Vol. 03, No. 02, pp. 46 –50 (2022)
ISSN: 2708-1818

JOURNAL OF LIFE AND BIO-SCIENCES RESEARCH

www.jlbsr.org

Meta-analysis with assessment of some Phylogenetic Relationship of *Entamoeba histolytica* of Iraq and Iran

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Abstract

Protozoans of *Entamoeba spp.* are globally distributed protozoan parasites that infect diverse hosts (human and animals) causing amebiasis with various symptoms ranging from abdominal discomfort, indigestion, diarrhoea, bloody diarrhoea, and even death. *Entamoeba histolytica* infection may be influenced by different strains which are already existing in our population. A meta-analysis was performed to evaluate the sequence comparison and geneflow of *E. histolytica* in Iraq and Iran. For this purpose, all reference sequences recorded from the aforementioned countries and deposited in the National Centre for Biology Information (NCBI) database of the mentioned countries (133 reference sequences, 110 from Iraq and 23 from Iran) were included in this study. After aligning and blasting all these sequences and considering the shared regions, eight unique sequences were obtained. According to the Codon-based Z-test of selections, they vary in degree of difference (p-value 0.05). Some records from the study area approached each other 100% which means that geneflow has occurred in the areas under investigation. Outstanding phylogenetic relationship of *Entamoeba histolytica* of both Iraq and Iran strains are related together and that is important in the molecular epidemiology aspect of amebiasis as it may influence the clinical and pharmacological orientation of the disease in both countries. Current meta-analysis was done for the first time in that approach in the place this study.

Keywords: *Meta-analysis, Entamoeba histolytica, Molecular Epidemiology, Phylogenetic, Amoebiasis.*

Received: June 20th, 2022/ Accepted: August 30th, 2022/Online: October 1st, 2022.

I. INTRODUCTION

Amoebiasis is attributed to a gastrointestinal protozoan *Entamoeba histolytica* which has a different disease manifestation. Genomic studies pointed the evolutionary relationship to this fascinating organism that could aid researchers to explain their pathogenicity and how that's related to the occur of the diseases (Das and Ganguly, 2014). Life cycle of amoebiasis organism is involved two interchangeable stages. These stages are the motile pathogenic trophozoite form and the infective cyst form. Infection by *E. histolytica* is endemic in many developing countries and that probably due to the poor sanitation and malnutrition (Haghighi *et al.*, 2002).

Entamoeba histolytica infection develops many variable disease outcomes. Only 10% of patients develop symptoms of the invasive amoebiasis while 90% of infected individuals remain asymptomatic (Stanley, 2003). Identification of the genetic features of protozoa is directly associated with the virulence or linked to the expected disease consequence. This is will be truly inspiring a significant range of amoebiasis investigations and researches. Intra and inter-specific genomic assessments have been used to detect the parasites' hereditary factor connected to the virulence or related to differential disease initiating abilities. These investigations also afford roughly thought that could provoking and clarified evidence explain many aspects of the evolution and population structure of this organism (Shah *et al.*, 2005).

There are several sub-species of *Entamoeba* infect a wide range of hosts (Weedall and Hall, 2011). The modest structural features such as the nuclear count per cyst have been considered to discriminate between these types. However, morphological differences do not constantly imitate species-level alterations and substantial genetic diversity occurs among morphologically undifferentiated or closely related parasites (Graham *et al.*, 2006).

The genome sequence of *E. histolytica* strain HM: IMSS was published and analyzed in 2005 (Loftus *et al.*, 2005). This genome assemblage consists of twenty, eight-hundreds, five hundred and sixty bp of DNA in one thousand four hundred and ninety-six scaffolds. The genome contains an elevated

AT content (nearly 75%). Fifty percent of the assembled sequence is expected to be coding, with eight thousand three hundred and thirty-three interpreted genes (Weedall and Hall, 2011).

