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# Detection of Calreticulin Gene EhCRT from Patients Infected with *Entamoeba histolytica* in Wasit

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## Abstract

*Entamoeba histolytica* is a major contributor to diarrheal illness and dysentery worldwide. This study aimed to employ a combination of conventional and molecular techniques to enhance the diagnosis of *E. histolytica* infected and to evaluate the expression of the calreticulin (EhCRT) gene . A total of 75 Patients' diarrheal stool specimens were gathered at the Al-Karama Hospital and health centers Wasit, Iraq during1st November to 30 February. The results of microscopic examination 30 samples out of 75, representing a percentage 40% .The Nested PCR aiming for the SSU rRNA gene yielded positive results in 21 out of the 30 samples who were identified as microscopically positive .The RT-qPCR analysis revealed a diverse pattern of EhCRT gene regulation among the 21 positive samples. Several samples exhibited significant up-regulation of the EhCRT gene, with fold changes as high as 51.934 and 22.449, indicating a potential role of calreticulin in the pathogenesis and *E. histolytica's* pathogenicity. Conversely, other samples displayed notable down-regulation of the EhCRT gene, with fold changes ranging from 0.015 to 0.120, which may be associated with a less virulent or less invasive phenotype of the parasite.

**Keywords:** *Entamoeba histolytica*, EhCRT, PCR, Microscopic examination, Diarrhea.

# الكشف عن جين EhCRT في المرضى المصابون ب *Entamoeba histolytica* في واسط

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**الخلاصة:** يعتبر طفيلي *Entamoeba histolytica* هو المسبب الرئيسي للاسهال والدزنتري في جميع انحاء العالم . هدفت هذه الدراسة الى استخدام التقنيات التقليدية والجزيئية لتشخيص عدوى ال *Entamoeba histolytica* وتقييم تعبير جين EhCRT .تم جمع 75 عينة براز من مرضى الاسهال في مستشفى الكرامة والمراكز الصحية في واسط / العر اق . خلال الفترة من 1 تشرين الثاني الى 30 شباط . كانت نتائج الفحص المجهري 30 عينة من اصل 75 بنسبة 40% , كانت نتائج تفاعل البلمرة المتسلسل الذي استهدف جين SSU rRNA 21 عينة من اصل 30 عينة تم تحديدها على انها موجبة في الفحص المجهري. كشف تحليل RT- qPCR عن نمط متنوع من تنظيم جين EhCRT بين 21 عينة موجبة , اظهرت عدة عينات تنظيم مفرط لجين EhCRT مع تغيرات تصل الى 51.934 و 22.449 مما يشير الى وجود دور محتمل لل Calreticulin في مسببات المرض والقدرة الممرضة للطفيلي . على العكس , اظهرت عينات اخرى انخفاض ملحوظ في تنظيم جين EhCRT مع تغيرات تتراوح بين 0.015 و 0.120 مما قد يكون مرتبط بنمط ظاهري اقل ضراوة او اقل غزو للطفيلي .

## 1.Introduction

An intestinal protozoan parasite called *E. histolytica* has the capacity to adhere to and kill epithelial tissue. In humans, It is the main reason why intestinal amebiasis occurs [1]. After schistosomiasis and malaria, the parasite *E. histolytica* is the reason of amoebic dysentery is among the most prevalent parasitic illnesses. This parasite invasion can cause colitis or liver amoebic , or it can be asymptomatic. Amoebic colitis has been identified as among the primary global reasons for acute diarrhea [2]. It ranks third globally as the most prevalent and deadly parasitic disease [3]. It is estimated that over 500 million people globally suffer from amoebiasis [4].The process of invasion starts when drinking or eating tainted with cysts and feces is consumed. It travels down the esophagus and into the stomach., where the stomach's acidic pH weakens the cyst's chitin wall, allowing it to enter the digestive tract where digestion takes place and the trophozoites proceed to the colon [5].The invasion process can cause serious tissue damage, such as toxic colon and liver abscesses, severe colitis, or amoebic colitis, depending on the strain's pathogenicity and the type of reaction of the host immune system [6].Frequent symptoms of this parasite-caused sickness include bloody diarrhea, fever, discomfort in the abdomen, colitis, malaise, tiredness, gas, and weight loss [7].

