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Original Research

Genetic variability of *E. histolytica* strains based on the polymorphism of the SREHP gene using nested PCR-RFLP in Erbil, North Iraq

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Abstract: *E. histolytica* is an intestinal parasite that causes asymptomatic infection mostly; however, it may also cause amoebic dysentery and liver abscess. Molecular identification is required in epidemiological studies due to the presence of morphologically identical nonpathogenic species. Therefore, this study was conducted to first evaluate the prevalence rate of *E. histolytica* among symptomatic individuals of Erbil city, and to investigate the genetic diversity of the parasite in a limited geographic area. Accordingly, a total of 2026 samples were examined microscopically, and confirmed by nested PCR for 18s rRNA gene. The results showed that the prevalence rate of *E. histolytica* was 1.97% (40 samples) among symptomatic patients. The SREHP gene was used as a marker to show the genetic polymorphism of *E. histolytica*; however, to compare the genetic diversity of symptomatic with asymptomatic isolates, 57 asymptomatic samples were obtained from our previous study. The amplified products of the SREHP gene were digested by *Alu*I endonuclease, and DNA banding patterns were analysed. Results showed 29 different DNA patterns among the 97 symptomatic and asymptomatic samples, 62 of which shared similar DNA patterns. However, 8 different DNA patterns were observed among asymptomatic samples, whereas 15 distinct patterns were observed among symptomatic isolates. In conclusion, this study found that the prevalence rate of *E. histolytica* was relatively low; relatively high genetic diversity was observed in a restricted endemic area; with higher rates of variability in symptomatic rather than in asymptomatic isolates, indicating a possible correlation between the genotype of *E. histolytica* and their clinical outcome.

Key words: *E histolytica*, genetic variability; SREHP; Polymorphism; RFLP; Molecular epidemiology.

Introduction

Entamoeba histolytica is a pathogenic parasite that inhabits the human gastrointestinal tract. This parasite may cause asymptomatic infections in approximately 90% of people or invasive amoebic dysentery and may also extend to extraintestinal locations, such as the liver, causing an amoebic liver abscess (1). The differences reported in the clinical outcome of the infections may be due to genetic variability and inter-strain-related virulence produced as a result of the presence of highly repetitive AT regions in the genomic sequence of *E. histolytica* (2). Isoenzyme analysis first depended on strain variation among isolates of *E. histolytica*, but due to the limited diversity of this method, it has been replaced by strain typing polymerase chain reaction (3). Several studies have reported several polymorphic markers of *E. histolytica,* such as short tandem repeats (STRs) in the tRNA, SREHP, and Chitinase genes and retrotransposons, using single nucleotide polymorphism and oligonucleotide microarray-based genotyping (2). Some studies have used the SREHP polymorphism to evaluate the existence of *E. histolytica* strains in certain geographic locations and to analyse the parasite population structure in nature (3-6). No study has been conducted in Iraq on the strains of *E. histolytica,* and there is limited information about the inter-species variation present in the region. Therefore, this study was conducted as the first report on the genetic diversity of *E. histolytica* that exists in Iraq and to show whether there is a correlation between the genetic makeup of the strains with virulence and the clinical outcome of the infection.

Materials and Methods

In the present study, stool samples were collected from symptomatic patients with gastrointestinal complaints and dysentery, while samples from asymptomatic individuals who did not show signs and symptoms were obtained from our previous study (unpublished data). A total of 57 positive samples from asymptomatic individuals were used in this study, which were confirmed to be positive for *E. histolytica* by PCR. Stool samples from 2026 symptomatic patients were collected from January 2019 to July 2019 and obtained from the central laboratory of the Kurdistan region. Samples were examined microscopically, and the positive samples were kept at -80°C for subsequent molecular analysis.

DNA was extracted from the positive stool samples using the QiaAmp Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's procedure. At the final step, DNA was eluted in 100 µl of a low salt buffer, and concentrations were read using a NanoDrop spectrophotometer. The positive control DNA of *E. histolytica* HM-1: IMSS standard strain was provided by Dr. Bahrami (Zoonoses Research Center, Kurdistan

University of Medical Sciences, Sanandaj, Iran) (7).

