Original Article

# Detection of *Eh*CRT Gene Expression in *Entamoeba histolytica*-Infected Children and its Correlation with Interleukin 25 and Tumor Necrosis Factor Alpha

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## Abstract

**Objectives:** *Entamoeba histolytica* is a human enteric protozoan, which is the causative agent of amebiasis. The host activates a series of immunological responses to protect against the parasite after contact with the ameba and further invasion of the gut epithelium layer. As a result, the ameba has developed a variety of evasion mechanisms to hold out the immune response and continue to survive and cause disease. The calreticulin (*Eh*CRT) is one of the immunogenic molecules of *E. histolytica* that induces an immune response in the human host. Increase in the expression of the *Eh*CRT gene could provide control mechanism that allows the parasite to adapt and survive in host tissues. **Aim of the Study:** This study was designed to detect the *Eh*CRT gene of *E. histolytica* by real-time polymerase chain reaction (PCR) in stool samples of children with amebiasis and its roles in host–parasite relationship via measuring the concentration of tumor necrosis factor alpha (TNFα) and interleukin 25 (IL25) by enzyme-linked immunoassay (ELISA) technique in their serum. **Materials and Methods:** A total of 86 diarrheal fecal samples were collected from children in age <1 year to 13 years suspected to be infected with *E. histolytica* during the period from December 30, 2020, to September 1, 2021. Microscopically positive samples were the subject to conventional PCR and real-time PCR for the detection of *E. histolytica* HM1:IMSS strain using (*Psp*) gene sequences and detection of calreticulin (*Eh*CRT) expression. Blood was withdrawn from each child included in the study for ELISA test to measure the level of IL25 and TNFα. **Results:** Fecal samples for microscopic examination revealed that 71 (82.6%) children had amebic colitis, *E. histolytica* gene was detected in 44 samples (71%) using conventional PCR, and the immunogene *Eh*CRT was expressed in 36 stool samples using real-time PCR. The results of the recent study showed highly significant elevation in the level of TNFα and IL25 in the amebic group (*Eh*+ve PCR). The majority of amebic children were in the age group of 1–4 years, had mucoid, acute, and with primary episodes of diarrhea. **Conclusion:** *E. histolytica* is a protozoan parasite highly prevalent among diarrheal children and is responsible for gastrointestinal amebiasis in the human host. The PCR is a useful tool in the diagnosis of *E. histolytica* infection. It is clear that the expression of the calreticulin gene (*Eh*CRT) concedes with the duration of diarrhea a virulence factor that plays a role in host pathogenic pathways. The findings of this study showed that the level of TNFα in the serum of children infected with amebic colitis (*Eh* gene + ve) is significantly increased during the course of infection and the cytokine IL25 exhibits a significant drops in the same children.

**Keywords:** *Eh*CRT, *Entamoeba histolytica,* interleukin 25, intestinal amebiasis, tumor necrosis factor alpha

## Introduction

*Entamoeba histolytica*, a protozoan parasite that causes amebiasis, infects the intestine. The symptoms include diarrhea, dysentery, and colitis. It is considered the third common parasitic cause of morbidity and mortality.[1] *E. histolytica* is thought to infect 50 million individuals worldwide each year, causing 70,000 fatalities.[2] While 90% of the infected people have no symptoms, the infection can cause serious

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consequences such as colitis, bloody diarrhea, liver abscesses, and colonic perforation.[3]

Ameba virulence is mediated by the number of mechanisms involving unique molecular interactions at both the cellular and molecular levels. These interactions between the host and the parasite take place in a succession of steps. Trophozoites link to intestinal epithelial cells via the parasite's surface Gal/GalNAc lectin, which binds to galactose (Gal) and/or N-acetyl-D-galactosamine (GalNAc) in the host cell membrane.[4] After sticking to host cells, an ameba uses a variety of cytotoxic processes, including apoptosis, phagocytosis, and trogocytosis to cause cell death and tissue invasion.[5]