The genomic structure of *E. histolytica* has been extensively studied by a collection of investigators and or researchers (Clark *et al.*, 2007). Numerous exciting evolutionary characteristics of *E. histolytica* genome have been emphasized. For example, the parasite has obtained an important number of metabolic genes (no less than sixty-eight) by the horizontal transfer of genes from bacteria (Loftus *et al.*, 2005). Orthologues of those genes originate in both the organism of interest and its evolutionary distant species *E. invadens* (Graham *et al.*, 2006) denoting the ancient nature of this gene transfer (Weedall and Hall, 2011). Initial characterization of *E. histolytica* genome showed some uncommon features in their organization. The genome of it is extremely repetitive (repetitive elements have been estimated to be 40 %). Among them, the genes coding for tRNA are extraordinarily plentiful; with an estimated four thousand and five hundred copies (around 10 times of human genome). Furthermore, these tRNA genes are gathered and organized into twenty-five discrete arrays. These arrays consist of tandemly repeated elements encoding between one and five tRNA acceptor kinds. The regions located between these tRNA genes involve short tandemly repeated sequences (STRs) which look like the micro/mini satellites of eukaryotic genomes. The one modification is that unlike randomly disseminated micro/mini satellites, STRs create a portion of a greater unit which is itself tandemly arrayed. The genes encoding the tRNA are assumed to be "hotspots" for mutation and recombination because of their exceptional structural organizations. The arrangement of tRNA gene showed inter-specific variation (Tawari *et al.*, 2008).

Dissimilar to *Plasmodium* parasite which has a steady genomic organization even among far correlated species, *Entamoeba* shows a high grade of genomic instability and elasticity or variation (Weedall and Hall, 2011). Genome reorganization related to the tissue invasion and organ tropism has been considered as a primary probable clarification for the unlike tRNA STR genotypes recognized in the hepatic abscess and stool derived microorganisms from the same infested victim (Ali *et al.*, 2008). Genome reorganization may be attributed to the abundance of Transposons and repetitive DNA molecules in the Entamoeba genome (Weedall and Hall, 2011). The organization of transposable elements takes cluster form, often present at syntenic breakpoints offering insights into their role in the instability of the chromosome and consequently, to the diversity of the genome and speciation in these parasites (Lorenzi *et al.*, 2008).

Since the genome of *E. histolytica* does not seem to involve any microsatellite similar elements, estimation of population structures and measurement of genetic diversity critically count on other genetic indicators like Serine Rich *E. histolytica* protein (SREHP) gene and chitinase (Weedall and Hall, 2011). SREHP is an immune dominant superficial protein, associated with phagocytosis of apoptotic host cells to avoid inflammatory reactions (Teixeira and Huston, 2008) whereas chitinase is solely produced during encystations of amoeba (De la Vega *et al.*, 1997). Both genes contain tandem repeats that referred to a high degree of inter isolate diversity based on their repeat types and arrangement patterns (Rivera *et al.*, 2006).

Many meta-analyses reviews were done other else (1,2,17) but not like current research categories of NCBI database which dependant on retrieved sequences.

This meta-analysis with review of some phylogenetic relationship between *Entamoeba histolytica* of Iraq and Iran will be investigated in this article.

II. Materials & Methods

Entamoeba histolytica parasite genome sequences that have been retrieved from the NCBI database (National Centre for Biology Information) were analyzed for sequence purity and multiple sequence alignment together and then contiged into single Fasta files through using the DNASTAR Lasergene/ SeqMan Pro software along with Blast tools. While all Fasta files were analyzed using MEGA Software (version 7.0.14) and then the phylogenetic tree was drowned and the Codon-based Z-test was calculated to find convergent sequences and the degree of rapprochement. A $p < 0.05$ was considered to indicate statistical significance. This meta-analysis was done for the first time in that approach in the place of this study.

III. Results

In searching of *E. histolytica* parasite sequences of Iraq and Iran submitted to the database of NCBI (National Centre for Biology Information) until 31st July 2021, 133 sequences have been retrieved (110 accession numbers from Iraq, and 23 accession numbers from Iran) (Table 1). The genome sequences ranged between 220 - 868 bps were elected for analysis.

Table 1. Sequences of *Entamoeba histolytica* registered in National Center for Biotechnology Information from Iraq and Iran.

