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RESEARCH ARTICLE

Molecular study to most important virulence factor of *Entamoeba histolytica* in Diwaniyah province

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Abstract

The present study was conducted to investigate the infection of *Entamoeba spp* by using molecular technique (Quantitative real time polymerase chain reaction) of primers and probes for gene 18S rRNA . The results revealed the presence of *Entamoeba histolytica* in the percentage (74%) of 200 *Entamoeba* microscopic positive samples. Also three primers were used in this study for detection the gene of virulence factor of *Entamoeba histolytica*. The Cysteine protease revealed the high percentage(86.6%) of infection then Amebopore gene (80.35%) whereas Gal/lectin gene gave the lowest (7 5%) ,without significant differences at ($p<0.05$).

INTRODUCTION

Amebiasis is defined as invasive intestinal or extraintestinal infection with the protozoan parasite *Entamoeba histolytica.* More than 50 million people worldwide are infected, and up to 110000 of these die every year (1). Only malaria and schistosomiasis surpass amebiasis as parasitic causes of death . Based on biochemical, immunological, and genetic data, *E. histolytica* has been reclassified into two morphologically identical but genetically distinct species: *E. histolytica*, which is potentially invasive (2).

Because this protozoan parasite is known as a pathogenic responsible of dysentery and liver abscess, the treatment of these individuals was systematically prescribed to interrupt parasite transmission and to avoid the progression from infected individuals to an invasive disease (3)

The virulence variability of trophozoites has been related to genome plasticity and *in vivo* changes in gene expression induced during host invasion. Some virulence factors have been identified as key factors in pathogenesis, however most molecular mechanisms relevant for infection establishment are still poorly understood(4).So the object of the present study was to detection the gene of virulence factor (cysteine , protease , amebopore , and Gal/lectin) in *Entamoeba histolytica*

Materials and methods

qPCR technique was used for amplification of conserved region in 18S rRNA gene that was used for detection of *Entamoeba histolytica* in 200 positive microscopic fecal samples .

Genomic DNA was extracted from fecal samples by using AccuPrep® Genomic DNA Extraction Kit (Bioneer , korea) and done according to the protocol described by the manufacturer instructions.

The primers were used in this study, was used for designed by using NCBI- Gene Bank data base and Primer 3 design online, and supported from (Bioneer, Korea) company .

Primer	Sequence

E.H-ssrRNA primer	F	**GAATTGACGGAAGGGCACAC**
	R	**AACTAAGAACGGCCATGCAC**
E.H-ssrRNA probe	**FAM-CAGGAGTGGAGCCTGCGCTT-BHQ1**	

qPCR master mix was prepared by using (AccuPower® 2×Greenstar qPCR kit, Bioneer. Korea), and done according to kit instructions as following table:

PCR master mix	**Volume**
DNA template	5µL
Forward primer 10pmol	1µL
Reverse primer 10pmol	1µL
TaqMan probe 10pmol	2µL
DEPC water	9µL
Total	**50µL**

Also three primers were used in this study for the gene of virulence factor, the first primer was used for cysteine protease gene the second primer was used for amoebapore gene while the third primer was used forGal/lectin gene .

Primer	**Sequence**	
cysteine protease	**F**	**GCTGTTGCTGGTACTTGCAAG**
	R	**ACAGCAACAGGTCCGTTTTC**
amoebapore	**F**	**TGCCTTTGCTGCAACAAGAG**
	R	**ACAGCTTGAGCACCATCAAC**
Gal/lectin	**F**	**GACGCACCAGGTACTCAAAATC**
	R	**AACCCATCTTCCACCCTGATTG**

These primers were designed by using NCBI- Gene Bank data base and Primer 3 design online, the primers used in quantification of gene expression using RT-PCR techniques based SYBER Green DNA binding dye, and supported from (Bioneer, Korea) company

PCR master mix	**Volume**
2X GreenStar master mix	25 µL
DNA template	5µL

Forward primer 10pmol	2.5 µL
Reverse primer 10pmol	2.5 µL
DEPC water	15µL
Total	**50µL**

After that, qPCR master Mix were added into AccuPower 2× GreenStar qPCR PreMix tube. Then, real-time PCR tubes sealed by the optical adhesive film and mixed by vigorous vortexing for resuspension of PreMix pellet. The tubes centrifuged at 3,000 rpm, for 2 minutes, and then the tubes placed in Miniopticon Real-Time Thermocycler.

Results :

- Quantitative Real Time PCR

The results revealed the presence of *Entamoeba histolytica* in the percentage(74%) of 200 *Entamoeba* microscopic positive samples. The results that presented in figure (1) show the Amplification of gene 18S rRNA concentrations of *Entamoeba histolytica* during reaction with syber dye inside the apparatus under threshold cycle in the Real-Time PCR.