The calreticulin of *E. histolytica* (*Eh*CRT) is one of the immunogenic molecules that stimulate an antibody response in the human host. However, *Eh*CRT interacts with C1q and C1 complex that inhibiting the complement system. *Eh*CRT also affects pathogenesis and the modulation of the host immunological response. *Eh*CRT works as an immunogenic for the specific activation of peripheral blood mononuclear cells *in vitro*, causing a Th2 cytokine profile during the acute phase and a Th1 profile during the resolution phase. Finally, excess of the CRT gene could constitute a regulatory mechanism that allows the parasite to adapt and survive in host tissues.[6]

As amebic binding to epithelium occurs, proinflammatory cytokines are secreted. The cytokine tumor necrosis factor alpha (TNFα) promotes inflammation. TNFα is a major mediator of mucosal inflammation seen across high concentrations in the gastrointestinal tract in some forms of inflammatory colitis. Increased TNFα release is linked to *E. histolytica* diarrhea, and an overly aggressive TNFα-induced immune response improves inflammation and hence disease.[7]

Inflammatory cytokines are secreted by epithelial cells, which signal and mobilize immune cells to kill parasites that have ready aliped through the wall of tuft cells are chemosensory cells in the epithelial lining of the intestines because they secrete interleukin 25 (IL25), a protective cytokine that can activate both innate and adaptive responses.[8]

## Materials and Methods

### Sample collection

This study was conducted on 86 children (between the ages of 1 month and 13 years) attending the Central Teaching Hospital of Pediatric and Al Mahmoudia General Hospital suffering from diarrhea and abdominal pain and suspected to be infected with *E. histolytica*. The sampling was collected during the time between December 30, 2020, and September 1, 2021. It was suspected of being infected with *E. histolytica*. After microscopic analysis, samples were transported on dry ice or ordinary ice. It was patient group divided into two parts. The first part is kept directly at -20 °C until to extraction DNA by treatment with PCR technique, and the second part was added to 300 ml TRIzol, after which it was stored in a freezer at -20°C until the process of extracting RNA from it began and subjecting it to real-time PCR.[7] For ELISA test to measure the level of IL25 and TNFα 2 ml of blood was with form each patient tube then centrifuge at about 5000 rpm for 5 min to separated the serum, which was aspirated using a micropipette and transfer into a sterile tube (Eppendorf tubes), and kept at −20 C° until the process of ELISA assays. The complete information for each patient was recorded in a questionnaire including name, age, sex, number of recurrence of infection, number of days of diarrhea, color of stool, and stool consistency. This form and personal interviews were applied to each patient participating in the current study.

### DNA and RNA extraction

The stool sample was divided into two parts: one part directly treated by the QIAamp DNA stool Mini Kit (Qiagen\Germany) to obtain the DNA for the diagnostic identification *psp* gene of *E. histolytica* and the second part conserved in TRIzol-utilized RNA extracted by Direct-zol™ RNA MiniPrep (ZYMO/USA) to obtain the RNA for the diagnosis of *Eh*CRT gene expression. Then, conversion of RNA to cDNA by cDNA synthesis was performed using PrimeScript™ RT Reagent Kit according to the manufacturer's instructions.

### Polymerase chain reaction identification of *Entamoeba histolytica*

*Psp* oligonucleotides were used to amplify DNA. These oligonucleotides amplified an 876-bp product. Amplification was performed in a MultiGene OptiMax Gradient Thermal Cycler (Labnet/USA). The amplification conditions were DNA denaturation for 5 minutes at 95°C, followed by 45 cycles of denaturation for 30 seconds for alignment at 53°C, and extension for 1 min at 72°C, followed by a final extension step of 10 min at 72°C.

### Real-time polymerase chain reaction for *Eh*CRT

For the quantification and expression of the specific oligonucleotides, *Eh*CRT5 TGGACCAGATGTATGTGGAGG and *Eh*CRT3 TGGTGCTTCCCATTCTCCATC primers were used in reverse transcriptase quantitative PCR (qPCR). In the qPCR, the Step KAPA SYBR® FAST qPCR Master Mix (2X) Kit (Kapa/USA) was performed. The amplification was carried out in 40 cycles with three stages: a denaturing stage at 95 °C for 30 seconds, an alignment phase at 51 °C for 30 seconds, an elongation phase at 72 °C for 30 seconds, and a final stage at 90 °C to complete the reaction. The amplification of *Eh*-α-actin reference gene (1000-bp) (the housekeeping gene) and an *E. histolytica* DNA control was included.[1]

### Enzyme-linked immunoassay test for interleukin 25 and tumor necrosis factor alpha

Serum levels of IL25 and TNFα were quantitatively determined in patients and control subjects by means of sandwich ELISA test using commercially available kits. It was used bioassay (China) kit the procedure Kit according to the manufacturer's instructions.

