Original Article

# Assessment of the Serum Level of IL-1B, IL-2, and IL-10 in Children Infected with *Enterobius vermicularis* in Babylon Province

**Aseel Sekar Naji AL-Bairmani, Hayam Khalis Al-Masoudi**
Department of Microbiology, College of Medicine, University of Babylon, Babylon, Iraq

## Abstract

**Background:** Children are most frequently infected with *Enterobius vermicularis*, a human-pathogenic intestinal parasite that belongs to the nematodes and causes enterobiasis. **Objectives:** Evaluation of interleukin (IL)-1B and IL-2 levels in pinworm-infected youngsters is the goal. **Materials and Methods:** A total of 87 children—including 30 control subjects—participated in this study. Their ages ranged from 2 to 13 years old, and both sexes (50 females and 37 males) were represented. IL-1B, IL-2, and IL-10 levels were measured using an enzyme-linked immunosorbent assay test. **Results:** When using the cellophane tape method, the results showed that 53 (60.92%) clinically infected patients had laboratory examinations, whereas all 87 (100.0%) clinically infected patients had positive diagnostic results. Additionally, the level of proinflammatory cytokines (IL-1B and IL-2) was slightly higher in the intestinal *E. vermicularis*, a human-pathogenic organism shown to be somewhat more prevalent in patients than in the control group in this investigation. Thirty children participated in the study. Additionally, the level of IL-10 did not increase much ($P = 0.005$). **Conclusion:** There was increasing serum of IL-1B, IL-2, and IL-10 in patients with enterobiasis.

**Keyword:** *Enterobius vermicularis*, IL-1B, IL-2, proinflammatory cytokines

## Introduction

*Enterobius vermicularis* is a nematode intestinal parasite that can cause disease in humans. The terms "threadworm" and "seatworm" are synonyms. Enterobiasis is the term used to describe symptomatic pinworm infection.[1]

Children are the most frequently affected; however, *E. vermicularis* infection can affect anybody. Children in school age and those from tropical regions are more at risk. Ingestion of pinworm eggs results in infection[2] through the fecal–oral route, and self-reinfection is common when the hand touches the perianal area and carries the infection to the mouth. Infection is closely associated with a dense population, socio-economic conditions, and the practice of sucking one's fingers.[3]

After ingestion, the embryonated eggs hatched in small intestine, and the larvae are released. The adult pinworms mainly attach themselves in the cecum and appendix.[4] Re-infection is common in enterobiasis, and public health and personal cleanliness are the key factors influencing the occurrence of this infection.[5]

Enterobiasis seems to be more prevalent in big animals because of its infectious nature members of the family and establishments like nurseries and kindergartens. Despite being quite common, this illness is frequently ignored as most infections are asymptomatic or crowded conditions.[6]

Infection with *E. vermicularis* commonly affects the gastrointestinal tract (GIT), and the ectopic infections are rare, especially in the liver.[7] Female genital tract and

Access this article online

Quick Response Code:

Website: www.medjbabylon.org

DOI: 10.4103/MJBL.MJBL_243_22

**Address for correspondence:** Mrs. Aseel Sekar Naji AL-Bairmani, Department of Microbiology, College of Medicine, University of Babylon, Babylon, Iraq.
E-mail: alghzalyasyl71@gmail.com

**Submission:** 12-Oct-2022 **Accepted:** 21-Nov-2022 **Published:** 27-Apr-2023



**How to cite this article:** AL-Bairmani AS, Al-Masoudi HK. Assessment of the serum level of IL-1B, IL-2, and IL-10 in children infected with *Enterobius vermicularis* in Babylon province. Med J Babylon 2023;20:59-63.

peritoneum are the common sites of ectopic infestation, and rarely they may also be seen in tissues such as kidney, male urinary tract, conjunctiva, spleen, and lung.[8] Severe adult worms may be seen excreting in the feces during an infection can be visualized by colonoscopy or proctoscopy.[9] The disease is highly correlated with a lack of cleanliness; a polluted bed linen and clothing might also contribute to transmission. Patients are often asymptomatic; however, female worms lay their eggs in large numbers in the perianal area. Pruritus is a typical symptom, especially at night. Complications such as extra-intestinal infection may occur but are uncommon.[10] The immune response caused by parasitic infection is complex and multiple. Strongly Th2-skewed reactions brought on by cytokines including interleukin (IL)-4, IL-5, and IL-13, as well as mastocytosis, eosinophilia, and antibody class flipping to create IgE, are brought on by helminths.[11]

