



Article

# Molecular Detection Of Intestinal Parasitic Infection In Children With Diarrhea And Assessment Of Serum TGF- $\beta$ and IL-13 level

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**Abstract:** Intestinal parasitic infections continue to be an important public health problem in children, especially in developing nations. The objective of this study was to investigate the presence of *E. histolytica* and *G. lamblia* in children with diarrhoea by microscopic examination, molecular methods and to assess the levels of cytokines such as IL-13 and TGF- $\beta$  in infected children versus healthy controls. From April to June 2026, 100 stool samples were obtained from 1-month-old to 12-year-old children at the Children's Hospital and Out Patient Laboratories. Macroscopic and microscopic examinations and DNA extraction and nested polymerase chain reaction (Nested PCR) of stool samples was performed in positive cases. The serum samples of infected patients and 50 healthy controls were analysed by enzyme-linked immunosorbent assay (ELISA). The microscopic examination revealed 55 positive samples (40 *E. histolytica*, 15 *G. lamblia*). The target gene of *E. histolytica* was identified in all 439 positive samples while *G. lamblia* was identified in 78.5% of the positive samples at 264 base pairs. As far as age distribution, *E. histolytica* was more commonly found in children between 2 and 7 years (42.5% positive) and *G. lamblia* was more commonly found in children between 2 and 12 years (41.5% positive). Serum IL-13 levels were highest in the *E. histolytica* group ( $321.12 \pm 88.0$ ), followed by the *G. lamblia* group ( $170.11 \pm 33.9$ ) and healthy controls ( $60.12 \pm 4.9$ ). Similarly, TGF- $\beta$  levels were  $6.54 \pm 3.87$ ,  $0.88 \pm 0.15$ , and  $3.50 \pm 1.90$ , respectively. In general, all the groups studied showed significant difference for both the markers ( $p < 0.001$ ). The results indicate that intestinal parasitic infection is associated with changes in the level of immune-related cytokines, but additional research is necessary to elucidate the roles of these cytokines in host-parasite interactions.

**Keywords:** *E. histolytica*, *G. lamblia*, Interleukin-13, Nested PCR.

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## 1. Introduction

Intestinal parasite infections are one of the most common public health issues in developing countries, especially in preschool and primary school children. These infections are responsible for higher morbidity and for affecting physical and immune growth as well as academic performance [1]. Intestinal parasites are a variety of protozoa including *Giardia lamblia* and *Entamoeba histolytica*, and intestinal helminths such as *Ascaris*, hookworms and tapeworms. The spread of each species of parasite is unique and each species exhibits its own pathological symptoms that are dependent on environmental and living conditions, water quality and sanitation [2]. Such infections are very prevalent in areas of high population density, where sanitation facilities are not well developed and where water is polluted. Children's vulnerability to infections and the adverse consequences of poor nutritional status is also further compounded by poor health awareness and malnutrition [3].

Amoebiasis (AMB) is a disease of the gut caused by a one-celled organism called *Entamoeba histolytica*. According to World Health Organization (WHO), amoebiasis is the third most important parasitic disease after malaria and schistosomiasis, causing 40,000 to 100,000 deaths each year [4]. It has been documented that as many as 10% of the world's population suffers from the presence of the parasite. About 90% of people who suffer from amoebiasis do not develop symptoms, and 10% develop an invasive infection. AMB affects primarily the stomach, causing cramps or pain, diarrhoea (which can be complicated by dysentery), nausea, colitis and (if left untreated) spread to extramucosal tissues [5]. The diseases should be distinguished from other diarrhoeal diseases (such as *Salmonella* spp, *Campylobacter* spp, and *Shigella* spp), and non-infectious causes (such as inflammatory bowel disease, carcinoma, ischaemic colitis, arteriovenous malformations, and diverticulitis) [6].

Giardiasis is one of the most prevalent protozoal infections in the world, causing millions of cases annually, due to the infection of the protozoal parasite *Giardia lamblia*. It causes an estimated 280 million cases of diarrhea a year, particularly in developing countries and developed countries [7]. *G. lamblia* resides within the small intestine and can result in symptoms of asymptomatic carriage to diarrhea and malabsorption [8]. Its pathogenicity relies on its ability to bind to the gut wall and to affect host immune system which helps it to evade recognition and remain infective. *G. lamblia* has several virulence mechanisms such as adhesion to intestinal epithelium, induction of apoptosis of enterocytes, secretion of proteases and decrease of the absorption and secretion of nutrients [9]. The parasite attaches to the intestinal cells through mechanisms such as a ventral disk and flagella, causing damage and shortening of the intestinal villi, thus reducing the body's ability to absorb nutrients, and stimulating inflammatory responses[10].