## Results

Positive amebiasis cases were revealed in 71 out of 81 fecal samples. The DNA extraction method was employed to extract the DNA and RNA of *E. histolytica* from stool samples. The DNA and RNA of *E. histolytica* were extracted from 62 (72.1%) out of 71 samples [Table 1]. The DNAs obtained from stool samples of amebiasis-infected children were evaluated by PCR using oligonucleotides specific (*Psp*). The result in Table 1 shows that 44 (71.0%) out of 62 samples were positive for *E. histolytica*. The real-time PCR assay was performed to measure the expression of calreticulin (*EhCRT*) in stool sample. The *EhCRT* was expressed in 36 (81.8%) out of 44 samples from the amebic group. The result of PCR amplification showed that the detection of gene *E. histolytica* was found in 84.1% (37 out of 44 +ve cases) of samples whom had *E. histolytica* acute infection. The gene of *E. histolytica* was isolated from the stool samples of all chronic *E. histolytica*-infected 7 (15.9%) children.

A highly significant ($P \leq 0.0001$) increase in the titer of TNFα was seen in amebic (478.20 ng/l) when compared to their titer in nonamebic group (307.67 ng/l). While significant ($P \leq 0.053$) decreases in the titer of IL 25 was seen in amebic (1013.19 ng/l) when compare with their titer in nonamebic group (1498.82 ng/l) [Table 2].

A significant elevation in the serum level of TNFα and IL25 in relation to the expression of *Eh*CRT Ag (delta value) in the stool samples of children whom strongly expressed *Eh*CRT Ag, their serum levels of TNF-α was 716.09 ng/l and of IL25 was 4052.64 ng/l than that of *Eh*CRT Ag not expressed [Table 3].

High concentration of TNFα was seen in the age groups 8–12 years (494.13 ng/l) and nonsignificant differences in the concentration of IL25 between the different age groups. Children in the age groups ≤1 years had (nonsignificant) the highest concentration (1685.60 ng/l) to IL25 [Table 4].

Regarding the gender, the concentration of TNFα in the serum of females (549.64 ng/l) was significantly ($P = 0.017$) higher than that in the serum of amebic males (425.33 ng/l) and both nonamebic groups; female (373.79 ng/l) and male (302.83 ng/l) respectively. On the other hand, the concentration of IL-25 in the serum of amebic group males (1181.31 ng/l) is none significantly ($P = 0.399$) higher than their level in serum of amebic female (752.63 ng/l) and lower than its level in the serum of the nonamebic male and female [Table 5].

The concentration of TNF α in the serum of patients with primary episodes of diarrhea due to *E. hitolytica* infection (489.50 ng/l) are significantly higher than their concentration in those with recurrent diarrhea in amebic group (431.11ng/l) and both nonamebic with primary (300.62 ng/l) and recurrent (321.78 ng/l) episodes of diarrhea. On other hand, the serum level IL25 in the serum of amebic child with primary episodes of diarrhea (1049.37 ng/l) was significantly higher than in the serum of those with recurrent diarrhea in amebic (882.93 ng/l) and nonamebic (956.65 ng/l), while it was significantly lower in those nonamebic with primary diarrhea (1769.90 ng/l) [Table 6].