Different cytokines have a crucial part in the regulation, which includes inflammatory processes and reactions in various metabolic diseases. Proinflammatory IL-10 and other anti-inflammatory cytokines, such as tumor necrosis factor alpha, IL-6, IL-8, IL-1β, and IL-12, modulate the immune response in the course of inflammation. Imbalance between inflammatory and proinflammatory cytokines leads to chronic inflammation, which is regarded as an essential factor throughout the beginning and development of different cytokines that are both pro- and anti-inflammatory IL-2, IL-1B, and IL-10 in children infected with *E. vermicularis*. The modulation of the immune response in living things is aided by cytokines. By secreting IL-2, IFN-γ, and lymphotoxin, Th1 cells help the defensive system of the host, whereas Th2 cells render it more vulnerable to infections by producing IL-4, IL-5, IL-6, and IL-10. IL-10 synthesis is also increasing in *E. vermicularis*.[12,13]

## Materials and Methods

### Research period and sample

The research started in September 2021 and ended in March 2022. A total of 87 youngsters and 30 controls took part, and the ages of them varied from 2 to 13 years, comprising both sexes (37 males and 50 females). Each participating kid in the study had a unique questionnaire form filled out by their mothers during interviews.

### Collection of cellophane tape samples

The samples were obtained by using the cellophane tape technique. Children's anal and perianal regions were repeatedly pressed with the tape's adhesive side before it was adhered to the labeled glass slide, placed in a sterile, clean nylon envelope, and then carefully sealed. Before defecating, using the restroom, or taking a bath, this procedure was carried out with the mothers of the children at night or in the early morning. The samples that were gathered were looked at using a light microscope.[14]

### Collecting blood samples

Three milliliters of venous blood was taken from each child who had *E. vermicularis* infection and was otherwise healthy (control group). The blood sample was placed in a gel tube, which was then centrifuged at 3000 rpm for 10 min to extract the serum after remaining at room temperature for 15–20 min to allow the blood to clot. To avoid freezing and thawing the samples repeatedly, which is not advised as it may impact the quality of the findings, the acquired serum was added in Eppendorf tubes (200 l) into many sections for various assays. All sera were kept at 200°C until immunological testing was done. All samples were simultaneously taken from the freezer and examined when the sampling was finished.

### Immunological test

Several tests were included, especially immunological tests involved IL-1B, IL-2, and IL-10. Blood samples of patients infected with *E. vermicularis* and healthy controls were collected (87 *E. vermicularis* and 30 samples as control).

### Human IL-1B ELISA test

The serum concentrations were assessed using the enzyme-linked immunosorbent assay (ELISA). The IL-1β level was measured according to manufacturer's instruction (ELISA). Human IL-1β antibody was pre-coated on the plate. The IL-1β content of the sample was added. The level of human IL-1β is then correlated with the development of color in the substrate solution. By adding an acidic stop solution, the process is stopped, and absorbance is measured at 450 nm

### Human IL-2 ELISA test

The serum concentrations were assessed using the ELISA. The IL-2 level was measured according to manufacturer's instruction (ELISA). Human IL-2 antibody was pre-coated on the plate. The IL-2 content of the sample is added. The level of human IL-2 then correlated with the development of color in the substrate solution. By adding an acidic stop solution, the process is stopped, and absorbance is measured at 450 nm.

### Human IL-10 ELISA kit

The serum concentrations were assessed using the ELISA. The level of IL-10 is measured according to manufacturer's instruction (ELISA). Human anti-IL-10 antibody has been pre-coated on the plate. When IL-10 from the sample is introduced, it binds to the antibodies that have been coated on the wells. Then human biotinylated conjugated IL-10 antibody is added to the immune complex and then added to the substrate. Addition of an acidic stop solution ends the process, and absorbance is measured at 450 nm.