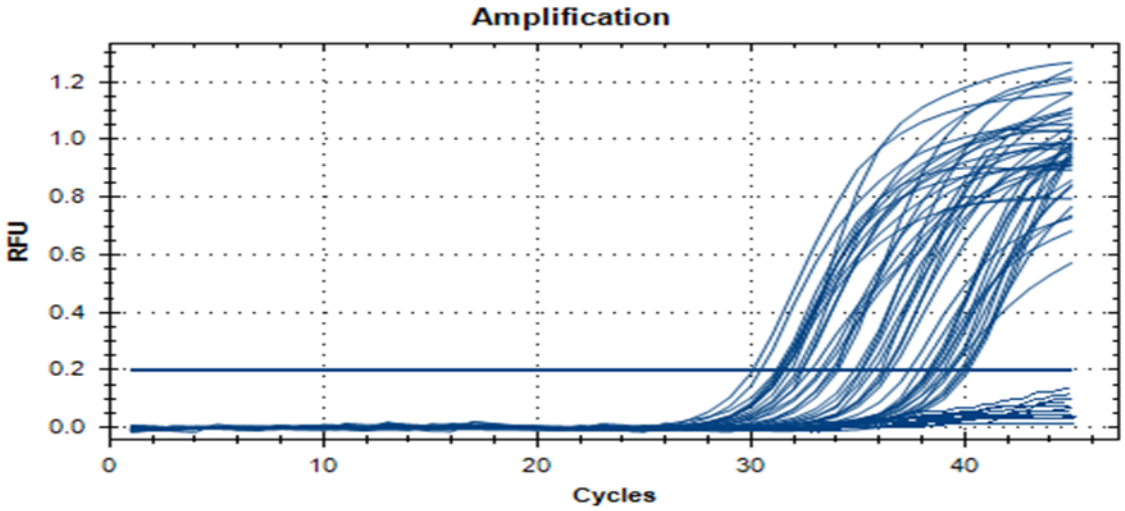

Figure (1):The Real -Time PCR Amplification plot of *Entamoeba histolytica* positive samples.

The present study demonstrated possession *E. histolytica* to three virulence factors under study using Real Time PCR using primers specific genes factors, also the molecular results confirmed the presence of virulence factor Cysteine protease in a sample of 97 out of 112 samples at a rate of 86.6% in figure (2) show the Amplification of positive samples of Cysteine protease.

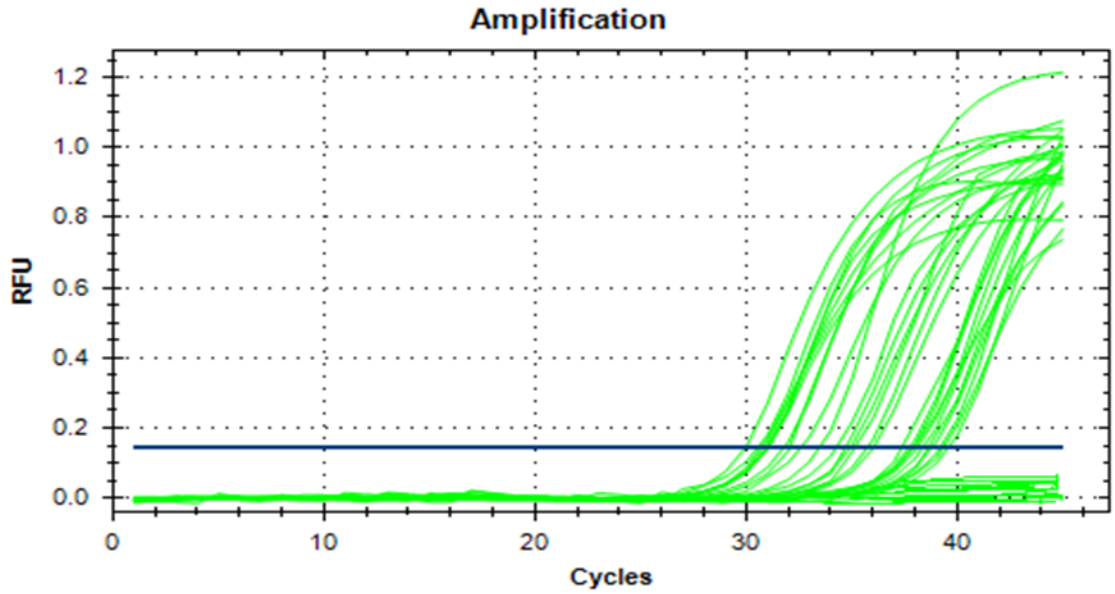

Figure (2):The Real -Time PCR Amplification plot for positive results for gene virulence factor Cysteine protease in *Entamoeba histolytica* .

Also the results showed the presence of the gene for virulence factor Amebopore in (80.35%) 90 samples out of 112 samples of *E. histolytica* Figure (3) .on other hand ,the Gal/lectin gene was detected in (75%)48samples out of 112 samples of *E. histolytica* Figure (4).