Molecular identification of *E. histolytica* targeted the 18S-ribosomal RNA gene by nested PCR amplification of the 439 bp final product as described by Khairnar and Parija (8, 9). After confirming that the samples were positive for *E. histolytica* by PCR, the genomic DNA of the positive sample was used for amplifying the SREHP gene (10). Nested PCR initially amplified 549 bp of the SREHP gene utilizing the first primer set SREHP5–5' GCT AGT CCT GAA AAG CTT GAA GAA GCT G 3', SREHP3–5' GGA CTT GAT GCA GCA TCA AGG T 3'. Then, the fragment from the product of the first PCR was targeted using the second set of primers nSREHP5–5' TAT TAT CGT TAT CTG AAC TAC TTC CTG 3', nSREHP3–5' TGA AGA TAA TGA AGA TGA TGA AGA TG 3 amplifying 450 bp. PCR was performed in a 20 µl reaction volume consisting of 10 µl of Hot Star Master Mix (Taq DNA polymerase 1 unit/10 µl, 2x reaction buffer, enzyme stabilizer, 4 mM MgCl2, sediment, 0.5 mM each of dATP, dCTP, dGTP, dTTP, pH 9 and loading dye) (GenNet Bio, Korea), 2 µl of template DNA, 2 µl of both primers (forward and reverse) and 6 µl of double deionized water. PCR conditions were optimized to consist of a single cycle of initial denaturation at 95 ºC for 10 minutes, followed by 30 cycles of 95ºC for 30ºC sec, 65ºC for 30 sec and 72ºC for 20 sec, with a final extension of 72 ºC for 5 minutes using a thermal cycler (Techne Ltd., Cambridge, UK). Similar conditions were applied for the second round of PCR except that the number of cycles rose to 40 cycles and the annealing temperature decreased to 62ºC. PCR products were electrophoresed on a 1.5% agarose gel in 1X Tris-boric acid-EDTA buffer (TBE), visualized by UV light after staining with 0.2 mg/ml ethidium bromide (Sigma), using a ladder size of 100 bp as a marker (Promega) (11).

All primers used in the study were subjected and aligned with the genomic database of all organisms through the National Center for Biotechnology Information (NCBI) and found to be specific for this research.

*Alu*I digestion

The nested PCR product of the SREHP gene was digested using restriction endonuclease *Alu*I (Promega, USA); 12 µl of PCR products were digested for 35 minutes at 37ºC, and then the enzyme was inactivated at 65 ºC for 15 minutes, following the manufacturer's procedure. The digested products were separated by electrophoresis in 2.5% agarose gels; then, ethidium bromide-stained gels were visualized by UV light, and fragment sizes were assessed using a 50 bp DNA ladder (Promega, USA).

Results

Among the 2026 examined stool samples from symptomatic patients, light microscopy showed 63 samples positive for trophozoites and/or cysts of *Entamoeba* species, which is equivalent to 3.1% based on their morphological features only. However, nested PCR analysis for the 18S rRNA gene revealed that the 439 bp band documented 40 positive samples for *E. histolytica,* which is equal to 1.97% of the total symptomatic samples (Figure 1). The 57 positive samples from asymptomatic individuals were confirmed to be positive for *E. histolytica* by PCR and used in the current study to show their genetic diversity.

The amplification products of the SREHP gene by nested PCR for both asymptomatic and symptomatic samples showed considerable polymorphisms; however, the majority of samples amplified the predicted product size, 450 bp (Figure 2, 3). Gel electrophoresis analysis for the SREHP gene showed the 450 bp band in 67 samples (69.07%) of 97 total samples, consisting of

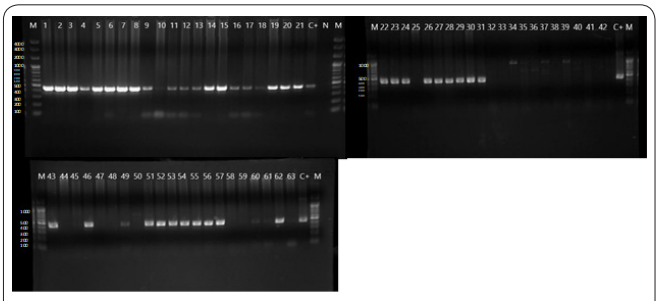

Figure 1. Agarose gel electrophoresis for nested PCR products of the 18S-rRNA gene for symptomatic samples. Positive samples for *Entamoeba histolytica* revealed a 439 bp band presented in lane numbers (1-9), (11-21), (22-24), (26-31), 43, 46, 49, (51-57) and 62. C+ represents the positive control, N represents the negative control, and M is a 100 bp DNA marker.