Because *E. histolytica* can pierce the wall of the intestine and invade the liver via the bloodstream portal, they can result in hepatic abscesses in extra-intestinal infections. These abscesses may be lethal if left untreated. Additionally, among additional organs , the brain and lungs may be affected by the abscesses [8]. One of the immunogenic substances that triggers a response of antibodies in the human host is the calreticulin of *E. histolytica* (EhCRT). Nevertheless, the complement system is inhibited by EhCRT's interactions with C1q and the C1 complex. Additionally, EhCRT influences host immune response regulation and pathology. In vitro, EhCRT functions as an immunogenic to specifically activate mononuclear cells from peripheral resulting in Th1 cytokines within the resolution stage and Th2 cytokines throughout the acute stage. Lastly, an overabundance of the CRT gene may represent a regulatory mechanism that enables the parasite for adaptation and endure in the tissues of host [9]. Together, the immune system's innate as well as adaptive components spontaneously eradicate this parasite. The body's initial, non-specific fight against pathogens is the innate immune system. During the invasion, amoebas will initially come into contact with innate immune responses such as stomach acid and a thick layer of mucus [10]. For many years, metronidazole was the preferred medication to treat amoebiasis. However, due to its toxic effects and recent ineffectiveness in treating a number of intestinal protozoan parasites, researchers are now looking for other medications and strategies to fight this parasite [11].

## 2. Materials and Methods

### *2.1.Samples Collection*

At the Al-Karama Hospital in Iraq, 75 diarrheal feces specimens from patients suspected of having an *Entamoeba histolytica* infection were first collected for the study,during1st November to 30 February . To preserve the samples' integrity, the stool samples were gathered in sterile receptacles and brought to the lab at the proper temperature. To link the laboratory results with the clinical presentation, the patients' clinical and demographic details, including age, gender, and symptoms, were documented. To find *Entamoeba* trophozoites and/or cysts, the fresh specimens of stool were examined under a microscope using direct saline/iodine wet mount

microscopy. For molecular analysis, Each specimen weighed around 0.2 g and was stored at -20°C.

*2.2. Molecular Detection*

DNA Extraction and PCR Targeting the SSU rRNA Gene DNA Extraction: The PRST Mini Kit of DNA Stool (Geneaid, Tawain) was used to extract genomic DNA from samples of feces following the guidelines provided by the manufacture. A 680-bp section of the *Entamoeba histolytica* RNA (SSU rRNA) gene that amplified by Nested PCR. The partial sequence of the *Entamoeba histolytica* strain HK9 17S ribosomal RNA gene (L36807.1) was used to generate the PCR primers. 12.5 µL of 2X PCR Master Mix, 1 µL of each primer (EhSSU-F: 5'-AGGACCATCAGAGATGCAAAGA-3' and EhSSU-R: 5'-TGTTTCTCGTGGGTTCTTTGAGA-3'), 5 µL from DNA template, and nuclease-free water on final volume of 25 µL make up the PCR master mix. Cycling conditions of PCR were: first denaturation at 95°C for 5 minutes, then succeeded by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 7 minutes. The amplified PCR products were examined by electrophoresis on a 1.5% agarose gel identified with ethidium bromide .

*2.3. Gene Expression Method*

The Accuzol Regent kit (Bioneer, Korea) was used for obtaining total RNA from the stool specimens . DNase I (Promega) was used to treat the isolated RNA in order to eliminate any remaining DNA contamination. The Prime Script RT Reagent Kit (Bionner) was then used to create complementary DNA (cDNA) of the RNA that had been processed with DNase. Japan). The expression level of the *Entamoeba histolytica* calreticulin (EhCRT) gene was measured using real-time PCR based on reverse transcription (RT-qPCR). The RT-qPCR master mix contained 12.5 µL of Promega GoTaq qPCR SYBR qPCR Master Mix (2X), 1 µL of each primer (EhCRT-F:5'-TGGACCAGATGTATGTGGAGG-3',EhCRT-R:TGGTGCTTCCCATTCTCCATC-3',Eh-α-actiTCACTGAAGCCCCACTGAATC3',EhαactinR:5'TGTTGAATGGGGCGAACATG-3'), 5 µL of cDNA template, and nuclease-free water to a final volume of 25 µL. The RT-qPCR cycling conditions were: initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds and annealing/extension at 60°C for 30 seconds.

*2.4.Data Analysis*

For additional evaluation and interpretation of the results, the data that extends from the real-time RT-qPCR and Nested PCR tests were analyzed using the proper statistical tools , involves determining the EhCRT gene's corresponding relative expression rates using the comparative Ct (ΔΔCt) method.