According to the stool consistency, the titration of TNFα in the serum of children with amebiasis and had bloody

**Table 1: DNA extraction, polymerase chain reaction and quantitative polymerase chain reaction identification of *Entamoeba histolytica* using (*Psp*) gene and (*Entamoeba histolytica* calreticulin) expression in stool sample from amebic and nonamebic groups**

| | | | *n* (%) |
|---|---|---|---|
| DNA extraction | | | |
| Amebic group | | | |
| Positive | | | 62 (72.1) |
| Negative | | | 9 (10.5) (12.7) |
| Total | | | 71 (82.6) |
| Nonamebic group | | | 15 (17.4) |
| Total | | | 86 (100.0) |
| PCR identification of *Eh* | | | |
| Amebic group | | | |
| Gene of *Eh* (HM1: IMSS) +ve | | | 44 (71.0) |
| Gene of *Eh* (HM1: IMSS) −ve | | | 18 (29.0) |
| Total *Eh* (HM1: IMSS) +ve | | | 62 (87.3) |
| DNA−ve | | | 9 (12.7) |
| Total | | | 71 (100) |
| Expression of calreticulin (*Eh*CRT) | | | |
| qPCR product (*Eh*CRT) +ve | | | 36 (81.8) |
| qPCR product (*Eh*CRT) −ve | | | 8 (18.2) (11.3) |
| Total | | | 44 (100) (62.0) |
| Total DNA−ve and EhCRT−ve | | | 27 (38.0) |
| Total amebic | | | 71 |
| **PCR for gene detection** | **Gene of *Eh* +ve, *n* (%)** | **Gene of *Eh* −ve, *n* (%)** | **Total, *n* (%)** |
| Acute infection | 37 (84.1) (67.3) | 18 (100) (32.7) | 55 (88.7) |
| Chronic infection | 7 (15.9) (11.2) | 0 | 7 (11.3) |
| Total | 44 (71.0) | 18 (29.0) | 62 |
| Fisher's exact test | | 0.096 | |

*Eh*: *Entamoeba histolytica*, PCR: Polymerase chain reaction, qPCR: Quantitative PCR, *Eh*CRT: *Eh* calreticulin

**Table 2: The serum concentration of tumor necrosis factor-α and interleukin-25 in amebic and nonamebic groups**

| Groups | *n* | Titer (ng/l) | SD | SEM | *P* |
|---|---|---|---|---|---|
| TNF-α | | | | | |
| Amebic (*Eh* +ve gene) | 44 | 478.20 | 202.92 | 36.45 | ≤0.0001 |
| Nonamebic (*Eh* −ve gene) | 15 | 307.67 | 86.02 | 22.21 | |
| IL-25 | | | | | |
| Amebic (*Eh* +ve gene) | 44 | 1013.19 | 657.69 | 137.14 | 0.053 |
| Nonamebic (*Eh* −ve gene) | 15 | 1498.82 | 832.77 | 215.02 | |

SD: Standard deviation, SEM: Standard error mean, *Eh*: *Entamoeba histolytica*, TNF-α: Tumor necrosis factor-α, IL-25: Interleukin-25

stool (549.64 ng/l) was seen to be significantly ($P < 0.022$) higher when compared with it is titration in serum of children infected with *E. histolytica* complaining mucoid (493.93 ng/l), liquid (429.58 ng/l) or the nonamebic group complaining mucoid (354.49 ng/l) or liquid (284.25 ng/l) diarrhea. The serum level of IL25 showed a nonsignificant ($P = 0.066$) increase in the nonamebic children complaining liquid diarrhea (1718.88 ng/l) when compared with those complaining mucoid (1058.69 ng/l) diarrhea in the same group or those complaining mucoid (1104.77 ng/l), liquid (662.89 ng/l) and bloody (845.71 ng/l) diarrhea in *E. histolytica*-infected group [Table 7].

The result in Table 8 showed an increase in the serum level of TNFα of amebic children with chronic infection (513.68 ng/l) which it was significantly ($P = 0.013$) higher than in the acute phase of infection of both amebic (472.94 ng/l) and nonamebic (307.67 ng/l) groups respectively. Serum level of IL25 in nonamebic acutely infected children (1498.82 ng/l) was found to be nonsignificantly increased when compared with amebic group (acute 984.54 ng/l and chronic 1204.12 ng/l infections).