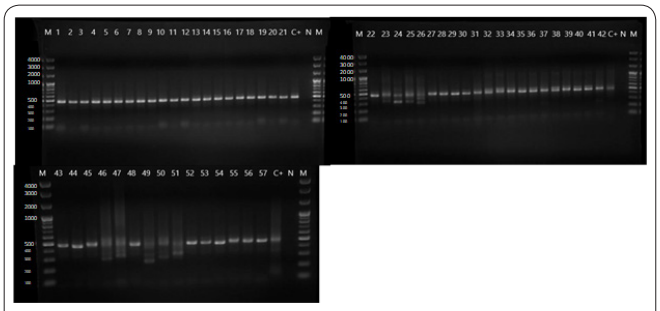

Figure 2. Nested PCR products of the SREHP gene for asymptomatic samples, analysed by agarose gel electrophoresis and visualized by UV light. Lane (1-22), (27-31), 34, 35, 37,39, (42-44), 48 and (52-57) reveal 450 bp band; lane 23, 47 and 51 reveal 350 and 450 bands; lanes 24, 25, 46 and 50 reveal 300 and 450 bp bands; lane 26 reveal 300, 400 and 450 bp bands; lanes 32, 33, 36, 38, 40, and 41 reveal 450 bp doublet bands; lane 45 reveals a 500 bp band; lane 49 reveals 250 and 450 bp bands; C+ represents the positive control, N represents the negative control, and M is 100 bp DNA markers.

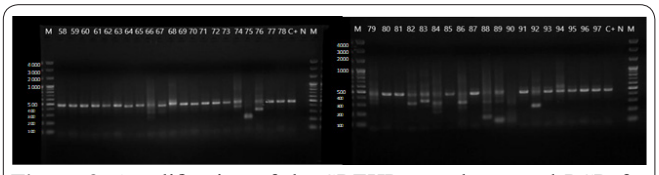

Figure 3. Amplification of the SREHP gene by nested PCR for symptomatic diarrhoeal isolates documented by UV visualization and analysed by agarose gel electrophoresis. Lanes (58-65), 67, (69-73), (77-81), 85, 87, 91, 93 and (95-97) reveal a 450 bp band; lanes 66, 82, 84, and 86 reveal 300 and 450 bp bands; lanes 68 and 94 reveal 450 bp doublet bands; lane 74 reveals 400 and 450 bp bands; lane 75 reveals a 250 bp band; lanes 76 and 83 reveal 350 and 450 bands; lane 88 reveals 150, 300 and 450 bp bands; lane 89 reveals 100, 300 and 450 bp bands; lane 90 reveals 100 and 300 bp bands; lane 92 reveals 250 and 450 bp bands; C+ represents the positive control, N represents the negative control, and M is 100 bp DNA markers.