*2.5.Statistical Analysis*

To carry out an analysis of statistics, the data was tabulated in IBM SPSS Spreadsheet Version 26.0. To determine whether there were any notable variations the chi-square test was utilized. A likelihood value ($p \leq 0.05$) suggested the statistics' importance [12].

## 3. Results and Discussion

### *3.1.Total Prevalence of Entamoeba histolytica in Stool Samples*

The current study included 75 Patients with diarrhea had their feces samples taken. Every sample examined under a light microscope underwent general stool inspection (direct smear approach). 75 stool samples were analyzed under a microscope. Intestinal parasites were found in 30 samples (40.0%) of cases, rely on specific characteristics, such as having a single nucleus, compact nuclear chromatin, and ingesting red blood cells for trophozoites, and having a single nucleus and a transparent cyst wall for cysts, Figure 1 and 2. The positive result of Nested-PCR was 21 from 30 (70 %), while negative results was 8 (30%), as shown in Figure 3 and 4.

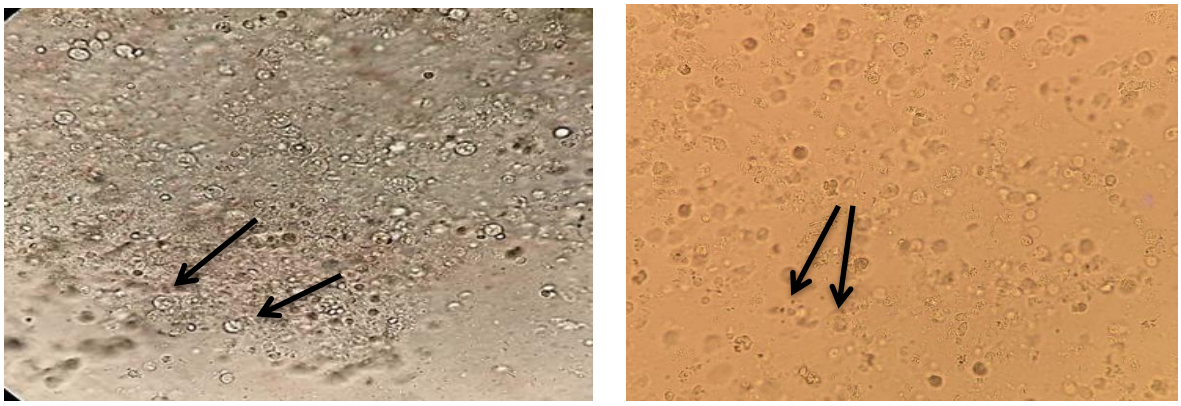

**Figure-1** Cyst of *Entamoeba histolytica* using Lugols iodine and normal saline examiner under 40x.

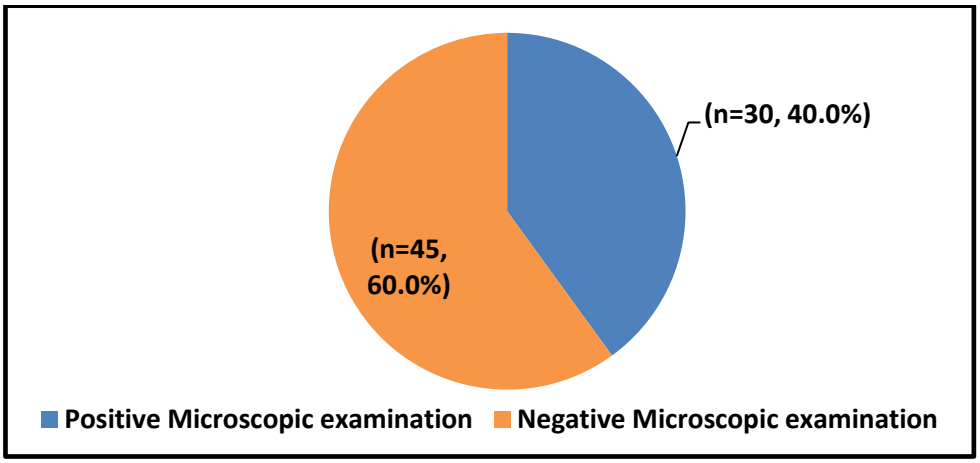


**Figure-2** The frequency percentage of positive and negative results of macroscopic examination.

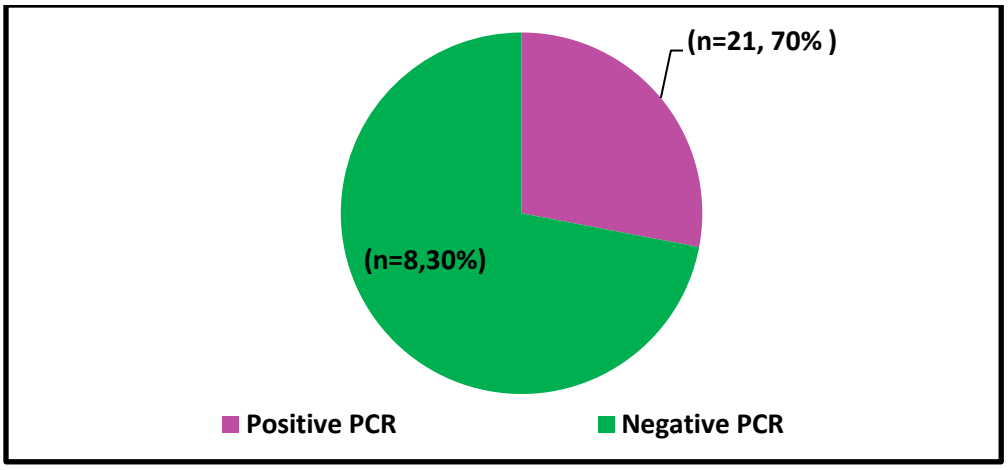


**Figure-3** The frequency percentage of positive and negative results of Nested PCR results.

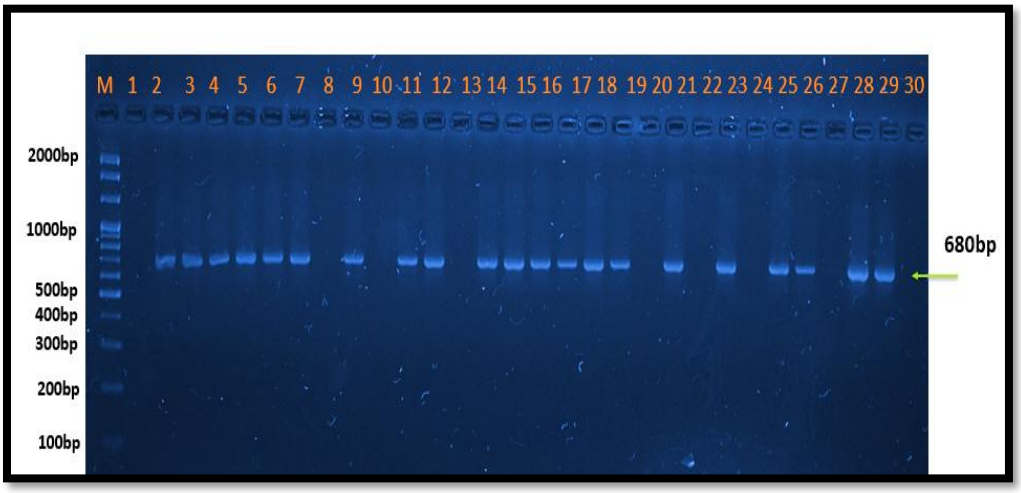


**Figure-4** Product electrophoresis of human DNA samples by Nested PCR .M: (DNA marker 2000 to 100bp. The line 1-30 at 680 bpproduct size show some positive *Entamoeba histolytica*.

The identification of *E. histolytica* microscopically is typically inaccurate and untrustworthy, especially when the stool material contains *Entamoeba* species that resemble *E. histolytica* in appearance , DNA was extracted from 30 stool samples that were microscopically found to have an *E. histolytica* infection in this experiment since microscopic examinations of *E. histolytic* are often inaccurate. Results show 21 samples (70%) were determined to be infection with *E. histolytica* by molecular detection; 11(52.3%) boys and 10 (47.6%) girls. The higher infection found in age between 1 to 10 years with percentage 38% , while lowest infection recorded in age 11-20 years with 9.5% years . Among the cases, stools of brown colour was found in 15 (71.4 %) subjects, mucoid stools 9 (42.8 %) and 8(38%) patients had liquid Table 1. However, the differences between the microscopic examination and molecular identification of *E. histolytica* infection by PCR technique may be due to the inaccurate storage condition that may lead to the destruction of the trophozoites stage of the parasite, small amount of stool that may contain very low parasitic load, treatment of patients with antiamoebic drugs.

