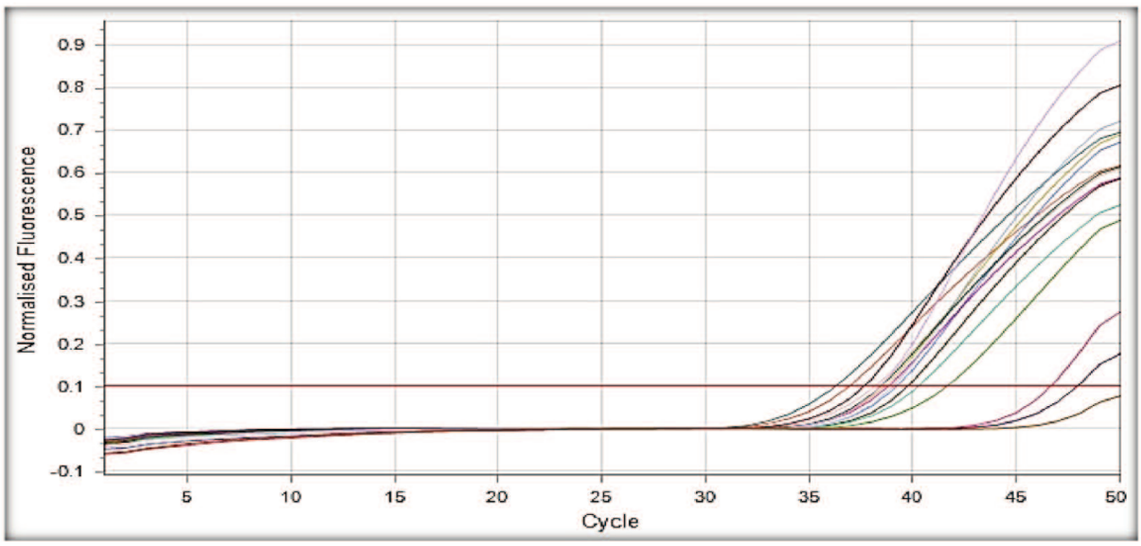

Figure 2. Melting curve analysis using real time PCR for recognitions of *E. histolytica* RD5 gene in liver abscess patients

histolytica in liver abscesses (aspiration) and healthy control by using qRT-PCR the findings of amplification, the findings of amplification were highly positive for the patients compared to the healthy control as demonstrated in figure 2.

Expression of Psp5 gene was investigated in *E. histolytica* in liver abscesses and healthy control by using qRT-PCR. The findings of amplification were highly positive for the patients compared to the healthy controls as illustrated in figure 3.

Discussion

E. histolytica is the intestinal parasite causing amoebic dysentery, but it has an access to the bloodstream through penetration into the intestinal tissue, leading to abscesses of the liver and other organs [1]. It was found that there are no significant differences for those infected with amoebic dysentery compared to the healthy control group, and these findings agreed with [13] who found there is no significant difference between those infected with the dysentery amoeba parasite at different ages compared to the control group, and that all ages are exposed to infection with this parasite without exception. Also, there were no differences between those infected in rural areas and those infected with *Entamoeba histolytica* in urban areas in Baghdad city. This is due to water contamination at the present time for cities and the corresponding rural