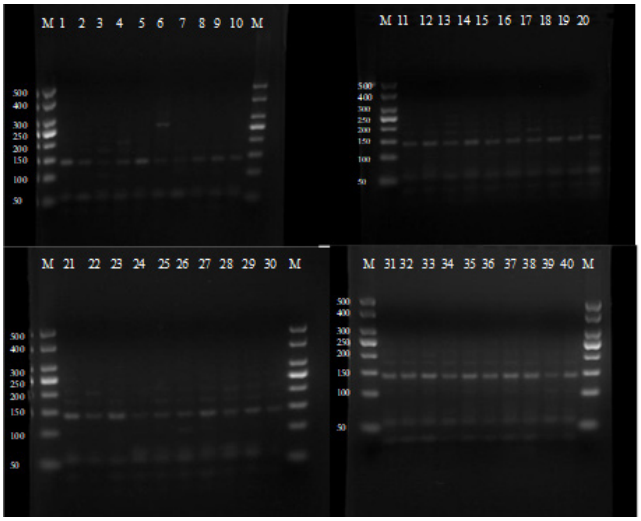

Figure 4. *Alu*I digestion of nested PCR products for asymptomatic samples from 1 to 40, analysed by gel electrophoresis. M represents 50 bp DNA markers. Lanes (1-5), (7-16), (18-23), 34 and 39 show 145-150 and 60 bands; lane 6 shows 250-260, 145-150 and 60 bands; lane 17 shows 180-190, 145-150 and 60 bands; lanes (24-29) show 145-150, and 60-70 bands; lane 30 shows a 145-150 band; lanes (31-33) (35-38) and 40 show 145-150 and 60 and 40 bands.

41 (71.92%) asymptomatic and 26 (65%) symptomatic samples. The remaining 30 (30.93%) samples showed different band sizes; among them, 23 (23.71%) samples revealed similar bands between symptomatic (9 samples) and asymptomatic (14 samples). Seven samples (7.21%) reported distinct bands: 2 (2.06%) samples from asymptomatic samples and 5 (5.15%) from symptomatic samples.

On the other hand, the *Alu*I digestion of the SREHP nested PCR product showed 29 different DNA patterns among the 97 samples of both asymptomatic and symptomatic samples (Figure 4-6). Shared DNA patterns between symptomatic and asymptomatic samples were seen in 62 (63.91%) of the 97 tested samples presented in 6 DNA patterns and consisted of 41 asymptomatic samples and 21 symptomatic samples. Moreover, 8 different DNA patterns for 16 (28.07%) samples were reported among the 57 asymptomatic samples, while 15 distinct patterns for 19 (47.5%) samples were observed among the 40 diarrhoeal samples (Table 1).

Discussion

Estimation of genetic diversity within species is vital in the pathogenesis and epidemiology of a pathogen; accordingly, genetic diversity of *E. histolytica* based on SREHP polymorphism among symptomatic and asymptomatic subjects in Erbil city was evaluated in the current study. The initial prevalence rate of *E. histolytica* was 1.97% among symptomatic patients based on the amplification of the 18S rRNA gene in Erbil city (North Iraq). This result is inconsistent with studies conducted in Diwanyha (south-central), Al-Najaf (southwestern) and Baghdad (central Iraq), which reported prevalence rates of 44.3%, 24% and 7%, respectively, among symptomatic patients (12-14). The high infection rates observed in Al-Najaf and Diwanyha cities (also sharing internal boundaries) may be due to the small sample

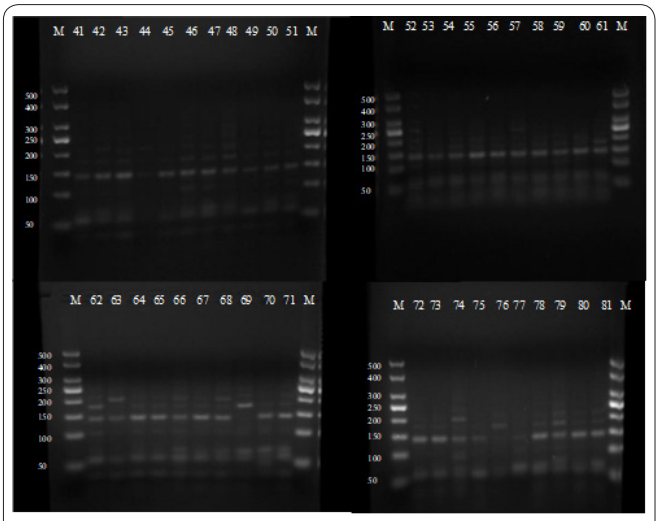

Figure 5. Agarose gel electrophoresis for the SREHP nested PCR product after digestion with *Alu*I restriction endonuclease for asymptomatic samples (41-57) and symptomatic isolates (58-81). M represents 50 bp DNA markers; lanes 41, 45, 49, 51, 72, 73, 75, 80 and 81 show 145-150 and 60 bands; lanes 42, 46, 47, 50,70, 77, and 78 show 145-150 and 60-70 bands; lanes 43, (52-60), 64, 65, 67 show 145-150 and 60 and 40 bands; lane 44 shows a 145-150 band; lanes 48 and 62 show 180-190, 145-150 and 60 bands; lane 61 shows 180-190, 145-150 and 60 and 40 bands; lane 63 shows 200, 145-150, 60 and 40 bands; lane 66 shows 145-150, 70, 60 and 40 bands; lanes 68 and 71 show 145-150 and 60-70 and 40 bands; lane 69 shows 170 and 60-70 bands; lane 74 shows 200, 145-150 and 100 and 60 bands; lane 76 shows 170 and 60 bands; lane 79 shows 180-190, 145-150, 70 and 60 bands.