The protozoan *E. histolytica* is the cause of amoebiasis, a human illness that has been found all over the world and results in significant morbidity and mortality. Parasite infections are influenced, either directly or indirectly, by ecological, biological, behavioral, social, and health-related variables [13]. Several studies have investigated the prevalence of *E. histolytica* in Wasit governorate. The current study's infection prevalence was highest when used Nested PCR 21 samples (70%). The outcomes of the present investigation are in agreement with those of an earlier study that looked at *E. histolytica* prevalence in stool specimens from Nasiriyah, Iraq's Dhi Qar Governorate. In this study PCR examination of 90 of the 2,639 stool samples used showed a very close *E. histolytica* infection rate was 74.44%, and 25.56% of the samples had negative results. In the same province, the results of the study conducted by [14] were similar to our results, as it recorded an infection rate 74% by using PCR . Also in the province of study Wasit, the results of the study conducted by [15] .were approach to our results, as it recorded an infection rate 80% by using PCR. These findings shed light on how common *E. histolytica* was among diarrheal patients in the study location over the specified time frame [16] . Our study's findings are at odds with those of [17] Where he found infection rate of *E. histolytica* was 38.1% prevalent in Thi-Qar, It is lower than our results. The results were also higher than what [18] achieved in Babylon for his record 34% about registration 24 positive samples from 70 samples.

Regarding age the maximum age of infection with *Entamoeba* species was 1>10 years recorded (38%). This may be connected to the age group's highest level of physical development, ongoing exposure to infections and pollution, weakened immune systems and inadequate sanitation. This result fits in study done in Duhok City, showed that children between the ages of 1 and 10 had the greatest infection rates (54.09%) [19]. Our study was identical to a study that was conducted in Eastern Kurdistan of Iraq found infection was (23.4%) in 1–3 years old [20].These outcomes match up with research by [8] in Jordan record 35 positive samples with percentage 50% in age 2-12. Contrary to our results, the lowest infection rate in Babylon Governorate was in the age group.1-10 (7%) [18] . Also in Erbil Northern Iraq, the results were the opposite of what we had concluded since the age group had the highest infection rate between 15-18 while highest infection in age group 26-35 [21].

As for Gender Our results were consistent with what was recorded in Babylon, where the infection rate was (54.3 %) and (45.7 %) in male and female respectivlly [18]. In Duhok province Iraq , males had a higher infection rate than females consistent with our study and at a rate of (67.43%) in male and (32.56%) in female [22]. However, several studies found that females had higher rates of amoebosis than males such as in Erbil [23] discovered that females had a greater incidence than males as show as follow (58.8% vs 41.4%). While [24], revealed rates that were almost same for men and women (49.6% versus 50.4 %). Because androgens make men enlist more leukocytes this makes immune-mediated disease more likely, hormonal fluctuations , immunological and behavioral may be responsible for the variation in rates of infection among males and females [25]. Additionally, working-class men are more likely to engage with their surroundings and consume food and beverages from street vendors or public places, which increases their chance of getting sick [26]. There are numerous immune-related genes on the X chromosome of which men only have one and women have two. Females may have an immunological edge over males as a result.

**Table 1-** Demographic characteristics of patients depend of Nested PCR.

| Characteristic | n (21) | % | P value |
|---|---|---|---|
| Age groups | | | |
| 1-10 years, *n* (%) | 8 | 38.1 % | |
| 11-20 years, *n* (%) | 2 | 9.6 % | |
| 21-30 years, *n* (%) | 4 | 19.0 % | |
| 31-40 years, *n* (%) | 3 | 14.3 % | 0.292 |
| ≥ 40 year, *n* (%) | 4 | 19.0 % | |
| Total | 21 | 100.0 % | |
| Gender | | | |
| Male, *n* (%) | 11 | 52.4 % | 0.827 |
| Female, *n* (%) | 10 | 47.6 % | |
| Total | 21 | 100.0 % | |
| Stool consistency | | | |
| Mucoid, *n* (%) | 9 | 42.8 % | |
| Liquid, *n* (%) | 8 | 38.1 % | |
| Bloody, *n* (%) | 1 | 4.8 % | 0.036* |
| Normal, *n* (%) | 3 | 14.3 % | |
| Total | 21 | 100.0 % | |
| Stool consistency | | | |
| Brown, *n* (%) | 15 | 71.4 % | |
| Yellow, *n* (%) | 3 | 14.3 % | |
| Bloody, *n* (%) | 1 | 4.8 % | 0.001* |
| Green, *n* (%) | 2 | 9.5 % | |
| Total | 21 | 100.0 % | |

A high proportion of patients in the amoebic group had mucoid (42.8 %) whereas very few patients (4.7%%) had bloody stool, these results similar to the [27] found depending of stool

consistency the higher infection in mucoid 33 sample from 44 and lowest infection in bloody 3 from 44 this is consistent with our findings. The researcher found himself in the following two years the same results, where he recorded in Baghdad mucoid stool in 55 samples with 77% and lowest infection also in bloody stool with percentage (4.23%). However, their results differed from ours regarding stool color, the highest incidence in our study was brown with percentage 71.4% while in their study it was yellow 50% [28].