**Table 3: Serum level of tumor necrosis factor-α and interleukin-25 in relation to the expression of calreticulin *Entamoeba histolytica* calreticulin antigen**

| Expression | *n* | Titer (ng/l) | SE | SEM | *P* |
|---|---|---|---|---|---|
| TNFα | | | | | |
| *Eh*CRT Ag up-regulated | 36 | 716.09 | 33.08 | 23.39 | 0.027 |
| *Eh*CRT Ag down-regulated | 8 | 432.11 | 109.15 | 54.57 | |
| IL-25 | | | | | |
| *Eh*CRT Ag up-regulated | 36 | 4052.64 | 200.70 | 141.91 | 0.002 |
| *Eh*CRT Ag down-regulated | 8 | 1584.10 | 464.13 | 232.06 | |

Ag: Antigen, SD: Standard deviation, SEM: Standard error mean, *Eh*CRT: *Entamoeba histolytica* calreticulin, TNF-α: Tumor necrosis factor-α, IL-25: Interleukin-25

## Discussion

Amebiasis is a disease caused by the protozoan *E. histolytica*, which has a worldwide distribution and causes widespread morbidity and mortality.[9] Environmental, biological, behavioral, social, and health-related factors all have an impact on parasite. It is a wide spread intestinal disease in Baghdad City and at all age groups.[10]

Because microscopic investigations of *E. histolytic* are frequently erroneous and unreliable, especially in samples including morphologically indistinguishable species such as *E. dispar, E. bangladeshi*, and *E. moshkovskii*, molecular methods are effective for identifying *Entamoeba* spp.[11] DNA was isolated from 62 of the 71 stool samples that were microscopically diagnosed as being infected with *E. histolytica* in this investigation. The gene of *E. histolytic* was detected in 44 stool samples out of 62 using PCR assay. Using real-time PCR, the *Eh*CRL were found to be expressed in 32 stool samples that improve to be diagnosed as *E. histolytic* conventional PCR. This result is identical with that of other studies.[12] However, the differences between the microscopic examination and molecular identification of *E. histolytica* infection by PCR technique may be due to the lysis of trophozoites that results from sample storage or may be due to the presence of other species of *Entamoeba* that have a morphology similar to *E. histolytic* which was microscopically diagnosed as *E. histolytic*. The small number of samples, treatment of patients with anti-amebic drugs, number of cycle for PCR assay, and the presence of other *Entamoeba* spp. inhabiting the human gut are factors that could affect the result of detection of *E. histolytica* and the expression of *Eh*CRL antigen by PCR technique.[13,14] The result of PCR showed that

**Table 4: Concentration of tumor necrosis factor-α and interleukin-25 in different age groups**

| | *n* | Titer (ng/l) | SD | 95% CI for mean | | Minimum | Maximum | *P* |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound | | | |
| TNF-α (years) | | | | | | | | |
| ≤1 | 8 | 234.76 | 69.54 | 124.10 | 345.41 | 136.61 | 299.41 | 0.026 |
| 1.1-4 | 22 | 462.56 | 193.58 | 387.50 | 537.62 | 208.67 | 925.73 | |
| 4.1-8 | 9 | 389.23 | 183.69 | 248.03 | 530.42 | 211.26 | 832.59 | |
| 8-12 | 4 | 494.13 | 205.83 | 17.18199 | 1005.45 | 281.73 | 692.69 | |
| >12 | 1 | 281.58 | 30.93 | 3.64 | 559.51 | 259.70 | 303.50 | |
| Total | 44 | 422.59 | 190.49 | 366.02 | 479.16 | 136.61 | 925.73 | |
| IL-25 (years) | | | | | | | | |
| ≤1 | 8 | 1685.60 | 954.105 | 167.40 | 3203.80 | 854.62 | 2800.45 | 0.39 |
| 1.1-4 | 22 | 1208.20 | 741.187 | 879.58 | 1536.82 | 316.35 | 3143.32 | |
| 4.1-8 | 9 | 1089.80 | 780.56 | 437.244 | 1742.36 | 115.76 | 2865.93 | |
| 8-12 | 4 | 1252.70 | 1001.70 | −7747.19 | 10252.59 | 544.39 | 1961.00 | |
| >12 | 1 | 619.55 | 332.43 | −2367.25 | 3606.35 | 384.48 | 854.62 | |
| Total | 44 | 1204.89 | 759.92 | 955.11 | 1454.67 | 115.76 | 3143.32 | |