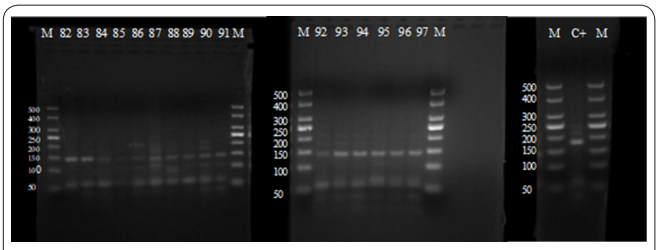

Figure 6. Restriction endonuclease *Alu*I digestion of nested PCR product for the SREHP gene analysed by agarose gel electrophoresis for symptomatic isolate (82-97). C+ represents HM-1:IMSS, and M represents 50 bp DNA markers; lanes (82-85), 87, 89 and 92 show 145-150 and 60 bands; lane 86 shows 200, 145-150, 100 and 60 bands; lane 88 shows 145-150, 100, and 60 bands; lane 90 shows 145-150, 100, 60-70 and 40 bands; lane 91 shows 145-150, 60 and 40 bands; lanes 93, 94 and 97 show 145-150, 60-70, 40 bands; lanes 95 and 96 show 145-150 and 60-70 bands; C+ represents HM-1:IMSS show 170 & 60-70 bands.

size, differences in environmental conditions, nutrition, the use of contaminated water supply and hygienic habits followed by people in these regions. On the other hand, the low prevalence rate reported in the current study could partially be attributed to seasonal variations, since infection rates with this parasite are highest during the hot summer season (15), while most samples of the present work were collected during winter and spring seasons when diarrhoea mostly resulted from viral or bacterial infections (16). However, consistent with the current study, a low rate of infection with *E. histolytica* was recorded in western Iran, the United Arab Emirates and Malaysia (17-19).

Amplification of the SREHP gene in the present

Table 1. List of isolates used for amplifying the SREHP gene, showing nested PCR products, *Alu*I digestion of these products, pattern number and their frequency.

Pattern NO.	Nested PCR product banding size (bp)	*Alu*I digestion of SREHP nested PCR product	Isolates	Frequency
1	450	145-150 & 60	A	22
2	450	250-260 & 145-150 & 60	A	1
3	450	180-190 & 145-150 & 60	A	2
4	350 & 450	145-150 & 60	A	2
5	300 & 450	145-150 & 60-70	A	4
6	300 & 400 & 450	145-150 & 60-70	A	1
7	450	145-150 & 60-70	A	4
8	450	145-150	A	2
9	450	145-150 & 60 & 40	A	10
10	450 d	145-150 & 60 & 40	A	5
11	450 d	145-150 & 60	A	1
12	500	145-150 & 60	A	1
13	350 & 450	145-150 & 60-70	A	1
14	250 & 450	145-150 & 60	A	1
1	450	145-150 & 60	S	6
3	450	180-190 & 145-150 & 60	S	1
4	350 & 450	145-150 &60	S	1
7	450	145-150 & 60-70	S	5
9	450	145-150 & 60 & 40	S	7
14	250 & 450	145-150 & 60	S	1
15	450	180-190 & 145-150 & 60 &40	S	1
16	450	200 & 145-150 & 60 &40	S	1
17	300 & 450	145-150 &70 & 60 &40	S	1
18	450 d	145-150 & 60-70 &40	S	2
19	450	170 & 60-70	S	1
20	450	145-150 & 60-70 & 40	S	3
21	400 & 450	200 &145-150 & 100 & 60	S	1
22	250	145-150 & 60	S	1
23	350 & 450	170 & 60	S	1
24	450	180-190 & 145-150 & 60 &70	S	1
25	300 & 450	145-150 & 60	S	2
26	300 & 450	200 & 145-150 & 100&60	S	1
27	150 & 300 & 450	145-150 & 100 & 60	S	1
28	100 & 300 & 450	145-150 & 60	S	1
29	100 & 300	145-150 &100 & 60-70 & 40	S	1
19	450	170 & 60-70	HM-1:IMSS	

d represents a doublet band, A indicates asymptomatic, S indicates symptomatic and HM-1:IMSS represents standard positive controls.