*3.2. Gene Expression Analysis*

In our work, the calreticulin gene was selected for investigation as a virulence factor of the parasite *E. histolytica*. The current study's findings demonstrated that, using RT-qPCR technology, the EhCRT gene was positive in 21 of the 21 samples that were examined, with a 100% prevalence. Several samples exhibited significant up-regulation of the EhCRT gene, with fold changes as high as 51.934 and 22.449, indicating a potential role of calreticulin in the pathogenesis and *E. histolytica's* virulence. Conversely, other samples displayed notable down-regulation of the EhCRT gene, with fold changes ranging from 0.015 to 0.120, which may be associated with a less virulent or less invasive phenotype of the parasite as shown in Table 2. Figure 5 and 6.

**Table 2-** Gene expression analysis

| Sample ID | EhCRT (Target) $C_T$ | Eh-α-actin (Ref) $C_T$ | $\Delta C_T$ (CRT - actin) | $\Delta\Delta C_T$ ($\Delta CT_{sample}$ - mean$\Delta$CT) | Fold Change ($2^{-\Delta\Delta CT}$) | Regulation |
|---|---|---|---|---|---|---|
| EH-01 | 29.81 | 25.29 | 4.520 | 0.301 | 0.811 | ↓ Down-regulated |
| EH-02 | 24.84 | 26.32 | -1.480 | -5.699 | 51.934 | ↑ Up-regulated |
| EH-03 | 27.34 | 24.44 | 2.900 | -1.319 | 2.494 | ↑ Up-regulated |
| EH-04 | 30.48 | 24.43 | 6.050 | 1.831 | 0.281 | ↓ Down-regulated |
| EH-05 | 25.22 | 25.49 | -0.270 | -4.489 | 22.449 | ↑ Up-regulated |
| EH-06 | 30.10 | 22.82 | 7.280 | 3.061 | 0.120 | ↓ Down-regulated |
| EH-07 | 29.29 | 24.35 | 4.940 | 0.721 | 0.607 | ↓ Down-regulated |
| EH-08 | 27.00 | 23.26 | 3.740 | -0.479 | 1.393 | ↑ Up-regulated |
| EH-09 | 28.05 | 24.85 | 3.200 | -1.019 | 2.026 | ↑ Up-regulated |
| EH-10 | 24.69 | 23.72 | 0.970 | -3.249 | 9.504 | ↑ Up-regulated |
| EH-11 | 32.10 | 24.80 | 7.300 | 3.081 | 0.118 | ↓ Down-regulated |
| EH-12 | 32.60 | 22.33 | 10.270 | 6.051 | 0.015 | ↓ Down-regulated |
| EH-13 | 30.83 | 25.93 | 4.900 | 0.681 | 0.624 | ↓ Down-regulated |
| EH-14 | 27.68 | 23.17 | 4.510 | 0.291 | 0.817 | ↓ Down-regulated |
| EH-15 | 27.55 | 23.92 | 3.630 | -0.589 | 1.504 | ↑ Up-regulated |
| EH-16 | 31.91 | 23.63 | 8.280 | 4.061 | 0.060 | ↓ Down-regulated |
| EH-17 | 25.00 | 22.13 | 2.870 | -1.349 | 2.547 | ↑ Up-regulated |
| EH-18 | 29.65 | 26.53 | 3.120 | -1.099 | 2.142 | ↑ Up-regulated |
| EH-19 | 28.77 | 24.64 | 4.130 | -0.089 | 1.063 | ↔Normalexpression |
| EH-20 | 23.82 | 23.41 | 0.410 | -3.809 | 14.012 | ↑ Up-regulated |
| EH-21 | 28.27 | 20.95 | 7.320 | 3.101 | 0.117 | ↓ Down-regulated |