Iraq (110 accession numbers)
KP233836.1, KP233837.1, KP233838.1, KP233839.1, KP233840.1, KT253450.1, KT253451.1, KT253452.1, KT253453.1, T253454.1, LC360750.1, LC360751.1, MF421529.1, MH882419.1, MH882420.1, MH882421.1, MH882422.1, MH882423.1, MH882424.1, MH882425.1, MH882426.1, MH882427.1, MH882428.1, MN061054.1, MN061055.1, MN227232.1, MN227233.1, MN227234.1, MN227235.1, MN227236.1, MN227237.1, MN307384.1, MN307385.1, MT250837.1, MT296770.1, MT296771.1, MT296772.1, MT296773.1, MT296774.1, MT296775.1, MT296776.1, MT296777.1, MT296778.1, MT296779.1, MT951203.1, MT951204.1, MT951205.1, MT951206.1, MT951207.1, MW029814.1, MW029815.1, MW164797.1, MW426045.1, MW426046.1, W426047.1, MW426048.1, MW426049.1, MW426050.1, MW426051.1, W426052.1, MW426053.1, MW426054.1, MW426055.1, MW426056.1, W426057.1, MW426058.1, MW426059.1, MW426060.1, MW426061.1, W426062.1, MW426063.1, MW426064.1, MW426065.1, MW426066.1, W426067.1, MW426068.1, MW426069.1, MW426070.1, MW426071.1, W426072.1, MW426073.1, MW426074.1, MW440565.1, MW440566.1, W440567.1, MW440568.1, MW440569.1, MW440570.1, MW440571.1, W440572.1, MW440573.1, MW440574.1, MW440575.1, MW440576.1,

W440577.1, MW440578.1, MW440579.1, MW440580.1, W440581.1, MW440582.1, MW440583.1, MW440584.1, MW624407.1, MW624408.1, W624409.1, MW624410.1, MW624411.1, MZ244206.1, MZ377020.1, MZ377021.1
Iran (23 accession numbers)
AB217859.1, AB217860.1, AB217861.1, AB217862.1, AB217863.1, AB217864.1, AB217865.1, AB217866.1, AB253474.1, DQ899178.1, DQ899179.1, KX528457.1, KX528458.1, KX528459.1, KX528460.1, KX528461.1, KX528462.1, KY823424.1, KY823425.1, KY823426.1, KY823427.1, KY884295.1, MW659191.1

Retrieved sequences were analyzed for sequence purity and multiple sequence alignment together through using the DNASTAR Lasergene/ SeqMan Pro software along with Blast tools, and then contiged into 41 sequences or single Fasta files, as a result, 8 sequences or single files (3 files from Iraq and, 5 files from Iran) were selected in which each package of sequences was represented by a single file (Table 2).

Table 2. Sequence of *Entamoeba histolytica* according to sequences batch with their references.

Iraq (3 accession numbers)
KP233839.1 (Al-Mayali and Al-Abodi, 2017), MT951205.1 (Unpublished), LC360751.1 (Unpublished)
Iran (5 accession numbers)
AB253474.1 (Haghighi *et al.*, 2009), KX528460.1 (Mohammadzadeh *et al.*, 2017), DQ899179.1 (Rostami *et al.*, 2017), KY823426.1 (Bahrami *et al.*, 2019), AB217860.1 (Razmjou *et al.*, 2006)

After these 8 contiged files were analyzed and supplied to MEGA Software (version 7.0.14) to have the phylogenetic tree the Codon-based Z-test (Figure 1), there was found that the sequence KP233839.1 (Al-Mayali and Al-Abodi, 2017) from Iraq has matched with the sequence KY823426.1 (Bahrami *et al.*, 2019) from Iran.

IV. Discussion

The differences between studied nucleotides sequence for one strain of *E. histolytica* vs. others in forward for Iranian strains against Iraqi strains and backward in opposite has shown in Table 3.

The values ranged from 0.000 which represented no difference, to a value of 1.000 which indicated complete difference that has been noticed in the forward direction (left part of Table 3). The first value was observed in strain Iran_AB253474.1 (Iranian strain) vs. strain, Iraq_LC360751.1 (Iraqi strain). In contrary for the second value which was observed in Iran_KX528460.1 vs. Iraq_KT253454.1, which meant full deference. Other values ranged between these values.

Table 3. Z-test differences between studied nucleotides sequence for one strain of *Entamoeba histolytica* vs others (p value 0.05).