study resulted in similar band sizes in most of the studied isolates, which is consistent with those reported in Bangladesh, Georgia, Africa and Turkey (6, 10, 20, 21). However, considerable polymorphism in SREHP nested PCR products was observed and presented as two or three bands in 30.93% of the analysed samples. SREHP is an abundant immunogenic surface protein that has been reported to be critical in phagocytosis and immune evasion, and it also plays a role in adherence to apoptotic cells; consequently, it participates in virulence of the parasite. Furthermore, the polymorphism that exists in the SREHP expressing variable numbers of tandem repeats might mediate adherence with wider range or variable affinity, suggesting that the tandem repeats are binding domains; thus, this polymorphism of the SREHP plays an important role in the pathogenic ability of different *E. histolytica* strains (5, 22, 23). The results in the current work reported that asymptomatic samples show less polymorphism than symptomatic samples, which may reflect a correlation between clinical outcome and SRHEP polymorphism. Additionally, the multiple bands observed in the present study could also be explained by the presence of polymorphisms between homologous loci on allelic chromosomes; otherwise, this feature could be either due to the presence of more than one strain of *E. histolytica* in a single sample or the presence of repeated loci at several locations in the genome of *E. histolytica,* each resulting in a different PCR product (24).

Genetic diversity depending on *Alu*I digestion of the

nested PCR product for the SREHP gene in the present study was relatively high, and a higher rate of variability was observed among symptomatic than asymptomatic isolates in a limited geographic region. A similar method was used in Georgia, Turkey, Africa, the Philippines and Bangladesh to estimate the genetic variability of *E. histolytica*, but none of the DNA patterns observed in the current study were identical or matched to those obtained from these regions, indicating the great diversity of the inter-species strains of *E. histolytica* among different geographic areas. Among 26 clinical isolates from Turkey, there were 12 different patterns. Although most of the tested isolates were from symptomatic individuals, a high level of genetic variability was observed (21). Genetic diversity in Georgia also seems to be extensive; among 7 symptomatic cultured samples, there were 4 different patterns; however, only 2 patterns were seen among 9 asymptomatic isolates; accordingly, and similar to the present study, genetic diversity is more common among symptomatic than asymptomatic samples (20). The presence of a high level of genetic diversity may reflect the existence of several different clones in a limited geographic location and/or rapid production of SREHP repeats. *Alu*I digestion of the nested PCR product from asymptomatic mentally retarded patients in the Philippines found only 4 DNA patterns among 74 samples, indicating a relatively low rate of genetic diversity in asymptomatic individuals in that region (25).

From 54 *E. histolytica* isolates in Bangladesh, 34 different DNA patterns were exhibiting extensive genetic diversity in restricted geographic areas; additionally, there was a distinct pattern of the liver abscess isolates from the intestinal isolate, suggesting a correlation between the parasite genotype and its clinical outcome (10). Evidence has suggested that diversity among *E. histolytica* strains based on gene polymorphism may result from the creation of new haplotypes due to the shuffling of alleles during the process of allele recombination and reassortment (26). Moreover, other evidence suggests the possibility of the presence of sexual reproduction in the natural population of *E. histolytica* due to the discovery of complement genes that are necessary for meiosis in the genome of *E. histolytica*. The existence of sexual reproduction is of great importance in terms of gene exchange, for example, in drug resistance and virulence genes, and consequently generating genotypes that spread rapidly; this may also explain the linkage disequilibrium patterns among genetic markers as that of SREHP gene polymorphism (27).

In addition to considering that the diversity of the genetic patterns is of biological origin, it may also to be immunologically mediated, since animals became protected from amoebic abscess after developing antibodies against repetitive or polymorphic portions of the SREHP gene (28).