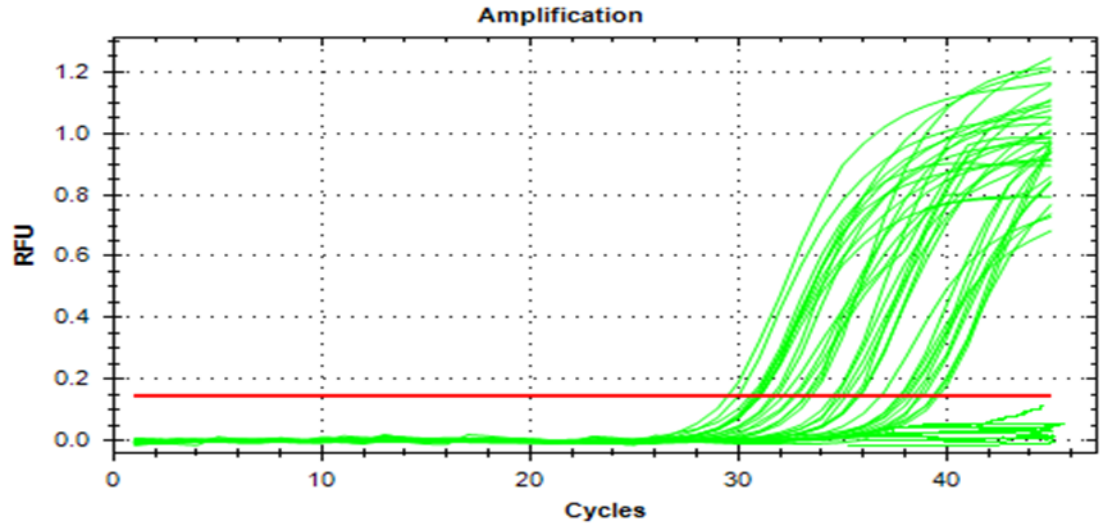

Figure (3):The Real -Time PCR Amplification plot for positive results for gene virulence factor Amebopore in *Entamoeba histolytica* .

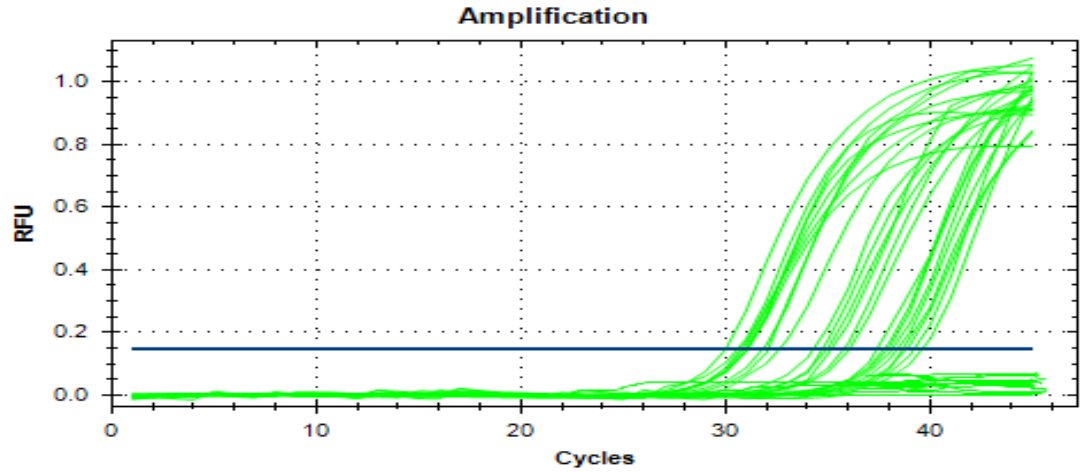

Figure (4):The Real -Time PCR Amplification plot for positive results for gene virulence factor Gal/lectin in *Entamoeba histolytica* .

Discussion:

The current study has been selected cysteine protenase gene to investigated *Entamoeba histolytica* . cysteine protenase was one of the three genes (Eh CP1, EhCp2, Ehcp5) that form about 90%of genes activity of the *E.histolytica* . There are evidences of the main role of cysteine proteinases as a virulence factor for *E. histolytica*, being involved in the breach of the mucus barrier, which is crucial in the pathogenesis of amoebiasis (5; 6). The proteolitic enzymes secreted by the parasite breach the mucus and the epithelial barrier, thus facilitating the penetration inside the tissue (7).

Consequently been investigating gene CP1 in the E.histolytica using Real time PCR, which is the most accurate. The results recorded presence gene CP1in the (97) sample out of 112 samples and this is consistent in of gene virulence factor which recorded by(8).

The results of Current study was targeted in one of its aspects the investigation of the Amebopore gene. The study recorded that Amebopore gene in the *E.histolytica* by 90 sample out of 112 with percentage 80.35% and this result is consistent in with (9) in their study about the pathogenesis of type *E.histolytica* because they knew this factor protein that is able to penetrate the bilateral fatty layers of the cell target and the formation of ion channels. Also Amoebapores, that are constitutively present at the cytoplasm of trophozoites (10), are capable of inducing apoptosis and necrosis of eukaryotic cells (11).

The present study recorded the Gal Lectin gene was detected in the *E.histolytica* by 84samples out of 112 samples with percentage 75% and this is consistent in terms of the genetic presence with(12) and (13) in their studies about the presence of this gene in *E.histolytica* and stated that the aim of the cell killing target depends on the adhesion of the parasite cell due to the possession of gene responsible for encryption Gal \ Gal Nac Lectin .

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