Based on 21 nested-PCR positive *Entamoeba histolytica* patients , EhCRT expression: High (fold change ≥ 1.5 up-regulated) vs Low (fold change < 1.5 down-regulated or normal). Data derived from RT-qPCR analysis (Table 3). The highest proportion of high EhCRT expression was seen in the youngest age group (1-10 years: 62.5%), while older patients (≥40 years) showed predominantly low expression (75.0%). Depending of gender high expression was slightly more frequent in males (54.5%) than females (40.0%).While Stool consistency

show high EhCRT expression was markedly more common in abnormal stool types mucoid (77.8%), liquid (75.0%) and the single bloody sample (100%). In contrast, all patients with normal stool consistency had low expression (100%). In Stool color the only bloody stool sample showed high expression (100%) , Brown stools had a moderate proportion of high expression (53.3%), while yellow and green stools were associated with low expression in most cases.

These findings indicate that up-regulation of EhCRT is strongly associated with abnormal stool characteristics (mucoid, liquid, bloody) and younger age, suggesting a potential role in more symptomatic and invasive disease.

**Table 3-** Relationship analysis EhCRT Gene Expression according to Demographic and Clinical Parameters

| Parameter | Category | Total n (%) | High EhCRT Expression n (% within category) | Low EhCRT Expression n (% within category) |
|---|---|---|---|---|
| Age groups | 1-10 years | 8 (38.1%) | 5 (62.5%) | 3 (37.5%) |
| | 11-20 years | 2 (9.6%) | 1 (50.0%) | 1 (50.0%) |
| | 21-30 years | 4 (19.0%) | 2 (50.0%) | 2 (50.0%) |
| | 31-40 years | 3 (14.3%) | 1 (33.3%) | 2 (66.7%) |
| | ≥ 40 years | 4 (19.0%) | 1 (25.0%) | 3 (75.0%) |
| Gender | Male | 11 (52.4%) | 6 (54.5%) | 5 (45.5%) |
| | Female | 10 (47.6%) | 4 (40.0%) | 6 (60.0%) |
| Stool consistency | Mucoid | 9 (42.8%) | 7 (77.8%) | 2 (22.2%) |
| | Liquid | 8 (38.1%) | 6 (75.0%) | 2 (25.0%) |
| | Bloody | 1 (4.8%) | 1 (100%) | 0 (0%) |
| | Normal | 3 (14.3%) | 0 (0%) | 3 (100%) |
| Stool color | Brown | 15 (71.4%) | 8 (53.3%) | 7 (46.7%) |
| | Yellow | 3 (14.3%) | 1 (33.3%) | 2 (66.7%) |
| | Bloody | 1 (4.8%) | 1 (100%) | 0 (0%) |
| | Green | 2 (9.5%) | 0 (0%) | 2 (100%) |

In our work, the calreticulin gene was selected for investigation as a factor that promotes of the parasite *E. histolytica*. According to the current study's findings, 21 samples had a 100% occurrence of the EhCRT gene. This results agree with [29] in Thi Qar discovered the pathogenicity factor 50 of the 50 samples that were evaluated tested positive for EhCRT with a 100% frequency utilizing PCR technique. [27] revealed that in 36 (81.8%) out of 44 samples from the amebic group of Baghdad's population expressed the EhCRT gene and they discovered that its expression produces a virulence factor that contributes to host pathogenic processes with the length of diarrhea. A study conducted in Baghdad also discovered that the EhCRT gene's production rate was high in the tested samples was found 84.1% (37 out of 44) [28].