Transforming growth factor- $\beta$  (TGF- $\beta$ ) is a protein synthesised by numerous immune cells. Its main roles encompass the modulation of inflammatory mechanisms, particularly inside the gastrointestinal tract [11]. TGF- $\beta$  is crucial to the development of stem cells and T cells., TGF- $\beta$  serves as a crucial element in the modulation of amoebic infections through the alteration and regulation of Mononuclear cell (MN) functionality[12].the study aimed to diagnosis the parasite that causes the diarrheal infection in children and evaluate the mean level of Transforming growth factor- $\beta$  and Interleukien-5 in patients and healthy participants serum.

## 2. Method

### Samples collection

One hundred stool samples were collected from children with moderate to severe diarrhea, including bloody diarrhea, at the Children's Hospital and outpatient laboratories. The study period extended from April to June 2026. The children ranged in age from 1 month to 12 years and included both sexes.

The samples were collected in sterile, wide-mouthed plastic bottles with tightly sealed lids to maintain moisture and prevent drying. Microscopic examination was performed within the first half hour of collection, focusing on areas containing mucus or blood, to detect the active stage of the parasite responsible for the infection. The active stage is most commonly observed in soft stools and less frequently in hard or semi-hard stools, which typically contain the encysted stage. The presence of the encysted stage is a common indicator of parasitic infection.

Blood was collected from patients and 50 healthy person as control using sterile wine syringes into a 15 ml test tube containing gel. It was then left to coagulate at room temperature and transferred to a centrifuge at 3000 rpm to separate the serum. The serum (0.8 ml) was then transferred by sterile pipette to three Eppendorf tubes and stored at -20°C until use.

### Sample Examination

#### Macroscopic Examination

The physical features of the stool, such as consistency, colour and odour, and the presence of blood or mucus, were checked in the laboratory. If more than one sample was to be analyzed, the ones which had mucus or blood were given priority followed by the rest.

#### Microscopic Examination

The diagnosis of the trophozoite stage of parasites was done by using microscopic examination using Direct Wet Mount technique in which the motion of amoebic dysentery parasite was observed. The directionality of the movement of the parasite was an important characteristic which distinguished it from the other species of amoebae. When samples were taken during active infection phases in acute amoebic dysentery, the examination showed the presence of red blood cells (RBCs) in the cytoplasm of the

parasite.

This test was done by putting a drop of physiological saline on one end of a glass slide and a drop of local iodine solution on the other end. Using a clean wooden stick, a match head sized sample of faeces were collected from a number of locations. On the slide, the physiological saline was added to this fecal sample. Feces were then immediately collected again, and mixed until the sample was uniform with local iodine solution. The slide was then covered and observed under light microscope using 100X oil lens to confirm the presence of the parasite[13].

#### **measurement of TGF- $\beta$ and IL-13 level**

Serum of all patients and healthy control were assessment for IL-13 and TGF- $\beta$  by ELISA method using commercially available (SUNLONG, China) by following the instruction of manufacturer.

#### **Molecular study**

A molecular study was conducted to detect *Entamoeba histolytica* and *G. lamblia* in stool samples, targeting specific genes. The procedure followed these steps:

##### **Genomic DNA Extraction**

Genomic DNA was extracted from samples that tested positive via microscopic examination, using the Presto™ Mini gDNA Kit (manufactured by Geneaid). This kit contains all the necessary components and reagents for efficient genomic DNA extraction—including buffers, spin columns, and collection tubes—specifically designed to yield high-quality, pure DNA suitable for subsequent molecular applications. The extraction was carried out according to manufacturer instructions.

##### **Purity and Concentration Measurement**

The purity and concentration of the extracted DNA were measured using a NanoDrop spectrophotometer—an instrument specifically designed for the detection and quantification of DNA. DNA is analyzed by determining its concentration (ng/ $\mu$ L) and assessing its purity through absorbance readings at wavelengths of 260 nm and 280 nm.