Genetic variability based on *Alu*I digestion of the SREHP nested PCR product observed in the present study seems to be less extensive than the polymorphism reported in Thailand, which was extremely high, with 21 different genotypes among 27 isolates depending on nucleotide sequencing. Similarly, in another study, 16 different DNA patterns among 18 cultured isolates were observed depending on the RFLP analysis of the SERHP gene. These differences observed between the current work and these two studies may be due to the presence of several genotypes of SREHP that are indistinguishable using RFLEP analysis or could be attributed to region specifications associated with strain variations as well as the origin of isolates (5, 29).

The present study concluded that there is relatively high genetic diversity within the examined isolates in a limited endemic area as well as a higher rate of genetic variability in symptomatic than asymptomatic isolates, indicated through 19 distinct DNA patterns among 40 symptomatic samples. This suggested an association between the genotype and the clinical outcome of the infection.

Ethical Approve

The study was conducted by the Declaration of Helsinki ethical standards, revised in 2008; and approved by the Ethics Committee of Hawler Medical University.

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Declarations of interest

The authors declare no conflict of interest in this research study.

References

1. Haque R, Huston CD, Hughes M, Houpt E, Petri WA. Amebiasis. New Engl J Med 2003; 348: 1565-73.

2. Paul J, Srivastava S, Bhattacharya S. Molecular methods for diagnosis of entamoeba histolytica in a clinical setting: An overview. Exp Parasitol 2007; 116: 35-43.

3. Rahman SMM, Haque R, Roy S, Mondal MMH. Genotyping of entamoeba histolytica by real-time polymerase chain reaction with cyber green I and melting curve analysis. Bangl J Vet Med 2006; 4: 53–60.

4. Elbakri A, Samie A, Ezzedine S, Odeh REA. Genetic variability of the serine-rich entamoeba histolytica protein gene in clinical isolates from the United Arab Emirates. Trop Biomed 2014; 31: 370–37.

5. Haghighi A, Kobayashi S, Takeuchi T, Thammapalerd N, Nozaki T. Geographic diversity among genotypes of entamoeba histolytica field isolates. J Clin Microbiol 2003; 41: 3748-56.

6. Samie A, Obi CL, Bessong PO, E. Houpt SS, Njayou M, Sabeta C, Mduluza T,Guerrant RL. Entamoeba histolytica: Genetic diversity of African strains based on the polymorphism of the serine-rich protein gene. Exp Parasitol 2008; 118: 354-61.

7. Turan H, Karkucak M, Yakut T, Ozsahin M, Gurlevik Z, Yanik ME, Ucgun T, Aliagaoglu C, Yaykasli KO. Does MBL-2 codon 54 polymorphism have a role psoriasis pathogenesis? Int J Dermatol 2014; 53(1):34-38.

8. Khairnar K, Parija SC. A novel nested multiplex polymerase chain reaction (PCR) assay for differential detection of entamoeba histolytica, e. Moshkovskii and e. Dispar DNA in stool samples. BMC Microbiol 2007; 7: 47.

9. Yaykasli KO, Yaykasli E, Ataoglu S, Ozsahin M, Memisogulları R, Çelebi E, Uçgun T, Ozcan ME, Uslu M, Yuce H. The frequency of omentin Val109Asp polymorphism and the serum level of omentin in patients with Rheumatoid Arthritis. Acta Medica Mediterranea 2013; 29:521-526.

10. Ayeh-Kumi PF, Ali IM, Lockhart LA, Gilchrist CA, Petri WA, Haque R. Entamoeba histolytica: Genetic diversity of clinical iso-