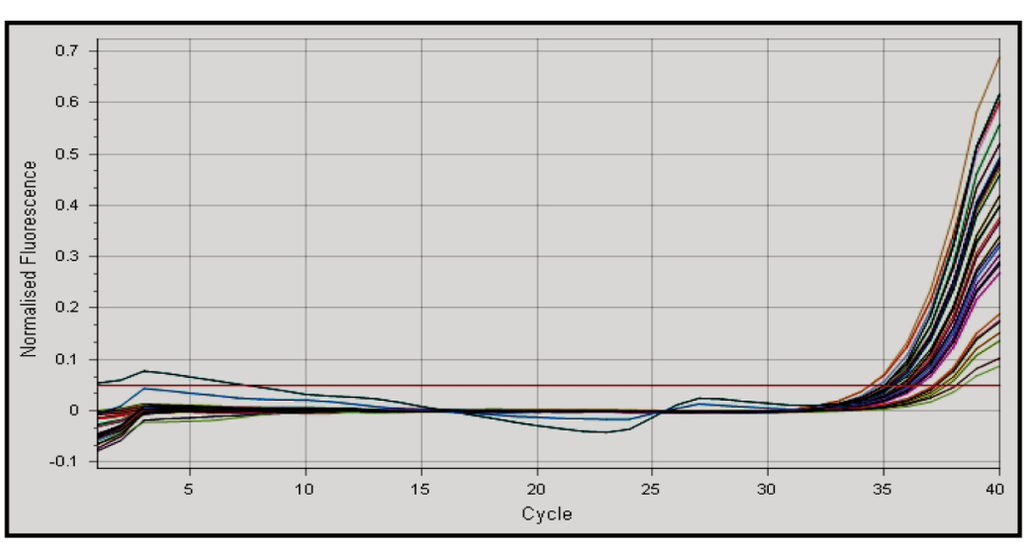

Figure 3. Melting curve analysis by real time PCR for recognitions of *E. histolytica* Psp5 gene in liver abscess patients

areas. Hammood and Bakr [14] mentioned in their report that the cause of the spread of the parasite, whether it is in the countryside or the city, is due to water pollution, and there are no standards for water sterilization in a proper manner that prevents infection with the parasite cysts. The results showed that there was a high rise in the levels of anti-*Entamoeba* IgA and anti-*Entamoeba* IgG for invasive *Entamoeba histolytica* in the same way as it is with the control group. Abd Al-Khaliq and Mahdi [15] reported that the levels of anti-*Entamoeba* IgA rises highly when infected with *Entamoeba histolytica* parasite when the parasite invades body tissues through the bloodstream. IgG levels also rise when the infection has reached its place and has become chronic. Genotyping of *Entamoeba* parasite was performed by agarose gel with product size 876 bp to identify RD5 gene. The results matched with [16], who stated that the genotyping of invasive *Entamoeba histolytica* by multiple genes was used by the region (228 bp) of the adh112 gene by electrophoresis on agarose gel. Also, Ibrahim et al. [17] reported that agarose gel with product size 580 bp was used to identify the specific genes of this parasite. For expression of the RD5 and Psp5, the gene was investigated in *E. histolytica* in liver abscesses and healthy control by using real time PCR, and the findings of amplification were highly positive for the patients compared to the healthy controls. The results of amplification were in a harmony with González-Rivas et al. [18] who showed the expression of gene of identified *Entamoeba* liver abscesses by using real time technique. Also Martínez-Hernández et al. [19] agreed with our results as he showed that the mechanism of the genetic expression on the Psp5 gene were giving positive results for all patients with liver abscesses ameobosis. We recommend works sequencing of both genes targeted in this study.

We concluded that hepatocellular amoeba parasite invasion of the liver cell was determined by the gene expression of the genes RD5 and Psp5 genes.

References

[1] Tharmaratnam Th., Kumanan Th., Iskandar M.A. et al. 2020. *Entamoeba histolytica* and amoebic liver abscess in northern Sri Lanka: a public health problem. *Tropical Medicine and Health* 48: article number 2. doi:10.1186/s41182-020-0193-2

[2] Carrero J.C., Reyes-López M., Serrano-Luna J., Shibayama M., Unzueta J., León-Sicairos N., de la Garza M. 2020. Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. *International Journal of Medical Microbiology* 310(1): article number 151358. doi:10.1016/j.ijmm.2019.151358

[3] González-Rivas E., Nieves-Ramírez M., Magaña U., Morán P., Rojas-Velázquez L., Hernández E., Serrano-Vázquez A., Partida O., Pérez-Juárez H., Ximénez C. 2020. Differential pathogenic gene expression of *E. histolytica* in patients with different clinical forms of amoebiasis. *Microorganisms* 8(10): article number 1556. doi:10.3390/microorganisms8101556

[4] Junaidi J., Cahyaningsih U., Purnawarman T., Latif H., Sudarnika E., Hayati Z., Muslina M. 2020. *Entamoeba histolytica* neglected tropical diseases (NTDs) agents that infect humans and some other mammals: a review. *E3S Web of Conferences* 151(1): article number 01019. doi:10.1051/e3sconf/202015101019

[5] Gonzales M.L.M., Dans L.F., Sio Aguilar J. 2019. Antiamoebic drugs for treating amoebic colitis. *Cochrane Database of Systematic Reviews* 2019(1): article number CD006085. doi:10.1002/14651858.CD006085.pub3

[6] Nakada-Tsukui K., Nozaki T. 2016. Immune response of amebiasis and immune evasion by *Entamoeba histolytica*. *Frontiers in Immunology* 7: article number 175. doi:10.3389/fimmu.2016.00175