CI: Confidence interval, SD: Standard deviation, TNF-α: Tumor necrosis factor-α, IL-25: Interleukin-25

**Table 5: Serum level of tumor necrosis factor-α and interleukin-25 according to the gender**

| | *n*=44 | Titer (ng/l) | SD | 95% CI for mean | | Minimum | Maximum | *P* |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound | | | |
| TNF-α | | | | | | | | |
| Amebic (*Eh* gene +ve) | | | | | | | | |
| Male | 24 | 425.33 | 116.59 | 360.7 | 489.9 | 259.7 | 647.1 | 0.017 |
| Female | 20 | 549.64 | 259.76 | 384.6 | 714.7 | 259.7 | 925.7 | |
| Nonamebic (*Eh* gene −ve) | | | | | | | | |
| Male | 8 | 302.83 | 78.00 | 242.8 | 362.8 | 208.7 | 430.1 | |
| Female | 7 | 373.79 | 184.07 | 242.1 | 505.5 | 136.6 | 832.6 | |
| IL-25 | | | | | | | | |
| Amebic (*Eh* gene +ve) | | | | | | | | |
| Male | 24 | 1181.31 | 681.91 | 787.5 | 1575.4 | 544.4 | 3143.3 | 0.399 |
| Female | 20 | 752.63 | 603.11 | 119.7 | 1385.6 | 377.7 | 1961.0 | |
| Nonamebic (*Eh* gene −ve) | | | | | | | | |
| Male | 8 | 1386.3 | 694.86 | 852.1 | 1920.4 | 419.16 | 2465.8 | |
| Female | 7 | 1361.60 | 992.95 | 598.4 | 2124.9 | 115.76 | 2865.9 | |

CI: Confidence interval, SD: Standard deviation, TNF-α: Tumor necrosis factor-α, IL-25: Interleukin-25, *Eh*: *Entamoeba histolytica*

**Table 6: Serum level of tumor necrosis factor-α and interleukin-25 according to the disease recurrences**

| | *n*=44 | Titer (ng/l) | SD | 95% CI for mean | | Minimum | Maximum | *P* |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound | | | |
| TNF-α | | | | | | | | |
| Amebic (*Eh* gene+ve) | | | | | | | | |
| Primary | 38 | 489.50 | 206.55 | 404.23 | 574.76 | 237.22 | 925.72 | 0.029 |
| Recurrent | 6 | 431.11 | 197.43 | 223.92 | 638.30 | 259.70 | 806.14 | |
| Nonamebic (*Eh* gene−ve) | | | | | | | | |
| Primary | 10 | 300.61 | 87.35 | 238.12 | 363.10 | 136.60 | 420.68 | |
| Recurrent | 5 | 321.78 | 91.41 | 208.27 | 435.29 | 208.66 | 430.07 | |
| IL-25 | | | | | | | | |
| Amebic (*Eh* gene+ve) | | | | | | | | |
| Primary | 38 | 1049.37 | 737.06 | 682.83 | 1415.90 | 115.76 | 3143.32 | 0.045 |
| Recurrent | 6 | 882.93 | 207.49 | 625.29 | 1140.57 | 544.39 | 1082.87 | |
| Nonamebic (*Eh* gene−ve) | | | | | | | | |
| Primary | 10 | 1769.90 | 780.46 | 1211.59 | 2328.21 | 956.60 | 2865.93 | |
| Recurrent | 5 | 956.65 | 711.05 | 73.76 | 1839.54 | 316.34 | 2105.30 | |

CI: Confidence interval, SD: Standard deviation, TNF-α: Tumor necrosis factor-α, IL-25: Interleukin-25, *Eh*: *Entamoeba histolytica*

detection the gene of *E. histolytic* was found 84.1% (37 out of 44) samples had *E. histolytic* acute infection. This result agree with that of previous studies.[15]