Z- test	Iraq_KT253454.1	Iraq_MT951205.1	Iraq_LC360751.1	Iran_AB253474.1	Iran_KX528460.1	Iran_DQ899179.1	Iran_KY823426.1	Iran_AB217860.1
Iraq_KT253454.1		1.088	-0.866	-1.109	0.000	-0.492	1.903	0.655
Iraq_MT951205.1	0.279		0.741	-2.504	1.253	0.798	0.127	-2.720
Iraq_LC360751.1	0.388	0.460		-4.541	0.967	2.246	0.047	-3.274
Iran_AB253474.1	0.270	0.014	0.000		-1.682	-4.568	0.026	-6.329
Iran_KX528460.1	1.000	0.213	0.336	0.095		0.967	1.561	0.033
Iran_DQ899179.1	0.623	0.426	0.027	0.000	0.336		0.047	-3.565
Iran_KY823426.1	0.059	0.899	0.963	0.980	0.121	0.963		-1.494
Iran_AB217860.1	0.514	0.007	0.001	0.000	0.974	0.001	0.138	

This arrangement reflected the change in the sequence by mutation over time and this mutation probably of substitution or an exchange type (Liò and Goldman, 1998; Strimmer and von Haeseler, 1996; Van de Peer *et al.*, 1996).

In table 3, it is shown that Iraqi strains have shown a various degree of variation as inter species in values of -0.866 to1.088 which confirmed the originality of these strains (Lemey *et al.*, 2009).

In the right side (backward direction) of table3 which represented the increase (Positive values) or the decrease (negative values) in the change of studied nucleotides sequence, the values of -6.329 to 2.246 were observed when comparing Iranian strains to Iraqi ones (Martin *et al.*, 2011) and these positive or negative values may reflect the positive selective pressure of proper allele to explain this variation (Vandamme, 2009).

The positive value was seen in Iran_DQ899179.1 vs. Iraq_LC360751.1 and this meant an increase in sequence differences. The negative value was seen in Iran_AB253474.1 vs. Iraq_LC360751.1. Other negative values were observed in arrangement between these two values, this could be related to pressure of selection that occurred in their distribution area (Vandamme, 2009).

Also, the inter species variation was observed among Iranian strains from value of -6.329 to 1.561 and this observation confirmed the originality of the studied nucleotide sequence as for the Iraqi strains; the later fact could be explained by the same above reference especially in large area with obvious separated regions in it where immigration or isolation provoke the evolutionary forces affecting speciation (Lemey *et al.*, 2009) and that is true for Entamoeba parasite as it eukaryote organism (Silberman *et al.*, 1999).

In figure (1) of phylogenetic tree which was prepared by Neighbour-Joining method (Salemi *et al.*, 2009) of aligned various Iranian and Iraqi *E. histolytica* strains. The tree clarified that these strains had various genetic distance from the ancestral root in various degree of genetic flow of their genetic elements at least for the studied nucleotides sequence (Weedall *et al.*, 2012).

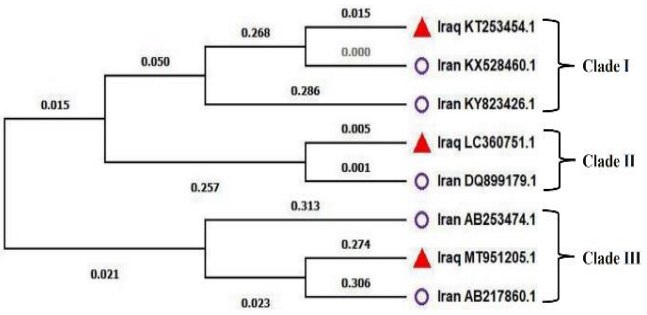

Figure 1. Phylogenetic relationships among gene sequences of *Entamoeba histolytica* in Iraq and Iran. The tree shown was inferred using the neighbor-joining method. The evolutionary distances were computed using the maximum composite likelihood method with rate variation among sites modeled using a gamma distribution (shape parameter 0.05).

Three clades were observed in figure 1. Clade I which showed that Iran_KX528460.1 (Iranian strain) had no difference from Iraq_KT253454.1 (Iraqi strain) as a value 0.000 was observed, and vice versa for the Iraqi strain vs. Iranian strain which showed a value of 0.015 in difference or variation, considering the probability of rapprochement together in the case of continuing sequencing. Both strains were varying from Iran_KY823426.1 (Iranian strain) in the same clade in about 0.286.