[7] Pawar S.V., Zanwar V.G., Gambhire P.A., Mohite A.R., Choksey A.S., Rathi P.M., Asgaonkar D.S. 2015. Unusual complication of amebic liver abscess: hepatogastric fistula. *World Journal of Gastrointestinal Endoscopy* 7(9): 916–919. doi:10.4253/wjge.v7.i9.916

[8] Morán P., Rojas L., Cerritos R. et al. 2013. Cutaneous amebiasis: the importance of molecular diagnosis of an emerging parasitic disease. *American Journal of Tropical Medicine and Hygiene* 88(1): 186–190. doi:10.4269/ajtmh.2012.12-0278

[9] Kantor M., Abrantes A., Estevez A., Schiller A., Torrent J., Gascon J., Hernandez R., Ochner C. 2018. *Entamoeba histolytica*: updates in clinical manifestation, pathogenesis, and vaccine development. *Canadian Journal of Gastroenterology and Hepatology* 2018: article number 4601420. doi:10.1155/2018/4601420

[10] Talamás-Lara D., Chávez-Munguía B., González-Robles A., Talamás-Rohana P., Salazar-Villatoro L., Duran-Diaz A., Martinez-Palomo A. 2014. Erythrophagocytosis in *Entamoeba histolytica* and *Entamoeba dispar*: a comparative study. In: Immunology and cell biology of parasitic diseases 2014. (Eds. L.I. Terrazas, A.R. Satoskar, M. Rodriguez-Sosa, A. Landa-Piedra). BioMed Research International vol. 2014: article ID 626259.

doi:10.1155/2014/626259
[11] Medina-Rosales M.N., Muñoz-Ortega M.H., García-Hernández M.H., Talamás-Rohana P., Medina-Ramírez I.E., Salas-Morón L.G., Martínez-Hernández S.L., Ávila-Blanco M.E., Medina-Rosales B., Ventura-Juárez J. 2021. Acetylcholine upregulates *Entamoeba histolytica* virulence factors, enhancing parasite pathogenicity in experimental liver amebiasis. *Frontiers in Cellular and Infectious Microbiology* 10: article number 586354. doi:10.3389/fcimb.2020.586354
[12] Ying L., Fang J., Lu T., Hua J. 2015. Advanced medical statistics. 2nd ed. World Scientific.
[13] Al-Shaibani W.S. 2020. Infection with *Entamoeba histolytica* and its effect on some blood parameters in Najaf City. *Journal of Physics: Conference Series* 1660(1): article number 012008. doi:10.1088/1742-6596/1660/1/012008
[14] Mahmood S.A.F., Bakr H.M. 2020. Molecular identification and prevalence of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* in Erbil City, Northern Iraq. *Polish Journal of Microbiology* 69(3): 263–272. doi:10.33073/pjm-2020-028
[15] Abd Al-Khaliq I.M., Mahdi B.M. 2018. Association between *Entamoeba histolytica* infection and human leukocyte antigen HLA-DRB1. *Annals of Medicine and Surgery* 36: 71–74. doi:10.1016/j.amsu.2018.10.019
[16] López-López P., Martínez-López M.C., Boldo-León X.M., Hernández-Díaz Y., González-Castro T.B., Tovilla-Zárate C.A., Luna-Arias J.P. 2017. Detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in clinical samples through PCR-denaturing gradient gel electrophoresis. *Brazilian Journal of Medical and Biological Research* 50(4): e5997. doi:10.1590/1414-431x20175997
[17] Ibrahim A.M., Abdulkaleq R., Al-Mayah Sh. 2019. Molecular identification and genetic diversity of *Entamoeba* species from diarrheic patients in Baqubah/Iraq. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 10(1): 951–960.
[18] Ghelfenstein-Ferreira T., Gits-Muselli M., Dellière S., Denis B., Guigue N., Hamane S., Alanio A., Bretagne S. 2020. *Entamoeba histolytica* DNA detection in serum from patients with suspected amoebic liver abscess. *Journal of Clinical Microbiology* 58(10): e01153-20. doi:10.1128/JCM.01153-20
[19] Martínez-Hernández S.L., Muñoz-Ortega M.H., Becerra-González V.M., Muñoz-Ortega M.H., Loera-Muro V.M., Ávila-Blanco M.E., Medina-Rosales M.N., Ventura-Juárez J. 2021. Evaluation of the PEΔIII-LC3-KDEL3 chimeric protein of *Entamoeba histolytica*-lectin as a vaccine candidate against amebic liver abscess. *Journal of Immunology Research* 2021: article number 6697900. doi:10.1155/2021/6697900

Received 04 May 2022
Accepted 23 July 2022