The present study showed an elevation in the serum levels of TNFα in the group that infected with amebiasis [Table 2] when compared with the nonamebic group. The first line of immune protection against *E. histolytica* is stomach acid which can kill the trophozoites while amebic cysts are very resistance. Cysts excyst in leumen of intestine then, trophozoites attach intestine tissues leading to disrupt the muscle layer to facilitate invasion of tissues. This stage leading to discharge powerful cytokines to employee immune cells to the location of invasion. The cytokines include TNFα and INFɤ which activate macrophages macrophages to discharge reactive oxygen species and nitric oxide that destroy the parasitic pathogen. IL25 is inhibited through the amebic colitis infection and the admission of IL25 in to infected mice while reduce the number of *E. histolytica* trophozoites, load of the parasitic Ag and disruption of epithelia in the large intestine.[16]

Table 3 was demonstrate that the over expression of the immunogenic protein *Eh*CRT Ag occur when trophozoite invasive into tissue, adaptation with environment inappropriate, modulation and protect of human immunity. Increase in the expression of *Eh*CRT progress toward the severity of pathogenesis. Secretion of high amount of TNFα was shown to be related to increase in the risk of developing diarrhea in children infected with *E. histolytica*. IL25 is one of the cytokines secreted by the epithelial cells of human intestine. This cytokine that is produced by intestinal epithelial cells has a role in preservation the function of bowel barrier and inhibiting the secretion of TNFα. In individual developing amebic colitis and diarrhea, the expression of

**Table 7: Serum level of tumor necrosis factor-α and interleukin-25 according to the stool consistency**

| | *n*=44 | Titer (ng/l) | SD | 95% CI for mean | | Minimum | Maximum | *P* |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound | | | |
| TNF-α | | | | | | | | |
| Amebic (*Eh* gene +ve) | | | | | | | | |
| Mucoid | 33 | 493.93 | 213.65 | 403.71 | 584.15 | 237.22 | 925.72 | 0.022 |
| Liquid | 8 | 429.58 | 177.70 | 243.09 | 616.08 | 259.70 | 653.46 | |
| Bloody | 3 | 549.64 | 259.76 | 384.6 | 714.7 | 259.7 | 925.7 | |
| Nonamebic (*Eh* gene −ve) | | | | | | | | |
| Mucoid | 5 | 354.49 | 71.11 | 266.18 | 442.79 | 261.16 | 430.07 | |
| Liquid | 10 | 284.25 | 86.22 | 222.57 | 345.94 | 136.60 | 420.68 | |
| IL-25 | | | | | | | | |
| Amebic (*Eh* gene +ve) | | | | | | | | |
| Mucoid | 33 | 1104.77 | 715.03 | 749.20 | 1460.4 | 115.76 | 3143.3 | 0.066 |
| Liquid | 8 | 662.89 | 201.32 | 342.54 | 983.23 | 384.48 | 854.61 | |
| Bloody | 3 | 845.71 | 711.05 | 73.76 | 1839.54 | 316.34 | 2105.30 | |
| Nonamebic (*Eh* gene −ve) | | | | | | | | |
| Mucoid | 5 | 1058.69 | 647.88 | 254.23 | 1863.2 | 316.34 | 2105.3 | |
| Liquid | 10 | 1718.88 | 854.87 | 1107.3 | 2330.4 | 419.16 | 2865.9 | |

CI: Confidence interval, SD: Standard deviation, TNF-α: Tumor necrosis factor-α, IL-25: Interleukin-25, *Eh*: *Entamoeba histolytica*

**Table 8: Serum level of tumor necrosis factor-α and interleukin-25 in relation to the acute and chronic infections**

| | *n*=44 | Titer (ng/l) | SD | 95% CI for mean | | Minimum | Maximum | *P* |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound | | | |
| TNF-α | | | | | | | | |
| Amebic (*Eh* +ve gene) | | | | | | | | |
| Acute | 37 | 472.94 | 202.72 | 392.74 | 553.14 | 237.22 | 925.72 | 0.013 |
| Chronic | 7 | 513.68 | 231.65 | 145.07 | 882.29 | 259.70 | 806.14 | |
| Nonamebic (*Eh* +ve gene) | | | | | | | | |
| Acute | 15 | 307.67 | 86.02 | 260.03 | 355.30 | 136.60 | 430.07 | |
| IL-25 | | | | | | | | |
| Amebic (*Eh* +ve gene) | | | | | | | | |
| Acute | 37 | 984.54 | 689.46 | 661.86 | 1307.23 | 115.76 | 3143.32 | 0.140 |
| Chronic | 7 | 1204.12 | 423.35 | 152.44 | 2255.80 | 854.61 | 1674.87 | |
| Nonamebic (*Eh* −ve gene) | | | | | | | | |
| Acute | 15 | 1498.82 | 832.77 | 1037.7 | 1959.99 | 316.34 | 2865.93 | |