### Analytical statistics

In order to perform statistical analysis, SPSS version 27 was used. Frequencies and percentages were used to present categorical information. The format for continuous variables was means ± SD. The means of the two groups were compared using Student's t-test. The means of the four groups were compared using the analysis of variance test. In order to determine the relationship between categorical variables, the Pearson $\chi^2$ test was performed. *P*-values less than 0.05 were regarded as significant.[15]

### Ethical approval

The study was carried out in conformity with the moral standards set forth in the Helsinki Declaration. Before a sample was obtained, it was done with the patients' verbal and analytical consent. A local Ethics Committee examined and approved the study protocol, subject information, and permission form in accordance with document number 6543 (containing the number and date in 10/6/2021) to receive this approval.

## Results

### Distribution of patients with *E. vermicularis* depending on diagnosis methods

The percentage of patients who often have *E. vermicularis* depending on diagnostic method has been done, and the outcomes are shown in Table 1. The present result showed all clinically infected patients: 87 (100.0%) have positive diagnostic results, whereas for those with laboratory examination, 53 (39.08%) have positive diagnostic results.

### Serum IL-1B level in patients with *E. vermicularis* and control groups

Serum levels of IL-1B between people with *E. vermicularis* and healthy control groups have been compared, and the outcomes are shown in Table 2. Serum average levels of IL-1β when patients have *E. vermicularis* were slightly greater in comparison to the mean amounts of the control groups 4545.88 ± 1493.48 versus 3439.56 ± 1965.60 pg/mL, but the change was not noteworthy ($P = 0.782$).

**Table 1: Patient distribution by *E. vermicularis***

| Diagnosis | Patients clinically diagnosis | | Patients with laboratory examination | | Healthy control | | *P*-value |
|---|---|---|---|---|---|---|---|
| | *N* | % | *N* | % | *N* | % | |
| Positive | 87 | 100.0 | 53 | 60.92 | 0 | 0 | 0.001 S |
| Negative | 0 | 0 | 34 | 39.08 | 30 | 100.0 | |
| Total | 87 | 100.0 | 87 | 100.0 | 30 | 100.0 | |

$\chi^2$ test; *n*: number of instances; significant at $P \leq 0.05$; S: significant

### IL-2 level in serum in patients with *E. vermicularis* and control groups

The contrasting of the serum IL-2 level between those who have *E. vermicularis* and control groups has been shown in Table 3. Typical amounts of serum IL-2 in patients with *E. vermicularis* were slightly higher in comparison to the mean levels of the control group, 971.16 ± 713.51 versus 862.93 ± 590.48 pg/mL, but the statistical analysis shows no significant difference ($P = 0.459$).

### Serum IL-10 level in patients with *E. vermicularis* and control group

The contrasting of the serum IL-10 level between those who have *E. vermicularis* and control groups has been shown in Table 4. Typical amounts of serum IL-10 in patients with *E. vermicularis* were increased in comparison to the mean level of control groups, 616.51 ± 418.03 versus 517.70 ± 364.43 pg/mL, but the increase was not significant ($P = 0.264$).

The correlations between serum IL-1 beta levels and some parameters in patients with *E. vermicularis* are shown in Tables 5–7. There was a highly significant correlation between serum IL-1β and IL-2 and IL-10 in patients with *E. vermicularis* infection.

**Table 2: Serum IL-1B level in patients with *E. vermicularis* control groups**

| IL-1B (pg/mL) | *E. vermicularis* patients | Healthy control | *P*-value |
|---|---|---|---|
| Mean ±SD | 4545.88 ± 1493.48 | 3439.56 ± 1965.60 | **0.782**† |
| Range | 2938.1–6392.3 | 1423.43–6782.12 | **NS** |

Standard deviation, number of instances, independent *t*-test, and non-significant at $P = 0.05$ are all part of the equation