##### **Electrophoresis of DNA in Agarose Gel**

To perform DNA electrophoresis and detection, a 1% agarose gel is prepared. This is achieved by dissolving 0.35 g of agarose powder in 35 ml of SB buffer and adding 3  $\mu$ l of "RedSafe" stain. The mixture is heated while being stirred continuously until it reaches a boil, then allowed to cool to a temperature of 50–60°C. The gel solution is then poured into the electrophoresis tray, with a comb positioned at one end to create wells; the solution should be poured gently to avoid the formation of air bubbles. If any bubbles appear, they should be removed using a pipette. Once the gel has solidified, the tray is placed into the electrophoresis tank, which contains an appropriate volume of SB buffer, and the comb is carefully removed.

Electrophoresis samples are prepared by mixing 7  $\mu$ l of the DNA sample with 3  $\mu$ l of loading buffer. The electrophoresis apparatus is then operated by applying an electric current at a voltage of 5 V/cm. The process takes 1.5 to 2 hours. Afterward, the gel is visualized under ultraviolet (UV) light using a gel documentation system to observe the DNA bands, including the products of the PCR reaction.

##### **Primers preparation**

All primers were obtained in a lyophilized (freeze-dried) form from the company Macrogen. The primer solutions were prepared by dissolving each primer in 300 or 320  $\mu$ l of nuclease-free water. ...a final concentration of 100 pmol/ $\mu$ l. The working solution was prepared by taking 10  $\mu$ l of the dissolved solution (100 pmol/ $\mu$ l) and diluting it by adding 90  $\mu$ l of nuclease-free water (free of DNAs and RNAs), resulting in a final working solution concentration of 10 pmol/ $\mu$ l.

##### **Nested Polymerase Chain Reaction (Nested PCR)**

Nested PCR was employed for the precise molecular detection of *\*Entamoeba\* spp.*, owing to its high sensitivity and specificity in amplifying target genetic segments. This technique involves two consecutive rounds of amplification using two sets of primers

targeting the 18S rRNA gene. The primers used for the first round of the polymerase chain reaction are listed in Table1.

**Table 1: Primers used in the detection of the parasite genus.**

| primer                               | Nucleotides sequences<br>5 → 3 | Size bp |
|--------------------------------------|--------------------------------|---------|
| EH-1                                 | F: 5'-AAGCATTGTTTCTAGATCTGAG-3 | 439 bp  |
| EH-2                                 | R: 5'-AAGAGGTCTAACCGAAATTAG-3' |         |
| Glutamate<br>DHase gene<br>G.lamblia | F: CAGTACAACCTCYGCTCTCGG       | 264 bp  |
|                                      | R: GTTRTCCTTGCACATCTCC         |         |

### Preparation of PCR Reaction Components

The PCR reaction mixture was prepared using a PCR Master Mix kit (Bioneer, South Korea) in accordance with the manufacturer's instructions. Once the reaction mixture was prepared, the tubes were sealed and centrifuged for 3 seconds. Subsequently, the tubes were placed into the thermal cycler to carry out the PCR amplification, as detailed in Table.(2)

**Table (2): Conditions used in the thermal cycler**

| Steps            | Temp  | Time   | Cycles   |
|------------------|-------|--------|----------|
| Pre-denaturation | 95 °C | 5min   | 1 cycle  |
| Denaturation     | 94 °C | 30 sec | 30cycles |
| Annealing        | 58 °C | 30sec  |          |
| Extension        | 72 °C | 30sec  |          |
| Final extension  | 72 °C | 5min   | 1 cycle  |

### Electrophoresis of Nested-PCR Products

1. The gene amplification products of the parasites were analyzed using 2% agarose gel electrophoresis.
2. The gel was prepared by dissolving 1 g of agarose in 50 mL of SB buffer and heating the mixture in a microwave until completely dissolved. After allowing it to cool slightly, 1 µL of RedSafe stain was added; the combs were then inserted, the gel was poured, and it was left to solidify.
3. Once solidified, the combs were carefully removed, and the gel was placed in an electrophoresis chamber containing SB buffer, ensuring the buffer covered the gel surface to a depth of 1–2 mm. Subsequently, 5 µL of each PCR product, along with a DNA ladder (up to 1200 base pairs), were loaded into the designated wells.
4. Electrophoresis was performed at the voltage specified in Table (3). Upon completion, the bands were visualized using a UV transilluminator, and the gel results were photographed for documentation and analysis.
5. The voltage for the electrophoresis apparatus was set in two stages, as shown below:

**Table (3) Stages of the electrophoresis procedure**

| First level |        | Second level |        |
|-------------|--------|--------------|--------|
| Voltage     | 40V    | Voltage      | 90 V   |
| Current     | 50 A   | Current      | 120 A  |
| Time        | 10 Min | Time         | 35 min |

Different concentrations of agarose gel were prepared, depending on the type of samples being subjected to electrophoresis, as shown below:

**Table (4): Materials used for preparing agarose gel in a 10 × 10 cm tray**

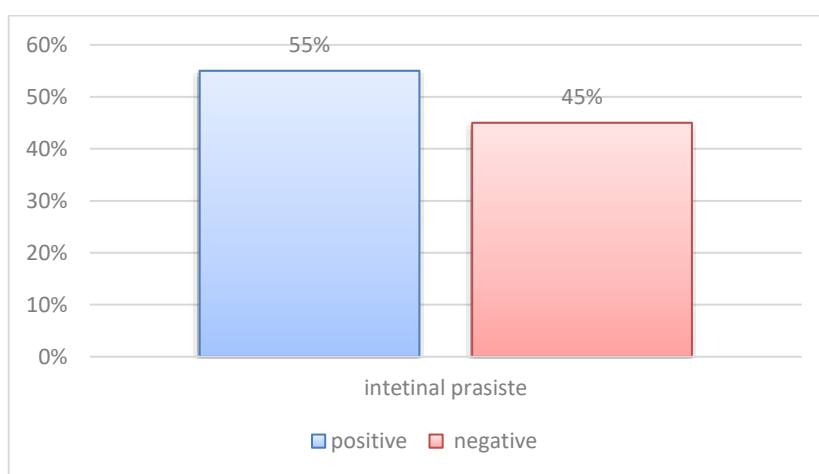
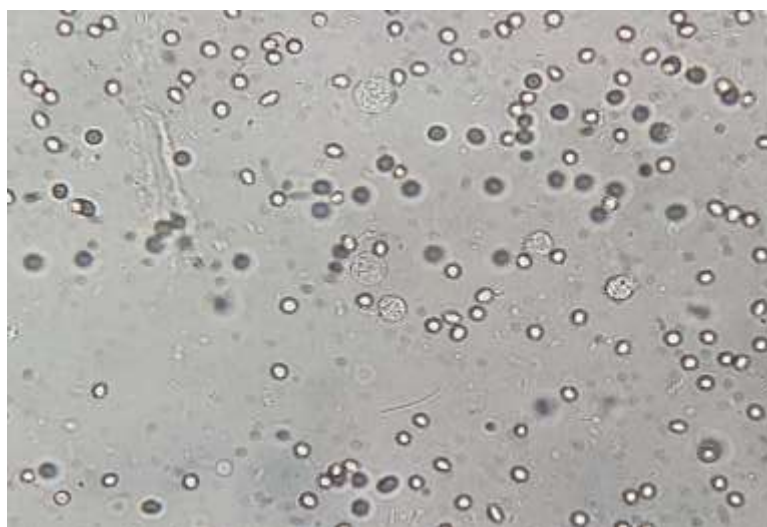
| materials                    | concentration1% | concentration 2% |
|------------------------------|-----------------|------------------|
| Agarose powder               | 0.5g            | 1g               |
| Sodium-borate (1X SB) buffer | 50 ml           | 50ml             |
| Safe red stain               | 1µl             | 1µl              |

**Statistical Analysis.**

The data of the study were statistically analyzed using Statistical Package for the Social Sciences (SPSS) software to estimate the averages.

**Results****Epidemiological Study**

The results of the study indicated that 55% of the 100 samples tested positive for *Entamoeba histolytica* and *G. lamblia* while 45% tested negative, as shown in Figure (1).

**Figure (1): Percentage of Samples Tested****Figure (2): The cystic stage of the intestinal Entamoeba spp parasite from a stool sample under magnification power (40X).****Distribution of parasitic infections by Age Group**