lates from Bangladesh as demonstrated by polymorphisms in the serine-rich gene. Exp Parasitol 2001; 99: 80-8.
11. Yaykasli KO, Kayıkçı MA, Yamak N, Soguktas H, Duzenli S, Arslan AO, Metin A, Kaya E, Hatipoglu OF. Polymorphisms in MMP-2 and TIMP-2 in Turkish patients with prostate cancer, Turk J Med Sci 2014; 44:839-843.
12. Hussein RA, Al-Mayah QS, Merdaw Ma-Z, Al-Bashier NT, Al-Abbas AA, Jasem IA. Evaluation of multiplex real-time PCR and ELISA in detection of intestinal protozoan parasites from children with diarrheal disease Inter J Adv Res 2015; 3: 782-8.
13. Al-Khalidi Kah. Detection of entamoeba histolytica in patients an infected infants with diarrhea in born and children's hospital by classic methods and real-time polymerase chain reaction. J Al Qadisiyah Pure Sci 2016; 21: 27-35.
14. Al-Hameedawi JJY. Molecular identification of entamoeba histolytica parasite by using actin and amebapore-a genes. Kufa J for Nursing Sci 2014; 4: 48-55.
15. Jaran AS. Prevalence and seasonal variation of human intestinal parasites in patients attending hospital with abdominal symptoms in northern Jordan. E Mediterr Health J 2016; 22: 756-60.
16. Nitiema LW, Nordgren J, Ouermi D, Dianou D, Traore AS, Svensson L, Simpore J. Burden of rotavirus and other enteropathogens among children with diarrhea in Burkina Faso. Int J Infect Dis 2011; 15: e646-52.
17. Bahrami F, Haghighi A, Zamini G, Khademerfan M. Differential detection of entamoeba histolytica, entamoeba disparandentamoeba moshkovskiiin faecal samples using nested multiplex PCR in west of Iran. Epidemiol Infect 2019; 147.
18. Elbakri A, Samie A, Ezzedine S, Odeh RA. Differential detection of entamoeba histolytica, entamoeba dispar and entamoeba moshkovskii in fecal samples by nested PCR in the United Arab Emirates (UAE). Acta Parasitol 2013; 58: 185-90.
19. Anuar TS, Al-Mekhlafi HM, Abdul Ghani MK, Azreen SN, Salleh FM, Ghazali N, Bernadus M, Moktar N. Different clinical outcomes of entamoeba histolytica in Malaysia: Does genetic diversity exist?. Korean J Parasitol 2013; 51: 231-6.
20. Simonishvili S, Tsanava S, Sanadze K, Chlikadze R, Miskalishvili A, Lomkatsi N, Imnadze P, Petri WA, Jr., Trapaidze N. Entamoeba histolytica: The serine-rich gene polymorphism-based genetic variability of clinical isolates from Georgia. Exp Parasitol 2005; 110: 313-7.
21. Tanyüksel M, Ulukanl‹G‹L M, Yilmaz H, Güçlü Z, Araz RE, Mert G, Koru Ö, William A. Petri J. Genetic variability of the serine-rich gene of entamoeba histolytica in clinical isolates from turkey. Turk J Med Sci 2008; 38: 239-44
22. Teixeira JE, Huston CD. Participation of the serine-rich entamoeba histolytica protein in amebic phagocytosis of apoptotic host cells. Infect Immun 2008; 76: 959-66.
23. Samuel L.Stanley J, Becker A, Kunz-Jenkins C, Foster L, Li E. Cloning and expression of a membrane antigen of entamoeba histolytica possessing multiple tandem repeats Proc Natl Acad Sci 1990; 87:4976-80.
24. Willhoeft U, Tannich E. The electrophoretic karyotype of entamoeba histolytica. Mol Biochem Parasitol 1999; 99: 41-53.
25. Rivera WL, Santos SR, Kanbara H. Prevalence and genetic diversity of entamoeba histolytica in an institution for the mentally retarded in the Philippines. Parasitol Res 2006; 98: 106-10.
26. Weedall GD, Clark CG, Koldkjaer P, Kay S, Bruchhaus I, Tannich E, Paterson S, Hall N. Genomic diversity of the human intestinal parasite entamoeba histolytica. Genome Biol 2012; 13: R38.
27. Weedall GD, Hall N. Evolutionary genomics of entamoeba. Res Microbiol 2011; 162: 637-45.
28. Zhang T, Cieslak PR, Stanley SL. Protection of gerbils from amebic liver abscess by immunization with a recombinant entamoeba histolytica antigen. Infect Immun 1994; 62: 1166-70.
29. Clark Cg, Diamond Ls. Entamoeba histolytica: A method for isolate identification. Exp Parasitol 1993; 77: 450-5.