Clade II showed very little difference (0.001) between Iran_DQ899179.1 (Iranian strain) vs. Iraq_LC360751.1 (Iraqi strain,) which showed a value of 0.005 and this meant that they have greater similarity regarding the studied nucleotides sequence of Entamoeba spp. in Al-Qadisiya province, Iraq (Al-Mayali and Al-Abodi, 2017).

Clade II showed variation to Clade I in about 0.257, while Clade I showed 0.050 difference and both had a distance of 0.015 from the common root.

The last clade (Clade III) showed the greatest variation in the nucleotides sequence either for Iranian or Iraqi strains as values of 0.313, 0.274, 0.306 and 0.023 were observed respectively. Total difference from common root of 0.021 value was observed.

Overall results reflected clear genetic flow among all strains, and this is a significant attribute to explain the role of molecular epidemiology in pathogenic microbial studies (Cui *et al.*, 2019). The study improved that meta-analysis is a magnificence approach to understand common molecular bases in neighbouring countries.

V. Conclusion

Phylogenetic tree between neighboured areas or even countries may lead to understand and planning to control the disease of *E. histolytica* and highlighted other aspects for further studies of pathophysiology of Amoebiasis.

Acknowledgements

Authors thank IT dept. of Duhok technical institute, Duhok Polytechnic University for providing all requirements to accomplish this article.

References

Al-Mayali, H., Al-Abodi, H. (2017). Molecular characterization of Entamoeba spp. parasite in Al-Qadisiya province, Iraq. *Al-Kufa University Journal for Biology*, 9(1), 374-386.

Ali, I., Solaymani-Mohammadi, S., Akhter J, Roy S, Gorrini C, Calderaro A, *et al.* (2008). Tissue invasion by *Entamoeba histolytica*: evidence of genetic selection and/or DNA reorganization events in organ tropism. *PLoS Negl Trop* 2:e219.

Bahrami, F., Haghighi, A., Zamini, G., Khademerfan, M. (2019). Differential detection of *Entamoeba histolytica*, Entamoeba dispar and Entamoeba moshkovskii in faecal samples using nested multiplex PCR in west of Iran. *J Epidemiology Infection*, 147.

Clark, C.G., Alsmark, U.C., Tazreiter, M., Saito-Nakano, Y., Ali, V., Marion, S., *et al.* (2007). Structure and content of the *Entamoeba histolytica* genome. *Adv Parasitol*, 65, 51-190. doi: 10.1016/S0065-308X(07)65002-7

Cui, Z., Li, J., Chen, Y., Zhang, L. (2019). Molecular epidemiology, evolution, and phylogeny of Entamoeba spp. *J Infection, Genetics Evolution*, 75, 104018.

Das, K., Ganguly, S. (2014). Evolutionary genomics and population structure of *Entamoeba histolytica*. *Comput Struct Biotechnol J*, 12(20-21), 26-33.

De la Vega, H., Specht, C.A., Semino, C.E., Robbins, P.W., Eichinger, D., Caplivski, D., *et al.* (1997). Cloning and expression of chitinases of Entamoebae. *Mol Biochem Parasitol*, 85(2), 139-147. doi: 10.1016/s0166-6851(96)02817-4.

Graham, C., Farrokh Kaffashian, Blessing Tawari, Jeffrey J Windsor, Anke Twigg-Flesner, Mina C G Davies-Morel, *et al.* (2006). New insights into the phylogeny of Entamoeba species provided by analysis of four new small-subunit rRNA genes *nt J Syst Evol Microbiol*, 56(Pt 9), 2235-2239.

Haghighi, A., Khorashad, A.S., Mojarad, E.N., Kazemi, B., Nejad, M.R., Rasti, S., *et al.* (2009). Frequency of enteric protozoan parasites among patients with gastrointestinal complaints in medical centers of Zahedan, Iran. *J Transactions of the Royal Society of Tropical Medicine*, 103(5), 452-454.

Haghighi, A., Kobayashi, S., Takeuchi, T., Masuda, G., Nozaki, T. (2002). Remarkable genetic polymorphism among *Entamoeba histolytica* isolates from a limited geographic area. *J Clin Microbiol*, 40(11), 4081-4090.