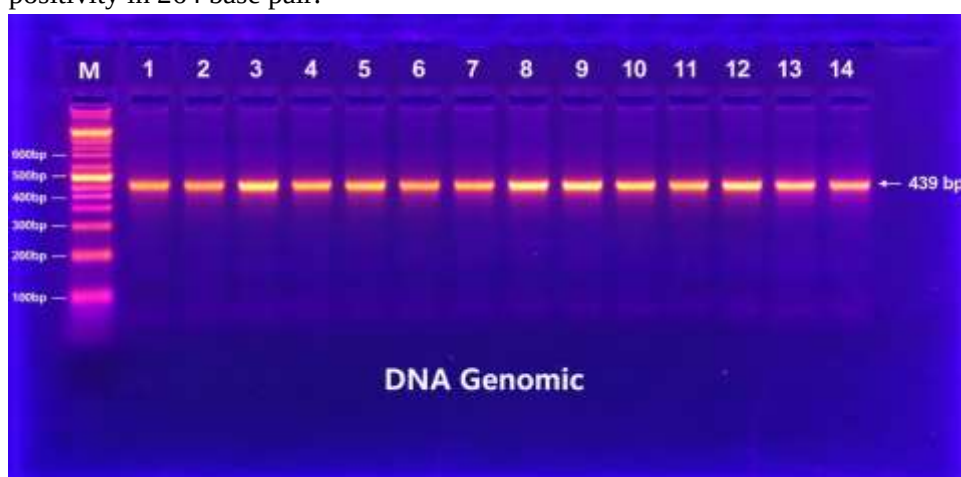
The results of the distribution of *E. histolytica* and *G. lamblia* by ages showed that age group 2-7 years showed high *E. histolytica* infection 42.5% while the age group below one years showed low percentage with 22.5%. while infection with *G. lamblia* showed high percentage in ages groups more than one years while showed low percentage 20% in ages below than one years as shown in table(5).

**Table (5): Distribution of parasitic infections by Age Group**

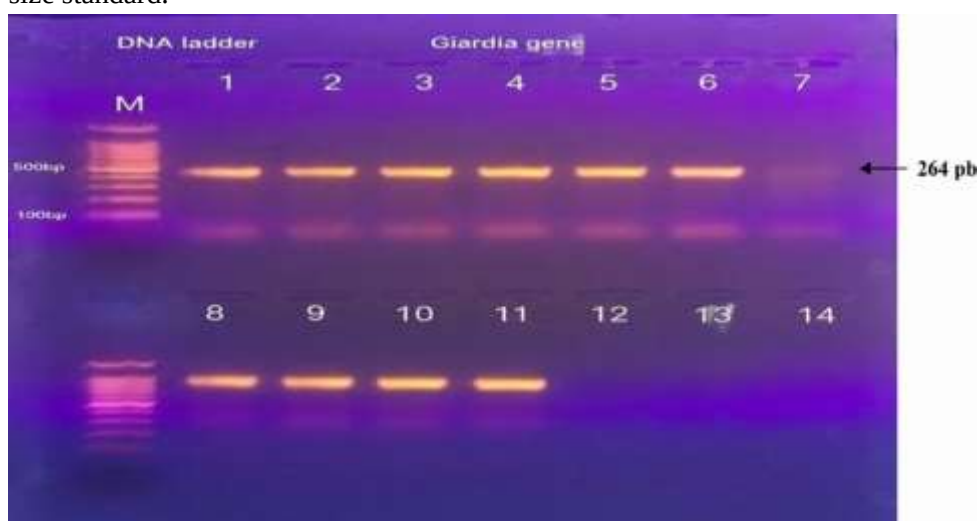
| Age group                     | Tested samples | <i>E. histolytica</i><br>n(%) | <i>G. lamblia</i><br>n(%) |
|-------------------------------|----------------|-------------------------------|---------------------------|
| 1month-1 years                | 15             | 9(22.5%)                      | 3(20%)                    |
| 2-7 years                     | 16             | 17(42.5%)                     | 6(40%)                    |
| 8-12 years                    | 24             | 14(35%)                       | 6(40%)                    |
| <b>Total positive samples</b> | 55             | 40(100%)                      | 15(100%)                  |

### DNA Extraction

Genomic DNA was extracted from 100 stool samples collected from patients exhibiting symptoms of a parasitic infection. This set included 55 samples that tested positive for the parasite via microscopic examination. The extraction process served as the initial step for amplifying target gene segments—used for diagnostic purposes—via Nested Polymerase Chain Reaction (Nested-PCR). A buffer-based extraction method was employed, and the resulting DNA extracts were subsequently analyzed using gel electrophoresis on a 1% agarose gel to confirm the presence of genomic DNA, as illustrated in Figure (3&4). The results showed that 100% of Eh1-Eh2 gene of *E. histolytica* showed positive results in 439 base pair while *G. lamblia* gene showed 78.5% positivity in 264 base pair.