**Table 3: Serum IL-2 level in patients with *E. vermicularis* and control groups**

| IL-10 (pg/mL) | Case–control comparison | | |
|---|---|---|---|
| | *E. vermicularis* patients | Healthy control | *P*-value |
| Mean ±SD | 971.16 ± 713.51 | 862.93 ± 590.48 | **0.459**† |
| Range | 231.64–1831.43 | 290.32–1460.34 | **NS** |

Standard deviation, number of instances, independent *T*-test, and non-significant at $P = 0.05$ are all part of the equation

**Table 4: Serum IL-10 level in patients with *E. vermicularis* and control group**

| IL-2 (pg/mL) | Case–control comparison | | |
|---|---|---|---|
| | *E. vermicularis* patients | Healthy control | *P*-value |
| Mean ±SD | 616.51 ± 418.03 | 517.70 ± 364.43 | **0.264**† |
| Range | 173.23–983.13 | 159.23–899.23 | **NS** |

Standard deviation, number of instances, independent *T*-test, and non-significant at $P = 0.05$ are all part of the equation

**Table 5: Correlations between serum IL-1β and demographic and some immunological parameters in patients *with E. vermicularis***

| Characteristic | Serum IL-1β level | |
|---|---|---|
| | *R*-value | *P*-value |
| Age | -0.207 | 0.137 |
| Gender | -0.088 | 0.580 |
| Residence | 0.206 | 0.139 |
| Family history | 0.055 | 0.697 |
| Treatment history | 0.036 | 0.799 |
| History of enuresis | 0.183 | 0.190 |
| IL-2 levels | 0.600 | 0.001* |
| IL-10 levels | 0.728 | 0.001* |

*R*: correlation coefficient

**Table 6: Correlations between serum IL-2 and demographic and some immunological parameters in patients *with E. vermicularis***

| Characteristic | Serum IL-1β level | |
|---|---|---|
| | *R*-value | *P*-value |
| Age | -0.228 | 0.101 |
| Gender | -0.283 | 0.400 |
| Residence | 0.020 | 0.888 |
| Family history | 0.091 | 0.516 |
| Treatment history | 0.075 | 0.596 |
| History of enuresis | 0.254 | 0.670 |
| IL-1β levels | 0.600 | 0.001* |
| IL-10 levels | 0.793 | 0.001* |

*R*: correlation coefficient

**Table 7: Correlations between serum IL-10 and demographic and some immunological parameters in patients *with E. vermicularis***

| Characteristic | Serum IL-10 level | |
|---|---|---|
| | *R*-value | *P*-value |
| Age | -0.205 | 0.141 |
| Gender | 0.243 | 0.179 |
| Residence | 0.159 | 0.256 |
| Family history | 0.102 | 0.467 |
| Treatment history | 0.234 | 0.092 |
| History of enuresis | 0.150 | 0.282 |
| IL-1β levels | 0.728 | 0.001* |
| IL-2 levels | 0.793 | 0.001* |

*R*: correlation coefficient

## Discussion

As scotch tape catches up any egg present in the perineal region, research has shown that it is the best method for detecting *E. vermicularis* eggs. The outcome is consistent with the findings of the majority of studies who found that using tests other than scotch tape resulted in a low rate of infection. The outcome is consistent with the findings of the majority of studies who found that using tests other than scotch tape resulted in a low rate of infection. One of them is in Sulaimania[16] and in Erbil.[17] This low incidence is due to the fact that the eggs are rarely observed in feces because during oviposition the female worm migrates out into the perianal region to deposit her eggs, which frequently happens at night. The Scotch tape approach was utilized in this investigation because it is well-known, extensively used, simple, safe, and dependable.[18,19]

The findings of this study have demonstrated a significant association between children's nighttime bedwetting and *E. vermicularis* infestation. After receiving therapy with antihelminthic medications from 31 patients, the percentage of patients with *E. vermicularis* infestation and enuresis is 48.38%. This study indicates that females are more likely than males to have enterobiasis. The rise in cure rates in female patients is around 64.28%, compared with 34.29% in male groups, which is practically an increase of about one-half.[20]

The present study was in agreement with the finding of Mohsen,[21] which showed that high levels of IL-1β were increasing in patients infected with intestinal parasite compared with healthy controls, but these results were not significant. IL-1B production by intestinal epithelial cells results in an influx of inflammatory cells into the intestinal mucosa and tissue damage.[22]

The finding of Habieb[23] showed that the mean level of IL-2 was decreased in patients infected with Toxoplasmosis. This explains that there is a reduction in the production of IL-2, which appears to mediate the downregulation of T-cell-derived cytokines.