Lemey, P., Salemi, M., Vandamme, A.-M. (2009). *The phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing*: Cambridge University Press.

Liò, P., Goldman, N. (1998). Models of molecular evolution and phylogeny. *J Genome research*, 8(12), 1233-1244.

Loftus, B., Anderson, I., Davies, R., Alsmark, U.C., Samuelson, J., Amedeo, P., *et al.* (2005). The genome of the protist parasite *Entamoeba histolytica*. *Nature*, 433(7028), 865-868.

Lorenzi, H., Thiagarajan, M., Haas, B., Wortman, J., Hall, N., Caler, E. (2008). Genome wide survey, discovery and evolution of repetitive elements in three Entamoeba species. *BMC Genomics*, 9, 595.

Martin, D.P., Lemey, P., Posada, D. (2011). Analysing recombination in nucleotide sequences. *J Molecular Ecology Resources*, 11(6), 943-955.

Mohammadzadeh, A., Spotin, A., Mahami-Oskouei, M., Haghighi, A., Zebardast, N., Kohansal, K. (2017). Gene migration for re-emerging amebiasis in Iran's northwest–Iraq borders: a microevolutionary scale for reflecting epidemiological drift of *Entamoeba histolytica* metapopulations. *J Parasitology research*, 116(1), 217-224.

Razmjou, E., Haghighi, A., Rezaian, M., Kobayashi, S., Nozaki, T. (2006). Genetic diversity of glucose phosphate isomerase from *Entamoeba histolytica*. *J Parasitology international*, 55(4), 307-311.

Rivera, W.L., Santos, S.R., Kanbara, H. (2006). Prevalence and genetic diversity of *Entamoeba histolytica* in an institution for the mentally retarded in the Philippines. *Parasitol Res*, 98(2), 106-110.

Rostami, S., Rezaeian, M., Jamali, R., Rezaie, S., Babaei, Z., Hooshyar, H. (2017). Differences in *Entamoeba histolytica* cysteine proteinase 5 gene isolated from Bandar Abbas and Tabriz, Iran. 5(2), 49-53.

Salemi, M., Vandamme, A.-M., Lemey, P. (2009). *The phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing*: Cambridge University Press.

Shah, P.H., MacFarlane, R.C., Bhattacharya, D., Matese, J.C., Demeter, J., Stroup, S.E., *et al.* (2005). Comparative genomic hybridizations of Entamoeba strains reveal unique genetic fingerprints that correlate with virulence. *Eukaryot Cell*, 4(3), 504-515.

Silberman, J.D., Clark, C.G., Diamond, L.S., Sogin, M.L. (1999). Phylogeny of the genera Entamoeba and Endolimax as deduced from small-subunit ribosomal RNA sequences. 16(12), 1740-1751.

Stanley, S.L., Jr. (2003). Amoebiasis. *Lancet*, 361(9362), 1025-1034.

Strimmer, K., von Haeseler, A. (1996). Accuracy of neighbor joining for n-taxon trees. *Systematic Biology*, 45(4), 516-523.

Tawari, B., Ali, I.K., Scott, C., Quail, M.A., Berriman, M., Hall, N., *et al.* (2008). Patterns of evolution in the unique tRNA gene arrays of the genus Entamoeba. *Mol Biol Evol*, 25(1), 187-198.

Teixeira, J.E., Huston, C.D. (2008). Participation of the serine-rich *Entamoeba histolytica* protein in amebic phagocytosis of apoptotic host cells. *Infect Immun*, 76(3), 959-966.

Van de Peer, Y., Rensing, S.A., Maier, U.-G., De Wachter, R. (1996). Substitution rate calibration of small subunit ribosomal RNA identifies chlorarachniophyte endosymbionts as remnants of green algae. 93(15), 7732-7736.

Vandamme, A. (2009). The Phylogenetic Handbook: Basic concepts of molecular evolution, 2, 3-32.

Weedall, G.D., Clark, C.G., Koldkjaer, P., Kay, S., Bruchhaus, I., Tannich, E., *et al.* (2012). Genomic diversity of the human intestinal parasite *Entamoeba histolytica*. 13(5), 1-13.

Weedall, G.D., Hall, N. (2011). Evolutionary genomics of Entamoeba. *Res Microbiol*, 162(6), 637-645.