Figure(3): The PCR amplification product for the Eh1-Eh2 gene in samples 1–14 representing positive samples—showed a gene size of 439 base pairs. Electrophoresis was performed on a 2% agarose gel for 1 hour, using a 1000-base-pair DNA ladder as a size standard.



Figure(4): The PCR amplification product for the glutamate dehydrogenases for *G. lamblia*.

lamblia gene in samples 1–14 representing positive samples—showed a gene size of 264 base pairs. Electrophoresis was performed on a 2% agarose gel for 1 hour, using a 1000-base-pair DNA ladder as a size standard.

### Mean level of IL-13 and TGF- $\beta$ in studied groups

The results in table (6) showed a significant difference ( $p < 0.001$ ) in mean level of IL-13 and TGF- $\beta$  in patients and control groups. *E. histolytica* infection showed high mean of IL-13 ( $321.12 \pm 88.0$ ) and TGF- $\beta$  ( $6.54 \pm 3.87$ ) compared with *G. lamblia* ( $170.11 \pm 33.9$ ,  $0.88 \pm 0.15$ ) and healthy group ( $60.12 \pm 4.9$ ,  $3.5 \pm 1.9$ ) respectively.

**Table (6): Mean level of IL-13 and TGF- $\beta$  in patients and control groups**

| Parameters   | <i>E. histolytica</i><br>Mean $\pm$ SD | <i>G. lamblia</i><br>Mean $\pm$ SD | Control<br>mean $\pm$ SD | P value     |
|--------------|----------------------------------------|------------------------------------|--------------------------|-------------|
| IL-13        | 321.12 $\pm$ 88.0                      | 170.11 $\pm$ 33.9                  | 60.12 $\pm$ 4.9          | $p < 0.001$ |
| TGF- $\beta$ | 6.54 $\pm$ 3.87                        | 0.88 $\pm$ 0.15                    | 3.5 $\pm$ 1.9            | $p < 0.001$ |

### 3. Discussion

In the present study, intestinal parasitic infections (IPIs) were investigated in diarrheal children, the involvement of two cytokines, namely, interleukin-13 (IL-13) and transforming growth factor beta (TGF- $\beta$ ) were evaluated and their association with the intestinal parasitic infections was investigated. The results showed 55% of the 55 stool samples examined were positive for intestinal parasites while 45% were negative. Of the positive cases, 40 cases of *E. histolytica* and 15 cases of *G. lamblia* were found. The high prevalence of *E. histolytica* indicates its significance in causing intestinal infection among children of the study population.

The current study was similar to that of Aziz et al. [14] who reported 58% parasite isolation in Salah al-Din and the same as Rashid [15] who reported 53% parasite isolation in Baghdad. Likewise, Alyasari et al., [16] reported a 61.93% prevalence. However, Lepore et al. [17] reported lower frequencies of detection of *E. histolytica* using microscopy (33 (8.3%) positive samples and 13 (4.28%) positive samples, respectively). Geographical variations, socioeconomic factors, sanitation practices, drinking-water quality, personal hygiene, age distribution, sample size and diagnostic techniques may be the reasons for the differences among these studies.

In terms of age distribution, the maximum number of children with positive results were found in the age group of 2-7 years (42.5%), followed by 8-12 years (35%) and 1 month – 1 year (22.5%). Conversely, the proportion of children positive for *G. lamblia* was similar for both 2 - 7 years old and 8 - 12 years old (40% each), and lowest (20%) for those 1 month to 1 year old. The results have shown that the age group distribution of infections was not uniform. This finding corresponds with the observation of Farhan [18] that intestinal parasites are more prevalent in children less than 10 years old. In other studies, Abdullah and Ali [19] found that *E. histolytica* infection was reported more frequently in children under 10 years of age and Hasan et al. [20] reported high prevalence of intestinal parasitic infection in children aged 1–10 years in Duhok, Iraq. A high prevalence of infection was also reported among children under 5 years in Kut by Rana [21]. The higher proportion of school-age children infected could relate to more contact with contaminated environments, and with contaminated food and water, as well as to variations in personal hygiene practices. When sanitation facilities and hygiene practices are not adequate, there is a possibility of getting exposed to the infectious stages of intestinal parasites at schools and other group settings [22].