The immune system uses IL-2 as a kind of IL and as a cytokine signaling molecule. It is a 15.5–16 kDa protein that controls the immune system-regulating functions of white blood cells (leukocytes, often lymphocytes). The body uses IL-2 as part of its normal defense against microbial infection and to distinguish between "self" and "non-self" substances. By attaching to lymphocyte-expressed IL-2 receptors, IL-2 exerts its effects. Activated CD4+ T cells and activated CD8+ T cells are the main sources of IL-2.[24]

Results of Liao *et al.*[24] confirm that levels of IL-10 were increasing in children infected with intestinal helminths when compared with children without intestinal helminth.

Many distinct myeloid and lymphoid cells generate the anti-inflammatory cytokine IL-10, which controls the inflammatory process. Multiple populations of IL-10-producing cells are stimulated during infection, which inhibits the activity of Th1 cells, natural killer cells, and macrophages. IL-10 is a down-modulatory factor that induces a modified Th2-cell phenotype in allergic disorders. Preschool- and school-aged children's higher IL-10 levels are consistent with findings of Sanchez *et al*.[25]

The present study discovered a negative correlation between age and blood IL-10 levels; as children aged, IL-10 levels tended to decline. This conclusion is unexpected for a nation with an endemic helminth problem, in which helminth exposure and immunological tolerance are supposed to rise with age.

The findings of this research study investigate the present significantly negative correlation between age and immunological marker (IL-1β, IL-2, and IL-10), whereas significant positive correlation among the ILs togethers.

Other study investigated that the correlation coefficients revealed a somewhat negative connection between age and IL-10 level, which was statistically significant ($r^2$=-0.483; $P$ = 0.002).[25]

As this medication is more effective for helminth, we suggested that these variances may have been caused by children having yearly single-dose albendazole treatments. This study's findings about the inverse relationship between IL-10 and age might possibly be impacted by deworming medication. Depending on the infecting species, albendazole decreases the worm load or shortens the duration of infections.[26] From the results of this study, we conclude that there were slightly elevated in proinflammatory cytokines in children infected with pin worm.