CI: Confidence interval, SD: Standard deviation, TNF-α: Tumor necrosis factor-α, IL-25: Interleukin-25, *Eh*: *Entamoeba histolytica*

IL25 was decreased and the administration of IL25 blocked *E. histolytica* infection and the disruption of the gut barriers, eosinophil elevation and inhibition of colonic TNFα secretion. The depletion of eosinophils with antibody will inhibit the production of IL25 mediated protection. In contrast, depletion of TNFα secretion can protect the host from *E. histolytica* infection.[17]

In Table 4, the results agree with.[18] High secretion of TNFα is now indicated to be linked with diarrhea in children infected with *E. histolytica*. The administration of TNFα blocker monoclonal Abs can reduce the inflammation and intestinal damage in amebic infection. The variation in the responses to stimuli inducing inflammation, decrease the adhesion ability to endothelial cells and poor chemotaxis in gradate age led to immature of innate and adaptive immunity, so improve immunity response in child must be through improve the nutritional supply, hygiene and inclusive vaccination to protects against pathogens.[19]

The results of Table 5 were agreed with that explained by.[20] These variation are commonly due to many factors such as functional and environmental which it is of hormonal origin. The environmental factors may be due to the differences in the exposure to the pathogenic agents. The infection with amebiasis is due to interactions between several environmental and genetic factors. The differences in Human Leukocyte Antigen (HLA) alleles may have an important role in the pathogenesis and immune responses of many diseases including *E. histolytica* infection in different persons to different antigens.[21]

According to the result of Table 6, it was agreed with that indicated by.[21] Peterson *et al*., 2010, was reported an

association between higher TNFα and low secretion of IL25 with an increase the risk of the first and recurrent episodes of diarrhea associated with *E. histolytica* infection. High secretion of TNFα and low secretion of IL25 may guess the upcoming susceptibility to E. histolytica diarrhea.[22]

The role of the cytokines in the development of amebic colitis and diarrheal processes it is recently known. The function of the intestinal lining epithelia is under the effects of many cytokines and the change in these cytokines during the infection with *E. histolytica* may aggravate intestinal secretion, permeability, motility and evoke amebiasis symptoms.[23]

The trophozoites of *E. histolytica* have the ability to escape the surveillance of immune elements since this parasite possesses an exclusive virulence factor. The interactions between the host immune system and the invading pathogen in acute diarrhea and dysentery result from the binding of *E. histolytica* Gal/GalNAc lectin which can recognized via host toll like receptor, which activates the nuclear factor kappa B. This process in turn stimulates the secretion of proinflammatory and inflammatory cytokines, like IL1β, IL6, IL8, IL12, TNFα and IFNγ in addition to chemoattractants of neutrophil and macrophage which lead to activate of innate immune response such as the epithelia of the intestine to produce IL25. This cytokine have a great role in protection against *E. histolytica* infection. The re-infection with E. histolytica can occur by inhibition of inflammatory response by hydrolases components produce by the trophozoite of the parasite.[24]

## Conclusion

*E. histolytica* is a protozoan parasite highly prevalent among diarrheal children and it is responsible for gastrointestinal amebiasis in the human host. The PCR is a useful tool in the diagnosis of *E. histolytica* infection. There was a significant strong positive correlation between delta value (*Eh*CRT) Ag and the inflammatory cytokines TNFα and IL25. On the other hand, a highly significant positive correlation was seen between TNFα and IL25. The majority of amebic diarrhea in children were at the age group of 1–4 years, had mucoid diarrhea, acute amebiasis, and with primary episodes of diarrhea.

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### Conflicts of interest

There are no conflicts of interest.

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