For microscopic identification of the parasites, molecular analysis was done. The *E. histolytica* target was found at the expected amplicon size of 439 base pairs and the *G. lamblia* target was detected at 264 base pairs. The expected molecular band was seen in all the samples reported as positive for *E. histolytica* and 78.5% of them were reported as positive for the molecular of *G. lamblia*. The discrepancy between the microscopic and

molecular detections may be attributed to variations in analytical sensitivity, quantity and distribution of the parasite material in stool specimens, efficiency of the DNA extraction process, or unknown chemicals that inhibit the PCR amplification process.

The present study showed that the serum level of IL-13 was different between the three groups studied. The infected children with *E. histolytica* had the highest mean concentration followed by *G. lamblia* and the lowest mean concentration was obtained in the control group. The results presented here indicate that intestinal parasitic infections may be linked to alterations in cytokine responses, but the differences noted do not prove that the parasites are directly responsible for the changes.

IL-13 is a significant cytokine of type 2 helper T-cell (Th2) responses and plays a role in the regulation of mucosal immunity. May affect goblet-cell activity, mucin synthesis, epithelial function and other factors related to intestinal defense[23]. In parasitic infections, activation of immunologic pathways associated with Th2 response can play a role in eliminating the parasites and restoring intestinal homeostasis. The high levels of IL-13 found in the *G. lamblia* group when compared with healthy controls is in line with the previous studies showing increased levels of IL-13 in giardiasis patients[24]. The elevated levels of IL-13 in the present study could be due to immune activation in reaction to the antigens of the parasite. The strength of the response may fluctuate depending on the severity of the infection, length of the disease process, host immune state, nutrient status and concurrent infections, however. There has also been previous experimental reports of increased IL-13 response upon exposure to *E. histolytica*, thus suggesting its role in host-parasite interactions[25].

The present study also showed significant differences between the serum levels of TGF- $\beta$  in the groups studied ( $p < 0.001$ ). Transforming growth factor-beta is a multifunctional regulatory cytokine involved in the regulation of inflammation, immune cell differentiation, tissue repair and mucosal immunity. TGF- $\beta$  plays a role in regulating immune responses in the gastrointestinal tract, and may protect against excessive tissue damage in infectious disease[26]. It has a differential effect depending on the immune cell type, the cytokines in the local environment and the stage of the infection. TGF- $\beta$  has been shown to inhibit some inflammatory responses and to affect the activity of macrophages, T cells and other immune-cell populations. Thus, fluctuations in the level of circulating TGF- $\beta$  could actually be due to variations in immune regulation, not necessarily due to changes in parasite load or disease severity.[27]

The increase of TGF- $\beta$  in *E. histolytica* group when compared with the control group is in line with the previous reports on change of TGF- $\beta$  during intestinal parasitic infections. Verma et al. [28] for instance, studied cytokine expression in symptomatic and asymptomatic amoebiasis and found that there were differences in the expression of TGF- $\beta$  between the groups studied. In addition, previous studies on *E. histolytica* have focused on how the expression of the cytokine TGF- $\beta$  affects the function of mononuclear cells, suggesting that this molecule could have effects on mononuclear-cell function in host-parasite interactions [29]

The significantly reduced TGF- $\beta$  concentration in the *G. lamblia* group compared to the control group, however, indicates that giardiasis may be linked with a different immune regulatory mechanism. A study conducted by Esra et al., [30] in Iraq found the mean TGF- $\beta$  concentration was low in patients with giardiasis in Kirkuk, as cited in the provided literature. This observation is generally consistent with the findings in the present study.

#### 4. Conclusion

The present study revealed the presence of intestinal parasitic infection in children who suffered from diarrhea and *E. histolytica* was more common than *G. lamblia*. The microscopic results were confirmed by molecular findings that revealed the presence of the target gene of *E. histolytica* at 439 bp and detection of *G. lamblia* with lower

molecular detection rates. There was variation in the prevalence of infection by age group, with a maximum number of *E. histolytica* cases occurring in the age group 2–7 years. Moreover, the concentration of serum IL-13 and TGF- $\beta$  were significantly different among the *E. histolytica*, *G. lamblia* and healthy control groups. Children infected with *E. histolytica* had the highest levels of IL-13, while the TGF- $\beta$  levels were significantly higher in the *E. histolytica* group than in the *G. lamblia* group and healthy controls. The results suggest a link between intestinal parasite infections and alteration of immune-regulatory cytokines. It is clear from the above, however, that further studies with larger numbers of specimens and with detailed molecular and immunological analyses are required to elucidate the roles of IL-13 and TGF- $\beta$  in the host immune response to these parasites.

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