## Financial support and sponsorship
Nil.

## Conflicts of interest
There are no conflicts of interest.

## References

1. Deplazes P, Eckert J, von Samson-Himmelstjerna G, Zahner H. Lehrbuch der Parasitologie für die Tiermedizin. Georg Thieme Verlag: NY, USA; 2012.
2. Yoon HJ, Choi YJ, Lee SU, Park HY, Huh S, Yang YS. *Enterobius vermicularis* egg positive rate of pre-school children in Chunchon, Korea (1999). Korean J Parasitol 2000;38:279-81.
3. Tomanakan K, Sanpool O, Chamavit P, Lulitanond V, Intapan PM, Maleewong W. Genetic variation of *Enterobius vermicularis* among schoolchildren in Thailand. J Helminthol 2020;94:e7.
4. Dunphy L, Clark Z, Raja MH. *Enterobius vermicularis* (pinworm) infestation in a child presenting with symptoms of acute appendicitis: A wriggly tale! Case reports. 2017:bcr-2017. doi: 10.1136/bcr_2.17-220473.
5. Moosazadeh M, Abedi G, Afshari M, Mahdavi SA, Farshidi F, Kheradmand E. Prevalence of *Enterobius vermicularis* among children in Iran: A systematic review and meta-analysis. Osong Public Health Res Perspect 2017;8:108-15.
6. Gunawardena S, Gunatilleke H, Ismail M. Prevalence of *Enterobius vermicularis* infection among schoolchildren attending four selected schools in the Hambantota district of Sri Lanka. Sri Lankan J Infect Dis 2013;3:106-10.
7. Zakharia K, Klair JS, Murali AR. Unusual etiology for transaminitis. Dig Dis 2019;37:518-20.
8. Nair GG, Balan P. Ovarian enterobiasis: a case report. Int J Res Med Sci 2018;6:1055.
9. Wendt S, Trawinski H, Schubert S, Rodloff AC, Mössner J, Lübbert C. The diagnosis and treatment of pinworm infection. Dtsch Arztebl Int 2019;116:213-9.
10. Djakovic A, Tappe D, Dietl J. [Diagnosis of and anthelminthic therapy for *Enterobius vermicularis* infections during pregnancy: Review of the literature and case report]. Z Geburtshilfe Neonatol 2006;210:147-52.
11. Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. Nat Rev Immunol 2011;11:375-88.
12. Ateş Y, Ergün H, Tüzün A, Bağcı S, Kurt İ, İnal A, *et al*. The lymphocyte subgroups and serum adenonise deaminase levels in the patients with familial Mediterranean fever. Ankara Acad J Gastroenteritis 2005;4:112-6.92-101.
13. Maizels RM, Pearce EJ, Artis D, Yazdanbakhsh M, Wynn TA. Regulation of pathogenesis and immunity in helminth infections. J Exp Med 2009;206:2059-66.
14. AL-Jaf EEH, Rahi ASA, Tofah JAR. Compartive study between molecular and classical methods for defection *Enterobius vermicularis* in Wasit Province. Master of Science in Biology-Zoology, College of Science, University of Wasit, 2018.
15. Al-Ukaelii SA, Al-Shaeb SM. Statically Analysis by Used SPSS Program. Jordan: Al-Shoroq House for Publishers and Advertisement Amaan; 1998.
16. Aziz Kadir MA, Amin OM. Prevalence of enterobiasis (*Enterobius vermicularis*) and its impact on children in Kalar/Sulaimania-Iraq. Tikrit Med J 2011;17:67-77.
17. Al-Daoody AAK, Al-Bazzaz ENH. Impact of *Enterobius vermicularis* infection on biochemical parameters in the blood of children in Erbil Province, Iraq. BMC Infect Dis 2020;20:1-6.
18. Jeandron A, Abdyldaieva G, Usubalieva J, Ensink JH, Cox J, Matthys B, *et al*. Accuracy of the Kato-Katz, adhesive tape and FLOTAC techniques for helminth diagnosis among children in Kyrgyzstan. Acta Tropica 2010;116:185-92.
19. Saed RS, Al-Masoudi HK. Estimation of some trace elements level in children with Entrobiasis in Babylon province, Iraq. Int J Health Sci 2022;6:1157-66.
20. AL-Quraishi MA. The relationship between the infestation with *E. vermicularis* and eneuresis in children. Med J Babylon 2006;3:1-2.
21. Mohsen RH. Assessment of the role of some immunological parameters in individual infected with *Entamoeba histolytica* in Babylon province. MSc thesis, College of Medicine, University of Babylon, 2020; p. 89.
22. Zhang Z, Wang L, Seydel KB, Li E, Ankri S, Mirelman D, *et al*. *Entamoeba histolytica* cysteine proteinases with interleukin-1 beta converting enzyme (ICE) activity cause intestinal inflammation and tissue damage in amoebiasis. Mol Microbiol 2000;37:542-8.
23. Habieb MHA. A study the correlation of some hormonal and immunological parameter in aborted women infected with toxoplasmosis in Babylon province. MSc Thesis, College of Medicine, University of Babylon, 2021; p. 143.
24. Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: New insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. Curr Opin Immunol 2011;23:598-604.
25. Sanchez AL, Mahoney DL, Gabrie JA. Interleukin-10 and soil-transmitted helminth infections in Honduran children. BMC Res Notes 2015;8:1-8.
26. Berner R, Bialek R, Forster J, Härtel C, Heininger U, Huppertz HI, *et al*. Vollständig Überarbeitete Auflage. In: Simon A, editor. DGPI Handbuch: Infektionen bei Kindern und Jugendlichen. Georg Thieme Verlag: Stuttgart, NY, USA; 2018. p. 7.