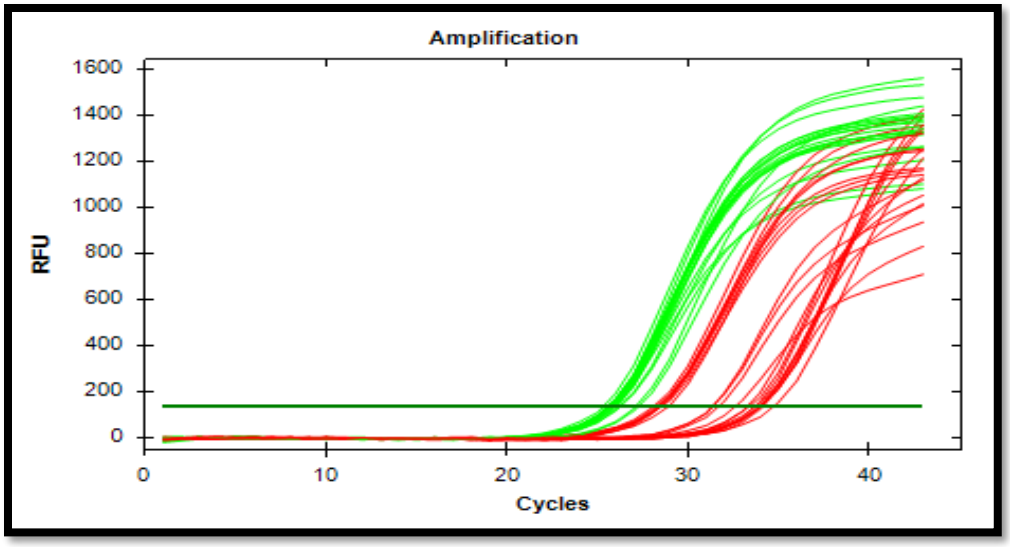


**Figure- 5** qPCR amplification plot of target gene (EhCRT) red plots and reference gene (actin) actin green plots**.**

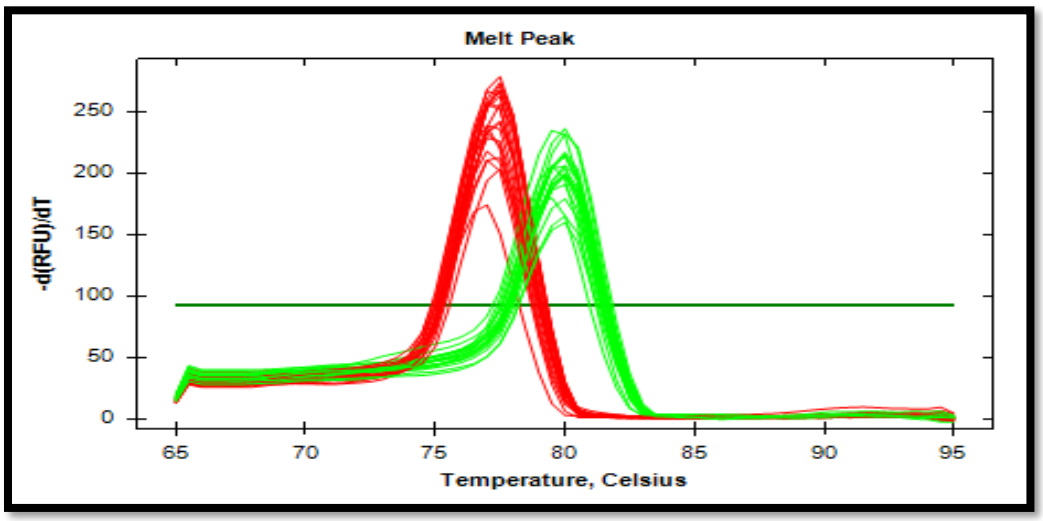


**Figure -6** qPCR melting curve of target gene (EhCRT) red plots and reference gene (actin) actin green plots

All eukaryotic cells, with the exception of fungi and erythrocytes, contain the multipurpose protein CRT. Mammalian CRT is mostly found in the endoplasmic reticulum, where it functions as a chaperone and calcium-binding protein. On the other hand, CRT for protozoa and metazoans seem to be secreted proteins that when trophozoites invade tissue, adapt improperly to their surroundings, modulate human immunity, and overexpress the immunogenic protein EhCRT Ag. EhCRT expression increases as the pathophysiology becomes more severe [29]. The highest proportion of high EhCRT expression was seen in the youngest age group in male , higher expression in children age related immune response causes by children often exhibit higher calreticulin expression due to an immature or developing immune system that relies more heavily on innate immune mechanisms this leads to stronger or less regulated inflammatory responses when exposed to pathogens like *E. histolytica* . Additionally, higher parasite burden and increased epithelial damage in pediatric cases further stimulate ER stress and immune activation pathways [30].

## 4.Conclusion

When diagnosing an *E. histolytica* infection, the PCR is a useful method . Most of amebic diarrhea Among kids between the ages of 1 and 10, with mucoid diarrhea, acute amebiasis, associated with initial diarrheal bouts. The accuracy and dependability of the diagnosis of *E. histolytica* infection can be improved by combining traditional microscopy with molecular methods, such as the assessment of EhCRT gene expression. The host-parasite interactions and the possible involvement of calreticulin in the pathophysiology of amebiasis in the Wasit, Iraq population are revealed by the patterns of EhCRT gene regulation. To clarify the mechanisms that underlie and clinical implications of the different expression levels of the EhCRT gene, more investigation is necessary.

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